HISTOCOMPATIBILITY & IMMUNOGENETICS

A COLLECTION OF BRIEF REVISION NOTES (2022)

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DEDICATION

To Charity for all her love, support and patience.

PREFACE

These brief notes are intended to be used as a prompt for further study and are not meant to be taken as the final word transplantation science. I will update these as I continue to develop my own knowledge of the subject.

Disclaimer: I am a scientist and not a medical doctor. Information provided in this booklet should not be taken as medical advice. Please consult your medical doctor for specialist advice.

All opinions expressed in this booklet are mine alone and do not necessarily reflect the views of any employer, associate or sponsor.

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A. CLINICAL

CHAPTER 1 TRANSPLANT IMMUNOLOGY

LEUKOCYTE POPULATIONS

B CELLS

B cells are one of the major forms of lymphocytes, the others being T cells and NK cells. Taken together, B and T cells make up make up 20 – 40% of circulating white blood cells (Leucocytes). Lymphocytes are immunologically competent cells that assist neutrophils and monocytes in the defence of the body against pathogenic infection and invasion by other foreign bodies. B cells play a major role in the humoral arm of the adaptive immune response.

B cells are small mononuclear cells with dense nuclei and very little cytoplasm. In adults, they mature mainly in the bone marrow and later migrate to the spleen. Maturation of B cells involves several stages in which gene rearrangement of V, D and J gene segments takes place to produce B cells with antigen specific 'B Cell Receptors (Ig)', which translates into a large antibody specificity repertoire, from a relative small number of genes. Immature B cells that produce Ig receptors which bind too strongly to self-antigen undergo further gene rearrangement or are clonally deleted. Mature B cells are initially naive until they encounter antigen. Naive mature B cells are characterised by cell surface expression of immunoglobulin (IgM and IgD) and CD19.

Subsets of B cells include Mature B cells, antibody secreting Plasma cells, Memory B cells and Follicular B cells.

The diversity of the B cell receptor is such that virtually all foreign molecules can present determinants that can be recognised by the antigen binding sites of at least some B cells. B cells recognize their cognate antigen in its native form unlike T cells which only recognise antigen as peptides presented by MHC. In the normal immune response, upon B cell receptors binding antigen, the bound antigen is internalised, processed and presented on the cell surface as peptides bound to HLA class II molecules. These peptide bearing B cells continuously circulate through the peripheral lymphoid organs where the peptide-MHC class II complex can be

recognised by antigen specific armed CD4+ helper T cells which provide the co-stimulatory signal needed alongside T Cell Receptor (TCR) binding, resulting in activation of the B cells, leading to proliferation and differentiation into antibody secreting plasma cells. The T cells are armed by being presented with peptide by professional antigen presenting cells recognising the same antigen initially recognised by the B cells.

In the alloimmune response such as results from pregnancy, transfusion or transplantation, B cell clones that are activated are primarily those with B cell receptors that recognise HLA antigens, leading to production of anti-HLA antibodies. Other antibodies, such as anti-endothelial antibodies, anti-HPA and anti-MICA antibodies are also produced depending on the sensitising antigen. The route of alloimmunisation influences the type of antibody produced. Transfusion tends to lead to transient production of IgM and IgG antibodies whilst pregnancy and transplantation leads to production of IgG antibodies and memory B cells.

T CELLS

T cells are one of the major forms of lymphocytes, the others being B cells and NK cells. Taken together, B and T cells make up make up 20 – 40% of circulating white blood cells (Leucocytes). Lymphocytes are immunologically competent cells that assist neutrophils and monocytes in the defence of the body against pathogenic infection and invasion by other foreign bodies. T cells play a major role in the humoral and cellular arms of the adaptive immune response.

T cells are small mononuclear cells with dense nuclei and very little cytoplasm. In adults, they mature mainly in the Thymus. Maturation of T cells involves several stages in which gene rearrangement of V, D and J gene segments takes place to produce T cells with antigen specific 'T Cell Receptors (TCR)'.

As part of their maturation, T cells undergo both Positive and Negative selection to generate a self-tolerant repertoire of T cells. Mature T cells are initially naive until they encounter antigen. Naive mature T cells are characterised by cell surface expression TCR, CD3 and in most cases either CD4 or CD8. The diversity of the TCR is such that virtually all foreign molecules can present determinants that can be recognised by the antigen binding sites of at least some TCRs. Unlike B cells which

recognize their cognate antigen in its native form, T cells only recognise antigen as peptides presented by MHC. That is, the T cell response is MHC restricted.

Subsets of T cells include Helper T cells (TH cells), Cytotoxic T cells (CTLs), Memory T cells, Regulatory T cells (Treg cells) and Natural Killer T cells (NKT cells).

TH cells provide help to other cells in the adaptive immune response including B cells and cytotoxic T cells. TH cells are characterised by cell surface expression of CD4. They recognise peptide presented by class II MHC molecules. Depending on the cytokine environment at activation, TH cells can differentiate into different types of helper cells, including Th1, Th2, Th3 and Th17. Presence of IL-12 leads to a Th1 response with secretion of proinflammatory cytokines including IFNγ which drives the cellular immune response. In the normal immune reaction, the Th1 response targets obligate intracellular pathogens. Aberrant elevation of the Th1 response can lead to autoimmunity. Presence of IL-4 leads to a Th2 response with secretion of IL-4, 5, 9, 10 and 13 which mainly drive the humoral immune response. In the normal immune reaction, the Th2 response targets extracellular pathogen. Aberrant elevation of the Th2 response can lead to allergy. Presence of IL-10 and TGFβ in vitro leads to a Th3 response. Th3 type cells have a regulatory phenotype and are involved in mucosal immunity, protecting mucosal surfaces from infection. They secrete IL-10 and TGFβ which suppress Th1 and Th2 responses. Presence of IL-21 or TGFβ + IL-6 leads to a Th17 response with secretion of IL-17 and IL-21 overproduction of which leads to autoimmunity.

CTLs are killer cells characterised by cell surface expression of CD8. They recognise peptide presented by class I MHC molecules. Depending on the cytokine environment at activation, CTLs can differentiate into different types of cytotoxic cells, including TC1 which secrete TGFβ and TC2 which secrete IL-4, IL-5, and IL-10. In the normal immune response CTLs are involved in the killing of cells infected with obligate intracellular pathogens. When exposed to infected/dysfunctional somatic cells, cytotoxic T cells release the cytotoxins, perforin, granzymes and granulysin. Through the action of perforin, granzymes enter the cytoplasm of the target cell and their serine protease function triggers the caspase cascade, which is a series of cysteine proteases that eventually lead to apoptosis.

Treg cells play an important role in the induction and maintenance of immunological tolerance. They are characterised by cell surface expression of CD4, CD25 and FoxP3. In the normal immune reaction, Treg cells suppress IL-2 and IFNγ production to achieve immune homeostasis.

NKT cells bridge the innate and adaptive immune responses. They share properties of both normal T cells and of NK cell and are characterised by cell surface expression of an invariant TCR with limited TCR specificities and NK cell markers. They are CD1d-restricted, lipid antigen-reactive, regulatory T cell that can promote immunity to tumours and infection.

In the alloimmune response, Th1 cells are involved in acute and chronic cellular rejection in solid organ transplantation and in GvHD induction in stem cell transplantation and Th2 cell drive humoral antibody mediated rejection (AMR). Th17 cells are implicated in steroid resistant rejection. They contribute to glomerulonephritis in kidney and to rejection of cardiac allografts. They have also been detected in bronchoalveolar in lung transplant patients after acute rejection. Cytotoxic T cells are involved in acute and chronic cellular rejection following activation by helper T cells, which are themselves activated in the Direct and Indirect pathways of allorecognition.

NK CELLS

Natural Killer (NK) cells are one of the major forms of lymphocytes, the others being T cells and B cells. They comprise approximately 15% of all circulating lymphocytes. NK cells are involved primarily in the innate immune response but also contribute to the adaptive immune response. NK cells are characterised by cell surface expression of a number of receptors including Killer cell Immunoglobulin like Receptor (KIR) and NKG2. They also express CD56. NK cells have a rapid effector function due to germline encoding of receptors. This contrasts with B and T cell which undergo gene rearrangement of V, D and J gene segments before producing antigen specific receptors. In contrast to resting T cells, NK cells express receptors for numerous cytokines constitutively and produce IFNγ and other NK-derived cytokines rapidly in response to stimulation.

NK cells interact with target cells through a complex competitive interaction of activatory and inhibitory signals of cell surface receptors, including KIR and NKG2, binding to their cogent ligands on the target cells. The 'missing self' hypothesis holds that NK cell reactivity occurs when the ligand for inhibitory receptors are down regulated or 'missing', leading to activation signals prevailing. The ligands for KIR receptors are HLA class I molecules. These include HLA-C locus antigens with either Asn (Group 1) or Lys (Group 2) at position 80, the HLA-Bw4 epitope and some HLA A antigens. The ligands for NKG2 include the MHC class I like antigens MICA and MICB. NK cells kill by releasing small cytoplasmic granules, perforins and granzymes, which cause target cell lysis or apoptosis. NK cells acquire their effector function by having receptors engaging their cogent ligands during maturation, a process known as 'licensing'.

NK cells can be divided into two subsets based on their level of cell surface expression of CD56. These are $CD56^{bright}$ and $CD56^{dim}$. There is some evidence to suggest distinct immunological roles. $CD56^{dim}$ NK-cells are more naturally cytotoxic and expresses higher levels of Ig-like NK receptors and FCγ receptor III (CD16) than the $CD56^{bright}$ NK-cells. $CD56^{bright}$ cells produce abundant cytokines but have low natural cytotoxicity.

In the alloimmune response following stem cell transplantation, donor versus recipient alloreactivity can potentially be generated in HLA-C allele group and/or HLA-Bw4 group mismatched transplantation. Where the recipient does not possess a HLA class I allele group that the donor NK cell inhibitory KIR ligands recognise, the donor NK cell KIR receptors 'sense' the missing ligand in the recipient and can mediate an allo response given the presence of the right activating signals. The Perugia group showed in a series of studies of haploidentical, T cell depleted stem cell transplants that NK cell KIR ligand mismatching in the GvH direction benefitted patients with AML. Patient who received these NK cell KIR ligand mismatched transplants had significantly reduced rates of relapse and improved incidents of engraftment.

In an EBMT study looking at the role of NK cell alloreactivity in over 200 AML patients transplanted with cord blood, NK cell KIR ligand mismatching in the GvH direction was shown to be significantly associated with a reduced incidence of relapse and better leukaemia free survival.

Another mechanism of action of NK cell cytotoxicity in stem cell transplantation is the 'missing ligand' model which postulates that in the stem cell transplant setting, donor NK cells bearing KIR receptors for HLA class I antigens missing in the donor and which would therefore normally be hypo responsive, upon transplantation, can become activated and exert an anti-leukemic effect.

In solid organ transplantation, NK cells are known to infiltrate allograft, suggesting that activation of NK cells may be critical in the immediate post-transplant period. However, one recent study found that there was no associated between NK cell KIR ligand mismatch and the incidence of acute rejection. This reflects the outcome of a much larger CTS study involving over 2,700 deceased donor kidney transplants which found no effect of NK cell ligand matching on graft survival.

MONOCYTES

Monocytes make up 2-6% of circulating leucocytes. They are the largest of the leucocytes. They are myeloid progenitor derived mononuclear phagocytic cells with bean or horseshoe shaped nuclei and abundant cytoplasm.

Monocytes circulate in the blood for 1 – 3 days before leaving to enter tissues where they mature into either macrophages or some dendritic cell subsets. Monocytes appear to be capable of taking up and processing antigen both from the bone marrow and also from the blood during their transit from the marrow through blood into tissues, for delayed presentation to T cells later in their cell cycle. Once they leave the circulation and enter tissues, the maturation process starts and they are no longer considered Monocytes but are instead considered Monocyte derived cells (macrophages or dendritic cells).

The effector functions of macrophages include phagocytoses and destruction of pathogens and cellular debris, production of cytokines which regulate and participate in haemopoiesis, inflammation and cellular responses and processing and presenting antigens to T cells, i.e. act as Antigen Presenting Cells (APC).

Monocytes can be divided into three main subsets based on their cell surface expression of CD14 (a component of lipopolysaccharide) and CD16 (FCγ receptor III). The Classical Monocyte subsets have high expression of CD14 and are CD16 neg., Intermediate Monocytes have high expression of CD14 and low expression of CD16 and Non Classical Monocytes have low expression of CD14 and high expression of CD16. In the normal immune response to inflammation, there is a gradual increase first of Intermediate Monocytes from Classical Monocytes, followed by an increase in the Non Classical Monocytes.

In the alloimmune response following kidney transplantation, activated monocytes in peripheral blood secrete proinflammatory cytokines which contribute to the development of transplant glomerulopathy. Following stem cell transplantation, the innate immune cells including granulocytes, NK cells and monocytes, reconstitute earlier than the adaptive immune cells including B and T cells. There is evidence to suggest that monocytes are the first cells to engraft. This reconstitution of the innate cell population, including monocytes, restores innate immunity, allowing bacterial prophylaxis to be lowered thus lowering the risk of post stem cell transplant infections.

MACROPHAGES

Macrophages are leukocytes produced by differentiation and maturation of monocytes in tissues. Monocytes and macrophages are phagocytes. Macrophages function in both the innate and adaptive immune responses. Macrophages are resident in many tissues of the body including lymph nodes and the spleen as well as in the liver, kidneys, brain, bones, lungs and gastrointestinal tract. Macrophages are also recruited into tissue through positive chemotaxis during inflammation.

The effector functions of macrophages include 1) phagocytoses and destruction of pathogens and cellular debris, 2) production of cytokines which regulate and participate in hemopoiesis, inflammation and cellular responses and 3) processing and presenting antigens to T cells, i.e. act as Antigen Presenting Cells (APC).

The antigen presenting role of macrophages involves take up and presentation of processed peptide through MHC class II antigens mainly to armed effector helper T cells. Macrophages can stimulate naive T cells but not as effectively as dendritic cells

can. Macrophages that phagocytose pathogens into intracellular vesicles become activated by Th1 T cells into secreting lysosomes into those vesicles thus destroying the pathogens. Cytokines produced by macrophages include TNFα, IL-1, IL-6, IL-8 and IL-12. These are proinflammatory cytokines which attract and activate neutrophils and promote fibrosis of endothelial cells.

Macrophages are characterised by cell surface expression of CD14 (a component of lipopolysaccharide), Epidermal growth factor module-containing Mucin-like Receptor 1 (EMR1) and CD68.

In the alloimmune response following renal transplantation, early post-transplant macrophage activation has been associated with poorer long-term graft and patient survival.

GRANULOCYTES

Granulocytes are myeloid progenitor derived polymorphonuclear cells with characteristic cytoplasmic granules as seen in blood films. They are highly motile phagocytic cells. They can be divided based on their cytoplasmic and nuclear appearances into Neutrophils, Basophils and Eosinophils. The most abundant of these are the Neutrophils. The name of Neutrophils derives from their characteristic staining when treated with haematoxylin and eosin. Whilst Basophils stain dark blue and Eosinophils stain bright red, Neutrophils remain a neutral pink.

Neutrophils play a central role in host defence to infection and tissue injury. They are recruited into tissue through positive chemotaxis during inflammation by chemokines including IL-8 and INFγ. They rapidly engulf and kill any antibody or complement opsonised pathogen, damaged cells or cellular debris. Once they have completed their function, their timely removal from sites of inflammation is essential for resolution of inflammation. Neutrophils do not re-circulate to the blood but instead die, perhaps through apoptosis and turn into Pus.

Basophils have been shown to play a role in the induction and maintenance of specific types of Th2 cytokine dependent immunity and inflammation, particularly

those that cause hypersensitivity and allergic symptoms. The granules of Basophils contain vasoactive substances such as histamine as well as anticoagulants such as heparin. The granules also contain peroxidases and platelet activating factors. Basophils express IgE receptors on their cell surface. Presence of Th2 cytokines or binding of IgE antibodies triggers activation which caused Basophils to de-granulate and secrete their content, causing the hypersensitivity reactions and allergic disorders.

Eosinophils are proinflammatory cells which are recruited from the circulation by diverse stimuli. They modulate immune responses through an array of mechanisms including the secretion of an array of proinflammatory cytokines. They proliferate during an allergic reaction such as asthma, hayfever or eczema. Eosinophils are capable of either protecting or damaging the host. They can initiate antigen-specific immune responses by acting as Antigen Presenting Cells (APCs) or they can serve as major effector cells inducing tissue damage and dysfunction by releasing toxic granule proteins and lipid mediators. Eosinophil granules contain toxic basic protein and cationic proteins such as Cathepsin, peroxidases, as well as common lysosomal enzymes. They also contain substances that can neutralize mast cell and Basophil secretions.

In the normal immune response, antibodies directed against antigens on the granulocyte membrane are known to cause a variety of disorders including neonatal alloimmune neutropenia, autoimmune neutropenia, and drug-induced immune neutropenia. In addition, alloantibodies to granulocyte antigens can contribute to transfusion related acute lung injury (TRALI), febrile transfusion reactions and refractoriness to granulocyte transfusions.

Granulocyte therapy contributes to stem cell transplantation and to transfusion. In stem cell transplantation, Granulocyte colony stimulating factor (G-CSF) is used in the mobilization of hematopoietic stem cells and progenitors prior to peripheral blood stem cell harvest. In transfusion, Granulocyte transfusion can be used as supportive therapy in patients with life-threatening neutropenia caused by bone marrow failure or in patients with neutrophil dysfunction.

NEUTROPHILS

Neutrophils represent the body's primary line of defence against invading pathogens such as bacteria and against and tissue injury. Neutrophils are the most abundant (50-60%) of the leukocytes and the most abundant (60-70%) of the granulocytes. Neutrophils, like other granulocytes, are myeloid progenitor derived polymorphonuclear cells with characteristic cytoplasmic granules as seen in blood films. The other granulocytes are Basophils and Eosinophils. The name of Neutrophils derives from their characteristic staining when treated with haematoxylin and eosin. Whilst Basophils stain dark blue and Eosinophils stain bright red, Neutrophils remain a neutral pink colour.

Neutrophils are recruited into tissue through positive chemotaxis during inflammation by chemokines including IL-8 and INFγ. They are highly phagocytic, rapidly engulfing any cellular debris and engulfing and killing any damaged cells and antibody or complement opsonised pathogen. Once they have completed their function, their timely removal from sites of inflammation is essential for resolution of inflammation. Neutrophils do not re-circulate to the blood but instead die, perhaps through apoptosis and turn into Pus.

The granules of neutrophils contain the active killing agents. The granules of the neutrophil have traditionally been divided into two types, Primary and Secondary. More recent studies have however shown that there are in fact a continuum of several subtypes of granules with differences in protein content and propensity for mobilization. The granules contain cationic proteins, defensins, proteolytic enzymes, myeloperoxidases, lysozymes and other acid hydrolases which contribute to cell lysis and destruction.

Neutrophils are implicated in a number of diseases. Neutropeania can result from some forms of leukaemia or as a side effect of medication or some types of infection and can leave individuals highly susceptible to infections. In inflammatory conditions such as Rheumatoid Arthritis (RA) a potential role for Neutrophils in the persistence of inflammation and progression of joint damage has been identified. Neutrophils are found in high numbers within the synovial tissue and in joint fluid of rheumatoid joints.

Recovery of Neutrophil count following stem cell transplantation is one of the traditional indicators of immune reconstitution. Number of days post transplant to

Neutrophil counts reaching 0.5 x 10^9/kg patient weight gives a measure of engraftment. Peripheral blood stem cell transplantation (PBSC) generally has the fastest recovery time of around 15 days, depending on a number of factors including induction therapy and cell dose transplanted. Bone marrow transplantation has a typical recovery time of around 21 days. Cord blood has the longest recovery time and can take up to 35 days. Patients are at increased risk of infection during this neutropaenic period.

In renal transplantation, Neutrophil activation appears to play a role in ischaemia-reperfusion injury (IRI) and the consequent delayed graft function (DGF), contributing to a reduction in overall graft function.

BASOPHILS

Basophils contribute to the body's response to allergens. Basophils are the least abundant cells in the circulation occurring at less than 1%. Basophils are myeloid progenitor derived polymorphonuclear (usually have a bi-lobal) granulocytes. They have characteristic cytoplasmic granules as seen in blood films. The other granulocytes are Neutrophils and Eosinophils. Basophils stain dark blue when treated with haematoxylin and eosin. Eosinophils stain bright red, while Neutrophils remain a neutral pink colour, hence the name.

Basophils have been shown to play a role in the induction and maintenance of specific types of Th2 cytokine dependent immunity and inflammation, particularly those that cause hypersensitivity and allergic symptoms. This initially caused Basophils to be considered a redundant mast cell like population. Studies later showed however that Basophils and mast cells had distinct differentiation pathways and cell surface markers.

The granules of Basophils contain vasoactive substances such as histamine as well as anticoagulants such as heparin. They also contain peroxidases and platelet activating factors. Basophils express IgE receptors on their cell surface. Presence of Th2 cytokines or binding of IgE antibodies triggers activation which caused Basophils to degranulate and secrete their content, causing the hypersensitivity reactions and allergic disorders.

One study into the potential role of Basophils in renal transplantation found evidence of sensitized Basophils in post-transplant patients but there have been very few follow up studies.

EOSINOPHILS**ERROR! BOOKMARK NOT DEFINED.**

Eosinophils have multiple biological functions and contribute to a variety of immune defence mechanisms, including defence against parasitic infections, particularly parasitic worms and protozoa. Eosinophils make up 1 – 4% of circulating leukocytes. Eosinophils are myeloid progenitor derived polymorphonuclear, usually 2 – 4 four lobed, granulocytes with characteristic cytoplasmic granules as seen in blood films. The other granulocytes are Neutrophils and Basophils. Eosinophils stain bright red when treated with haematoxylin and eosin. Basophils stain dark blue, while Neutrophils remain a neutral pink

Eosinophils are proinflammatory cells which are recruited from the circulation by diverse stimuli. They modulate immune responses through an array of mechanisms including the secretion of an array of proinflammatory cytokines. They proliferate during an allergic reaction such as asthma, hay fever or eczema.

Eosinophils are capable of either protecting or damaging the host. They can initiate antigen-specific immune responses by acting as Antigen Presenting Cells (APCs) or they can serve as major effector cells inducing tissue damage and dysfunction by releasing toxic granule proteins and lipid mediators. Eosinophil granules contain toxic basic protein and cationic proteins such as Cathepsin, peroxidases, as well as common lysosomal enzymes. They also contain substances that can neutralize mast cell and Basophil secretions, thereby down modulating the allergic response. Eosinophils predominantly secrete their granule protein by regulated exocytosis and degranulation, thereby avoiding tissue damage by releasing granular content while migrating to effector sites.

Eosinophils participate in the process of acute rejection in solid organ allografts and their levels in kidney allografts do have a diagnostic value for acute rejection. Graft eosinophilia (high numbers of Eosinophils) is a sensitive and specific marker of acute rejection in liver allografts.

MAST CELLS

Mast cells are derived from the same common myeloid progenitor as the myeloblasts that differentiate into granulocytes and monocytes. Mast cells are resident in many tissues, particularly near surfaces exposed to the environment. They are involved in allergy reactions and in anaphylaxis. They also play an important role on wound healing, in clearance of enteric pathogens, visceral hypersensitivity and in intestinal cancer.

The granules of Mast cells contain vasoactive substances such as histamine as well as anticoagulants such as heparin. This characteristic is shared with Basophils, which initially caused Mast cells to be considered Basophils with specialised functions. Studies later showed however that Basophils and Mast cells had distinct differentiation pathways and cell surface markers. Mast cells express IgE receptors on their cell surface. Binding of IgE antibodies and many other stimuli, including products derived from either pathogens or the host during innate immune responses, triggers activation which causes Mast cells to degranulate and secrete their content, causing allergy reactions and anaphylaxis.

Mast cells are at the interface between the innate and acquired immune systems. They are capable of producing a vast array of both pro- and anti-inflammatory molecules, of acting as antigen presenting cells (APC) and of expressing a spectrum of costimulatory molecules. Thus depending on circumstances, mast cells are capable of either protecting or damaging the host or both.

In the alloimmune response in renal transplantation, Mast cells are believed to have a protective effect with regards to acute alloreactive responses through activation of Treg cells. However Mast cells are thought to have an adverse role in the progression of chronic organ rejection through the release of different profibrotic mediators and through increased collagen deposition.

DENDRITIC CELLS

Dendritic cells are the most potent of the Antigen Presenting Cells (APC) responsible for priming the immune response. Distributed throughout the tissues of the body, they possess surface pattern recognition receptors that recognize pathogen associated molecular patterns making them capable of initiating an immune response to infection. Two main subsets of dendritic cells have been described, the myeloid dendritic cells (mDC) and plasmacytoid dendritic cells (pDC) with the mDC being the more abundant. These are traditionally believed to derive from myeloid and lymphoid precursors respectively, though more recently both cells lines have been proposed to derive from both cell types of precursors. Dendritic cells are capable of stimulating naive T cells and are therefore known as professional APC's. More recently, an additional role for dendritic cells as important mediators of peripheral immune tolerance and maintenance of immune homeostasis has been described.

In the normal state, with absence of inflammation, dendritic cells reside in the peripheral tissue as immature interstitial cells. In this state, their main characteristics are an efficient capability of ingesting exogenous antigen and expressing them on the cell surface in association with MHC antigens as well as low levels of expression of costimulatory molecules. During an inflammatory response, maturation of dendritic cells is triggered leading to a reduced endocytotic capability as well as an increased level of expression of costimulatory molecules and distinct chemokine receptors and upregulation of intercellular adhesion molecules required for interaction with T cells. Mature dendritic cells migrate to T cell areas of the secondary lymphoid tissue where they stimulate naive T cells.

Dendritic cells play a central role in the alloimmune response. After kidney transplantation, donor derived dendritic cells, triggered into maturation by the transplant Ischemic and Reperfusion Injury (IRI) as well as locally released pro-inflammatory cytokines, migrate from the kidney to the recipient lymphoid organs where they stimulate the host immune response through the direct and indirect routes of allorecognition. In stem cell transplantation, the number of circulating plasmacytoid and myeloid dendritic cells and their origin, donor or recipient, have been shown to be associated with the initiation of acute Graft versus Host Disease (aGvHD), relapse and graft failure. The recognition of alloantigen presented by residual host dendritic cells to donor T cells in the direct pathway of allorecognition initiates GvHD. Ongoing antigen presentation involves donor derived dendritic cells presenting host antigen to donor T cells in the indirect pathway of allorecognition. Some studies have shown that the absolute numbers of circulating dendritic cells

post stem cell transplantation is an independent predictor of aGvHD. Patients with aGvHD after stem cell transplantation have lower numbers of circulating mDC and pDC compared to healthy individuals but do have a higher number of dendritic cells in affected areas such as the skin.

LANGERHANS CELLS

Langerhans cells are a specific kind of white blood cell. They are found largely in the squamous epithelia of epidermis - the outer layer of the skin, as well as in lymph nodes. Langerhans cells are the prototypic non-lymphoid members of the dendritic cell family. The cells in this family exhibit prominent dendritic morphology and potent antigen-presenting cell capabilities. These features make this family of cells distinct from monocytes, macrophages and other bone marrow-derived cells. Langerhans cells originate from the bone marrow and then migrate into the epithelium to perform the function of antigen recognition and presentation.

Langerhans cells express lower levels of cell surface MHC class II molecules and are less capable of stimulating resting T cells in mixed lymphocyte reactions when compared to 'mature' dendritic cells. However, upon culturing in vitro for 2–3 days, Langerhans cells upregulate MHC class II expression, as well as costimulatory molecules and become functionally 'mature' and potent stimulators in mixed lymphocyte reactions.

Langerhans cells, like other APCs, must quickly respond to the invading bacterial structures. This function is accomplished through toll-like receptors (TLRs), which are a major class of signalling receptors. Receptors involved in antigen recognition are toll-like receptors (TLRs) and C-type lectin receptors. TLRs are pattern recognition receptors associated along with pathogen-associated molecular patterns (PAMPs). Langerhans cells express C-type lectin receptors including membrane bound C-type lectins (CD207), Mannose receptors (CD206), CD205 and CD209.

After the antigen recognition, signal transduction is mediated via the interleukins and MyD88 resulting in the release of NFk-β, which is an important factor in Langerhans cells and dendritic cell maturation. Interaction of the PAMPs with the C-type lectin receptors and toll-like receptors and resultant stimulation of Langerhans cells brings about antigen presentation and elicitation of cell-mediated immune response.

B CELL RECEPTOR (IGG)

STRUCTURE AND FUNCTION OF THE B CELL RECEPTOR

The IgG or Antibody molecule is a large, roughly 'Y' shaped molecule, composed of two types of polypeptide chains, a 50KDa heavy (H) chain and a 25KDa light (L) chain. Each IgG molecule consists of two heavy chains and two light chains. The heavy chains are linked to each other by disulphide bonds and each heavy chain is linked to a light chain by disulphide bonds. The two heavy chains and two light chains of each IgG molecule are identical to each other.

The light chain is made up of two domains, each folded into a structure known as the immunoglobulin fold. The immunoglobulin fold is a structure that is mimicked by many other molecules. It essentially consists of two sheets of anti-parallel β-strands folded in a Greek-Key motif and sandwiched together.

The variability in the sequence of the light chain is mainly limited to the first domain, also known as the Light chain Variable domain (VL). The other domain is known as the Light chain Constant domain (CL). The heavy chain is made up of one Variable domain (VH) and three Constant domains (CH1, CH2 and CH3).

The Variable Domains of the Light and Heavy chains (VL and VH) fold together to make up the Variable region of the IgG molecule and confer on it its antigen binding specificity. The VL and VH and CL and CH1 domains fold together to make up the Fab or antibody binding fragment of the molecule. The CH2 and CH3 domains of the two heavy chains fold together to form the Fc or crystallizable fragment of the molecule.

Sequence variability is not distributed throughout the Variable domain but is concentrated instead into three hypervariable regions each of the VL and VH domains. These regions are named Hypervariable regions 1 – 3 (HV1, HV2 and HV3). Together, these Hypervariable regions code for hypervariable loops at the tips

of the Variable domains. These loops determine the specificity of the IgG molecule and are therefore known as Complementarity Determining Regions (CDR1, CDR2 and CDR3).

IgG has several effector functions including:

- The neutralization of pathogen by coating the pathogen and occupying its bind sites so that it cannot affect cells
- The opsonization of pathogen by marking it for phagocytoses by phagocytes including macrophages
- The recruitment of Complement to lyse pathogen cells

GENERATION OF B CELL RECEPTOR DIVERSITY

The total number of antibodies that humans can potentially produce exceeds 10^{11}. Before the sequences of the immunoglobulin genes were known, there were two theories put forward to explain how this level of diversity could be obtained. The germline theory held that all the possible variations were coded for by separate genes, whilst the somatic diversification theory held that immunoglobulin was coded for by a limited number of sequences which were rearranged throughout an individual's lifetime. The somatic diversification has proved to be essentially correct.

The vast majority of the diversity of an immunoglobulin molecule lies in the variable domain. The variable domain is coded for by a set of gene segments which are randomly selected and rearranged in a process known as somatic recombination. Further diversification is later introduced when the B cell is activated. This consists of point mutations and is known as somatic hypermutation.

The immunoglobulin molecule is made up of a light and heavy chain. The light chain variable domain is coded for by a set of V gene segments (VL) and a set of joining or J gene segments (JL). The heavy chain variable domain is coded for by a set of V gene segments (VH), a set of diversity of D gene segments (DH) and a set of J gene segments (JH).

The somatic recombination process of the light chain involves the random selection of a VL gene segment which is then joined to a randomly selected JL gene segment. This is catalysed by recombination activation genes (RAGs). VL genes are prevented from accidentally joining to other VL gene segments by the use of recombination signalling sequences (RSS) which consist of a heptamer, a 12 or 23 nucleotide spacer and a nonamer. This is the 12/23 rule and it prevents gene segments with a 12 nucleotide spacer being joined to other 12 spacer segments and gene segments with a 23 nucleotide spacer being joined to other 23 spacer segments. This same process prevents J, D and VH gene segments from self-joining. The joining process is not precise and introduces further Junctional diversification.

The variable heavy chain is created by a DH gene segment, randomly selected, joining to a JH segment and then the combination joining to a randomly selected VH gene segment. Junctional diversity is introduced during these joins.

Further combinational diversity is introduced by the different combinations of heavy and light chain variable regions that pair to form the antigen binding site.

Finally, upon activation, further diversification is introduced in a process known as somatic hypermutation.

PRODUCTION OF ALLOGENIEC ANTIBODIES

Allogeneic HLA antibodies can be produced in response to exposure to foreign HLA through blood transfusion, pregnancy or transplantation. Allogeneic HLA antibodies are produced in response to foreign HLA when B cell surface immunoglobulin receptors specifically bind the foreign HLA and the B cell is triggered by armed CD4+ T cells into activation and differentiation into antibody secreting plasma cells. The antibodies secreted have the same specificity as the surface immunoglobulin of the activated B cell.

When a B cell immunoglobulin receptor binds to allogeneic HLA, the bound HLA is internalised, processed and presented on the cell surface as peptides bound to HLA class II molecules. The peptide-MHC class II complex can be recognised by antigen

specific armed T cells, resulting in activation of the B cells, leading to proliferation and differentiation into plasma cells. The T cells are armed in the direct and indirect pathways of allorecognition by presentation of peptide from the same allogeneic HLA by professional antigen presenting cells. The direct pathway involves donor derived dendritic cells presenting allogeneic HLA peptides to host CD4+ helper T cells. The indirect pathway involves allogeneic HLA being internalised, processed and presented to host CD4+ helper T cells as peptide-MHC complexes by host dendritic cells. Both the direct and indirect routes result in antigen specific activated CD4+ T cells which are capable of providing the second signal that the B cell requires, along with binding of antigen, in order to become activated. The trapping of B cells in the T cell zone of secondary lymphoid tissues raises the probability that the otherwise low frequency of armed T cells of the right specificity would make such as encounter.

T cell are triggered to synthesis and secrete a number of cytokines such as IL-4 and other effector molecules including CD40 ligand which binds the B cell CD40 receptor and helps drive the resting B cell into the cell cycle, by the recognition of the allogeneic HLA peptide in the context of HLA class II on the B cell by the armed T cell triggers.

The activated B cells proliferate for several days before eventually differentiating into Plasma cells capable of secreting antibodies. Plasma cells can have a wide range of life span with some living for only days to weeks but others are long lived and result in persist antibody production.

Allogeneic antibodies produced following transfusion can be IgG or IgM and are not generally long lasting. Antibodies developed as a result of pregnancy or transplantation are however generally IgG and are long lasting.

Other allogeneic antibodies that be produced include anti-HPA antibodies, anti-endothelial cell antibodies and anti-MICA antibodies.

Female transplant recipients who subsequently have children are at risk of developing anti-paternal antibodies and need to be carefully managed if these antibodies are also donor specific.

T CELL RECEPTOR

STRUCTURE AND FUNCTION OF T CELL RECEPTOR

The T cell receptor (TCR) is an antigen receptor molecule on the surface of T cells, responsible for the recognition of antigen presented to T cells by MHC molecules leading potentially to the activation of the T cell and an immune response to the antigen. The T cell receptor is a heterodimer consisting of two transmembrane glycoprotein chains, the α and β chains (there are also a small proportion of γδ chains) each with two domains, which are linked by a disulphide bond.

The TCR bears some structural similarity to the Fab fragment of an antibody molecule. The α and β chains each posses a constant (C) domain proximal to the cell membrane and a variable (V) domain distal from the membrane. This gives a Vα and Cα domain for the α chain and a Vβ and Cβ domain for the β chain. The variable and constant domains of the α chain dimerise with the variable and constant domains of the β chain. All domains adopt the classical immunoglobulin fold with two anti-parallel β-sheets adopting a Greek key motif held together into a 'sandwich' by a disulphide bond. The Cα domain does differ a little in having part of the β sheet replaced by loosely packed strands and a short segment of α helix. Like the variable region of the Fab fragment of antibodies, the variability of amino acids in the V region of the TCR is not evenly distributed throughout the sequence but is concentrated into hypervariable regions which code for the hypervariable loops. These are brought together by the α and β chain at the tip of the TCR to form the Complementarity Determining Regions (CDRs) which make contact with the MHC. The alignment of the TCR CDRs differs slightly from those of antibody molecules. The Vα CDR2 loop for instance is oriented roughly at 90 degrees for the equivalent antibody CDR. The Vβ domain includes a fourth hypervariable region which does not have an equivalent in antibodies.

The short cytoplasmic tail of the TCR means it cannot directly signal when it binds to a peptide-MHC complex. Instead the TCR is associated on the cell membrane with a group of non polymorphic signalling molecules collectively called CD3 which transmit an intracellular signal when the TCR binds to a peptide-MHC complex.

CD3 is made up of one γ and δ and two ε molecules which all have in their extracellular domains some limited sequence homology to the immunoglobulin domain. These molecules have small cytoplasmic domains and transmembrane domains with negatively charged residues. In the membrane, these negatively charged residues form salt bridges with the positively charged residues in the transmembrane region of the TCR. The TCR-CD3 receptor complex is completed by two other invariant proteins ζ and η which form dimmers linked by disulphide bonds. At the T cell surface therefore, the TCR-CD3 complex is expressed as an αβ (or γδ) heterodimer, in association with CD3γε and CD3δε dimmers with an intracellular ζζ homodimers or a ζη heterodimer.

GENERATION OF T CELL RECEPTOR DIVERSITY

T cell gene rearrangement takes place in the thymus. The mechanism used to generate T cell diversity is essentially the same as that used for the generation of B cell diversity but without the somatic hypermutation on activation.

The T cell receptor is made up of α and β chains. The α chain is coded for by a set of V gene segments (Vα) and a set of joining or J gene segments (Jα). The β chain variable domain is coded for by a set of V gene segments (Vβ), a set of diversity or D gene segments (Dβ) and a set of J gene segments (Jβ).

The somatic recombination process of the α chain involves the random selection of a Vα gene segment which is then joined to a randomly selected Jα gene segment. This is catalysed by recombination activation genes (RAGs). Vα genes are prevented from accidentally joining to other Vα gene segments by the use of recombination signalling sequences (RSS) which consist of a heptamer, a 12 or 23 nucleotide spacer and a nonamer. This is the 12/23 rule and it prevents gene segments with a 12 nucleotide spacer being joined to other 12 spacer segments and gene segments with a 23 nucleotide spacer being joined to other 23 spacer segments. This same process prevents J, D and Vβ gene segments from self joining. The joining process is not precise and introduces further Junctional diversification.

The variable β chain is created by a randomly selected Dβ gene segment joining to a randomly selected Jβ segment and then the combination joining to a randomly selected Vβ gene segment. Junctional diversity also is introduced during these joins.

Diversity is concentrated in the CDR3 loop, contributed to by the joining of the β chain DJ gene segments.

THE MAJOR HISTOCOMPATIBILITY COMPLEX

INTERNATIONAL HISTOCOMPATIBILITY WORKSHOPS

The Major Histocompatibility Complex (MHC), known as the Human Leukocyte Antigen (HLA) system in humans, is the most polymorphic region in humans. Since the identification of what came to be named the MHC in 1958, there are now, as of Mar 2021, are a total of 29,417 HLA and related alleles described - see http://hla.alleles.org/alleles/index.html. The extensive polymorphism of the HLA region is believed to have been driven by the evolutionary pressure to detect and mount an immune response to infectious pathogens.

This huge advance in our knowledge of the MHC system has been achieved largely due to the very early appreciation of the scope of work necessary to elucidate the HLA system and the willingness of the earlier researchers in this field to collaborate. This international group of investigators were willing to share reagents and unpublished data. The first HLA antigens were defined by individual groups using their own reagents, antisera and cell panels, identified locally. An exchange of reagents was necessary to compare antisera to standardize the definition of antigens and to establish a common nomenclature. The researchers agreed to come together in a workshop to create the opportunity to exchange reagents for mutual study.

The first Histocompatibility Testing Workshop was organized by Dr Bernard Amos and held in his laboratory at Duke University in Durham, North Carolina in 1964. It involved scientists from several countries. It was at this workshop that Paul Terasaki introduced the lymphocytotoxicity test for serologic typing and crossmatching,

describing the first positive leukocyte antibody crossmatch test associated with hyperacute renal graft rejection.

This first workshop proved very successful, leading to more extensive collaborations in the following years.

In addition to providing a mechanism for exchanging reagents, the International HLA Workshops have also been on the forefront of promoting new technology and disseminating both reagents and technical skills worldwide. This has been an invaluable resource for stimulating immunogenetics research and facilitating rapid translation of new technology and knowledge to patient care.

MHC GENETICS

MHC genes are encoded on the short arm of chromosome 6 (6p21.31). The MHC is the most polygenic region in humans with over 220 genetic loci identified. The MHC genes are located in clusters, with the genes that code for the class II α and β chains centromeric of the gene that codes for the class I α chain. The gene that codes for the class I β chain is located outside of the MHC on chromosome 15. The class I and II genes are separated by a cluster of class III genes which code for several components of the complement system and for other molecules involved in the immune response such as TNF.

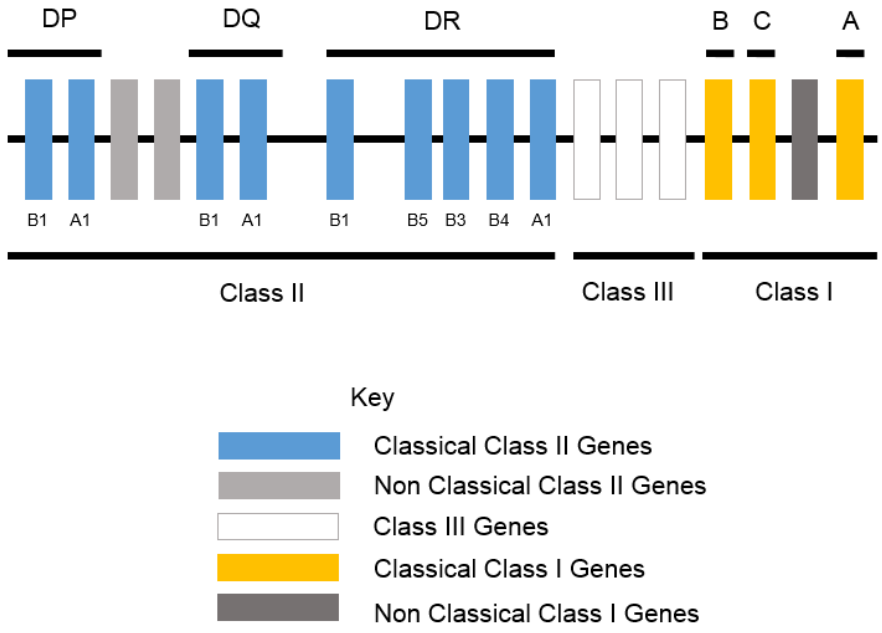


Figure 1 - MHC Genes

GENERATION OF MHC DIVERSITY

The huge diversity of the HLA system has been driven evolutionarily by survival advantage to disease, the so-called Pathogen Mediated Selection theory. Different populations in different parts of the world have been exposed to different pathogens, thus driving the survival of certain HLA types which confer protection, over other HLA types. Without HLA diversity, a single disease would be able to wipe out a whole population.

Proposed types of Pathogen Mediated Selection theory include:

- The Heterozygous advantage theory which proposes that heterozygous individuals will be able to present a wider array of peptides to T cells
- The rare allele theory which proposes that pathogen may evolve to overcome the common alleles but not a given rare allele
- The fluctuating selection hypothesis which states that HLA diversity is generated as the frequency of various pathogens in the population fluctuate over time

The mechanism by which diversity is generated include point mutation and gene conversion. *de novo* MHC sequences are mostly likely generated by point mutations. New haplotypes on the other hand are likely generated by gene conversion in which transferring of sections of DNA within and across MHC loci takes place.

STRUCTURE AND FUNCTION OF MHC

In humans, the MHC molecules are arranged into two classical sets - MHC class I and II both with different functions.

HLA class I molecules are heterodimeric, membrane bound glycoproteins made up of a polymorphic 43 kDa α or heavy chain, in non-covalent association with a non-polymorphic β2 microglobulin (12 kDa) protein. The class I molecule is anchored to the cell membrane by the α-chain. The α-chain is made up of three extra cellular domains, α1, α2 and α3, a transmembrane region and a cytoplasmic chain.

The α3 domain and β2 microglobulin are proximal to the cell membrane and have a folded structure that resembles that of an immunoglobulin C domain. The α1 and α2 domains are distal to the cell membrane and fold together into a structure consisting of a floor of anti-parallel β pleated sheets flanked by a pair of anti-parallel α helix side walls. The folding of the α1 and α2 domains creates a groove into which peptides can bind. Peptides typically of 8 – 10 amino acids in length bind into this groove through a series of hydrogen bonds and ionic interactions at each end of the peptide. The vast majority of the polymorphism of the HLA class I gene codes for amino acids that line this peptide binding groove and therefore have a direct impact on the nature of the peptide that can bind.

The main function of HLA class I molecules is to present cytosolically derived (intracellular) peptides to the T cell receptor of CD8+ cytotoxic T cells. Where the peptides are pathogen derived, this can lead to an immune response resulting in the killing of the infected cell.

HLA class II molecules are heterodimeric membrane bound glycoproteins made up of two non-covalently associated polymorphic chains, α (34 kDa) and β (29 kDa).

Both chains contribute two domains each, α1 and α2 and β1 and β2 respectively. The α2 and β2 domains are proximal to the cell membrane and have a folded structure that resembles that of an immunoglobulin C domain. The α1 and β1 domains are distal from the cell surface and fold together into a structure consisting of a floor of anti-parallel β pleated sheets flanked by a pair of anti-parallel α helix side walls. The folding of the α1 and β1 domains creates a groove into which peptides can bind. The groove is open at both ends which allowing long peptides to bind. Peptides bound by HLA class II molecules are typically longer than peptide bound by HLA class I molecules. The vast majority of the polymorphism of the HLA class II gene codes for amino acids that line this peptide binding groove, mainly in the β chain but also in the α chain.

The main function of HLA class II molecules is to present extracellular derived peptide to the T cell receptor of primarily CD4+ helper T cells thereby eliciting an immune response, including B cell activation for production of antibodies and cytotoxic T cell activation.

ANTIGEN PROCESSING AND PRESENTATION

MHC class I molecules bind cytosolically derived peptides within the endoplasmic reticulum (ER) and present them at the cell surface to CD8+ cytotoxic T cells. Binding of peptide is crucial to the folding of the class I molecule and its stability. The folding of the MHC class I molecule and the binding of the peptide involves the coordinated action of a number of proteins. The assembly of the 'MHC class I molecule - peptide complex' begins with the interaction of the heavy chain containing the α1, α2 and α3 extracellular domains with the chaperone molecule calnexin which retains the molecule in a partly folded state in the ER (7). This complex then assembles with the β2 microglobulin. Upon assembly with β2 microglobulin, the partly folded 'α chain - β2 microglobulin heterodimer' dissociates from calnexin and binds to the calnexin homologue calreticulin which has a similar chaperon function. The partly folded 'α chain - β2 microglobulin - calreticulin MHC class I molecule' is then recruited into a peptide loading complex (PLC) which involves the protein tapasin forming a bridge between the MHC class I molecule and the Transporter Associated with antigen Processing (TAP). The PLC also includes the ER oxido-reductases ERp57 which is involved in disulphide bond formation. The MHC class I molecule is retained in the ER until released by the binding of peptide.

The PLC maintains the molecule in a state which allows it to bind peptides transported into the ER by TAP. Molecules within the PLC contribute to a peptide editing function, replacing low affinity peptide with higher affinity ones. Peptides bound by MHC class I are generally 8 – 10 amino acids long and are generated from cytosol-derived endogenous proteins that are processed into peptides by the protease complex Proteasome. These peptides are optimised at the C terminus for binding to MHC class I but are often extended at the N terminus. Once in the ER, an ER resident enzyme, the ER aminopeptidase associated with antigen processing (ERAAP) cleaves N-extended peptides at the N terminus, optimising the peptide for binding to MHC class I. Upon binding high affinity peptide, the MHC class I molecule completes its folding, the PLC disassembles and the 'MHC class I – peptide complex' is able to leave the ER and is transported to the cell surface.

The MHC class II molecules bind extracellularly derived peptides for presentation to CD4+ T helper cells in the endocytic pathway at a site that is known as the MHC class II containing compartment (MIIC). The peptides are generated from extracellular antigen which are taken up into intracellular vesicles in the cell, typically macrophages and dendritic cells. The pH of the endosomes progressively decreases resulting in activation of proteases and cleavage of the antigen into peptide fragments which bind to MHC class II in MIIC for presentation at the cell surface.

The MHC class II molecule however starts its transport towards the MIIC via the ER and therefore needs to be protected in the ER from prematurely binding peptide. Peptide binding by MHC class II in the ER is prevented by a protein known as the Invariant chain (Ii). The Ii forms a trimer, with each of its subunits binding non-covalently with an MHC class II heterodimer, with the Ii polypeptide chain lying within the peptide binding groove thus blocking it. Whilst the 'MCH class II – Ii complex' is forming, it is associated with the chaperon molecule calnexin. Once fully folded, the 'MHC class II molecule – Ii complex' is released from calnexin and invariant chain chaperones MHC II either directly into MIIC or indirectly by internalising 'MHC class II – Ii complexes' from the cell surface into MIIC. In the MIIC, Ii is degraded by a series of late endosomal proteases, including cathepsin S and L, to leave a fragment of peptide known as the Class II associated Invariant chain peptide (CLIP) in the peptide binding groove. HLA-DM then assists in the exchange of CLIP for relevant exogenous peptide prior to transport of the stable 'MHC class II – peptide complex' to the cell surface. HLA-DM is an MHC class II homologue which stabilises the MHC class II molecule as it releases CLIP and binds high affinity peptide.

Autoimmunity develops when there is an imbalance between the immunological effector mechanisms and the regulatory mechanisms originating from defective elimination and/or control of self-reactive lymphocytes. All autoimmune diseases are believed develop through phases of initiation, propagation and resolution.

One proposed hypothesis for initiation of autoimmunity is that polymorphisms in various genes result in defective regulation or reduced threshold for activation of autoreactive lymphocytes. This then allows environmental factors such as infections, trauma etc. to initiate or augment activation of self-reactive lymphocytes that have escaped thymic control.

Autoimmune diseases normally manifest during the propagation phase. Propagation of the autoimmunity is maintained by the fact that it is driven by self-antigens. Tissue damage and alterations in self-proteins then leads to generation of new antigenic epitopes i.e. epitope spreading. In addition, the autoimmune reaction creates an inflammatory environment, with cells of the immune system interacting to produce cytokines and other mediators that amplify the reaction. On going propagation of the autoimmune response is propagated and sustained by an accumulation of effector cells in tissues.

Finally, autoimmune reactions can be controlled though they can flare up again. This control involves the induction and activation of regulatory mechanisms that limit the effector response and restore the balance between effector and regulatory mechanisms, with T regs playing a key role.

THE HLA NOMENCLATURE SYSTEM

HLA alleles are named using a convention which encodes the genetic and protein differences between the alleles within the designation of each allele as well as encoding the gene expression. The allele designation is prefixed with ‘HLA’ to indicate that it is a HLA type, followed by a hyphen separator and then the gene, i.e. -A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5, -DQA1, -DQB1, -DPA1, -DPB1. The allele designation is then made up of up to four sets of digits or fields, each separated by a colon. Finally, the allele designation may be suffixed by a letter.

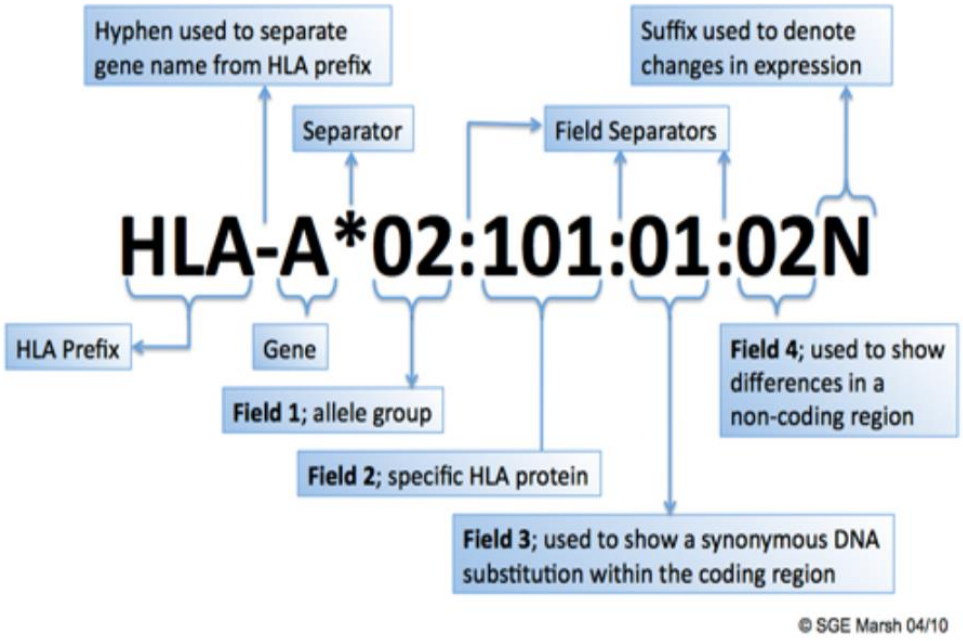


Figure 2 - Nomenclature image from http://hla.alleles.org

The first field describes the HLA type. This often corresponds to the serological antigen encoded by this allele. The next field describes the specific protein encoded by the allele. Alleles whose designations differ in the first two fields have nucleotide substitutions that change the amino acid sequence of the encoded protein.

HLA alleles that differ only by synonymous or silent mutations within the coding sequence are given different third field designations.

HLA alleles that only differ by sequence polymorphisms in the introns or in the 5' or 3' untranslated regions are given different forth field designations.

The letter suffixes indicate the level of expression of the HLA allele. Those alleles which have been shown not to be expressed are known as 'Null' alleles and are given the suffix 'N'. The suffix 'L' indicates 'Low' cell surface expression. The 'S' suffix indicates 'Secreted' molecules not present on the cell surface. The suffix 'C' indicates

presence of the antigen in the 'Cytoplasm' and not on the cell surface. The suffix 'A' indicates an 'Aberrant' expression. The suffix 'Q' indicates 'Questionable' expression.

REGISTERING NEW HLA ALLELES

H&I laboratories that identify new alleles can submit them to the European Bioinformatics Institute (EBI) for inclusion in the Immuno Polymorphism HLA Database (IPD-IMGT/HLA) of the international ImMunoGeneTics project (IMGT) at https://www.ebi.ac.uk/ipd/imgt/hla/subs/submit.html. Once accepted the allele will be assigned a new name. New allele submissions must meet a number of mandatory requirements for acceptance (reproduced below):

1. Where a sequence is obtained from cDNA or where PCR products are subcloned prior to sequencing, several clones must have been sequenced
2. Sequencing must always be performed in both directions
3. If direct sequencing of PCR amplified material is performed, products from at least two separate PCR reactions must have been sequenced
4. In individuals who are heterozygous for a locus, where one of the alleles is novel, the novel allele must be sequenced in isolation from the second allele. Thus an allele sequence which is derived using a sequence based typing (SBT) methodology, where both alleles of a heterozygous individual are sequenced together, is insufficient evidence for assignment of an official designation
5. Sequence derived solely from the primers used to amplify an allele must not be included in the sequence that is submitted
6. Where possible, a novel sequence must be confirmed by typing of genomic DNA using a method such as PCR-SSOP or PCR-SSP. Where a new sequence contains either a novel mutation or a previously unseen combination of nucleotides (sequence motif), this must be confirmed by a DNA typing technique. This may require the use of newly designed probes or primers to cover the new mutation; these reagents should also be described.
7. An accession number in a databank must have been obtained. Sequences may be submitted to these databases online at the following addresses:
   EMBL: /embl/Submission/index.html
   GenBank: http://www.ncbi.nlm.nih.gov/Genbank/submit.html
   DDBJ: http://www.ddbj.nig.ac.jp/submission-e.html
8. Full length sequences are preferable though not essential; the minimum requirements are exons 2 and 3 for an HLA class I sequence and exon 2 for an HLA class II sequence

9. Where a novel sequence differs only within an intron or other non-coding part of the gene, a full-length sequence must be obtained, which covers all coding and non-coding regions. In the absence of a full-length genomic sequence from the most closely related allele, it may be required that this also be sequenced and submitted before a name can be assigned to the novel sequence
10. Where possible, a paper in which the new sequence is described should be submitted for publication. Draft publications can be submitted to the database by email or fax (+44 20 7284 8331)
11. Sequences derived solely from tumour material will not be considered for nomenclature
12. The complete HLA type for HLA-A, -B, -DRB1 genes must be submitted for the material in which a novel allele has been defined. In addition, the sample should have been characterised for the second allele at the locus of interest in a heterozygous individual.
13. DNA or other material, preferably cell lines, should, wherever possible, be made available in a publicly accessible repository or alternatively, at least in the originating laboratory. Documentation on this will be maintained by the WHO Nomenclature Committee
14. Submission of a sequence to the Nomenclature Committee must be performed using the online tool available at /imgt/hla/subs/submit.html. Researchers will be expected to complete a questionnaire/checklist relating to the sequence and provide a comparison of their new sequence with known related alleles. If the sequence cannot be submitted using the online web tools researchers should contact IPD-IMGT/HLA directly for details of alternative submission methods.

NON-MHC GENES

THE KIR RECEPTOR COMPLEX

The adaptive immune response recognises infection through presentation of pathogen derived peptides in association with MHC to the host T cells. One of the mechanisms which pathogens use to evade this immune response is to down regulate their MHC cell surface expression. Natural Killer (NK) cells are able to detect altered expression of MHC through a number of cell surface receptors leading to target cell lysis. These receptors include the killer immunoglobulin like receptors (KIR), which are also expressed on some effector T cells. In humans, the

KIR gene cluster is located on chromosome 19. KIR genes are both polygenic and polymorphic. The KIR gene cluster codes for 15 expressed KIR genes and 2 pseudo genes.

The ligands for KIR receptors are HLA class I molecules. These include HLA-C locus antigens with either Asn (Group 1 HLA-C antigens) or Lys (Group 2 HLA-C antigens) at position 80, the HLA-Bw4 epitope and some HLA-A antigens.

KIR receptors binding to HLA class I are either inhibitory or stimulatory with the overall effect of NK cell interaction with the target cell dependent on the balance between these inhibitory and stimulatory signals. It is thought that the inhibitory KIR's bind class I with greater affinity than the corresponding activating KIR with the effect that under normal circumstances the inhibitory signal prevails. The 'missing self' hypothesis holds that NK cell alloreactivity occurs when the ligand for inhibitory KIR receptors is down regulated or 'missing', leading to activation. This however requires that KIR receptors engage their cogent HLA class I molecules during maturation to acquire effector function. NK cells that express only inhibitory KIRs for absent HLA class I molecules are hypo responsive in the non-transplant setting.

Inhibitory KIR receptors possess long cytoplasmic tails with immunoreceptor tyrosine based inhibitory motifs (ITIMs). Activating KIR receptors have short cytoplasmic tails that pair with adaptor molecules with immunoreceptor tyrosine based activating motif (ITAMs). The nomenclature for KIR receptors therefore includes an 'L' (long tail) for inhibitory KIR's and an 'S' (short tail) for activating KIR's. The nomenclature also includes 'P' for pseudo genes. The inhibitory and activating KIR receptors share sequence and structural similarities in their extracellular domains. KIR's have either 2 or 3 extracellular immunoglobulin domains and this is reflected in their nomenclature as either '2D' or '3D', giving KIR receptors nomenclature such as KIR2DL1, KIR2DS2 and KIR3DL1, where the final digit indicates the order in which the genes were described.

Different KIR genes have been identified - KIR2DL1-3, KIR2DL5, KIR3DL1-3 are inhibitory, KIR2DS1-5 and KIR3DS1 are activating, KIR2DP1 and KIR3DP1 are pseudo genes and KIR2DL4 has both inhibitory and activating properties. KIR2DL2 and KIR2DL3 recognize HLA-C1 with an Asn80 residue. KIR2DL1 recognizes HLA-C2 alleles with Lys80 residue. KIR3DL1 is the receptor for HLA-B alleles sharing the

Bw4 specificity. Finally, KIR3DL2 was shown to function as a receptor for HLA-A3/-A11 alleles when bound to Epstein–Barr virus (EBV) peptides.

The KIR genes assemble into haplotypes with two haplotypes described, 'A' and 'B'. The 'A' haplotype has only one activating KIR (2DS4), while the 'B' haplotype has a higher number of activating KIRs and generally possess more KIRs than the 'A' haplotype.

MICA/MICB

The major histocompatibility complex class I related chain (MIC) was first described in the 1990's. The genes are located centromeric to the HLA class I B gene. The MIC gene family consists of seven members MICA–MICG. The only two MIC genes which are expressed are MICA and MICB, the others are pseudo genes.

MICA and MICB genes are polymorphic but not as much as the classical HLA class I genes. A total of 107 MICA alleles and over 47 MICB alleles have been described as of Nov 2018. MICA has six exons separated by five introns. Exon 1 encodes the leader peptide, exons 2–4 encode the three extracellular domains, exon 5 encodes the transmembrane domain and exon 6 encodes the cytoplasmatic tail. MICA genes are in linkage disequilibrium with HLA-B alleles

Unlike HLA class I where the polymorphic residues are located mainly in the region that forms the peptide binding groove, polymorphism in MIC is more dispersed throughout the α2 and α3 domains. There is also polymorphism in the trans-membrane region. Many MIC antigens have the same extracellular domains with the only differences lying in the trans-membrane regions.

MICA and MICB antigens are constitutively expressed on epithelial cells, especially those of the gastrointestinal tract and on fibroblasts, monocytes, dendritic cells and on endothelial cells. They are not constitutively expressed on lymphocytes. They are however up regulated in stressed cells and act as a marker of cell stress.

The structure of MICA is similar to that of HLA class I but has some sticking differences. Like HLA class I, MICA has three extracellular domains (α1, 2 and 3), a transmembrane region and a cytoplasmic domain. Unlike HLA class I, the MICA protein does not associate with β2 microglobulin. The MICA α1 and 2 domains form a platform that is analogous to the platform formed by HLA class I α1 and 2 domains. In HLA class I, this platform forms the peptide binding groove. The MICA molecule however has extensive disordering of sections of the alpha helix in the α2 domain resulting in a very shallow groove, incapable of binding peptide. The MICA α1 and 2 platform domains do not interact with the α3 domain except for being linked together through a short linker chain. This allows for some flexibility in the structure.

MIC antigens serve as ligands receptors on NK cells and on some T cells. The MICA molecule interacts with NK cells, γδ T cells, and αβ CD8+ T cells, which express NKG2D, a common activating NK cell receptor. The NKG2D receptor forms a complex with MICA by binding orthogonal to the alpha helices of the platform α1 and 2 domains.

NKG2D GENETIC ORGANISATION AND ANTIGEN STRUCTURE

Natural Killer (NK) cells are one of the major forms of lymphocytes, comprising approximately 15% of all circulating lymphocytes. NK cells are involved primarily in the innate immune response but also contribute to the adaptive immune response. NK cells are characterised by cell surface expression of a number of receptors including Killer cell Immunoglobulin like Receptor (KIR) and NKG2D. NKG2D is also expressed on γδ T cells and on αβ CD8+ T cells.

NKG2D is a major recognition receptor for the detection and elimination of cells either as a result of infection or genomic stress such as in cancer. In NK cells, NKG2D serves as an activating receptor, able to trigger cytotoxicity. The function of NKG2D on CD8+ T cells is to send co-stimulatory signals to activate the T cells. The ligand for the NKG2D receptor is the MICA molecule. The NKG2D receptor forms a complex with MICA by binding orthogonal to the alpha helices of the platform α1 and 2 domains.

The NKG2D gene (also known as KLRK1), is located in the natural killer complex (NKC) on chromosome 12. The NKG2D gene is highly conserved with only a few alleles described. In humans, the NKG2D gene has 10 exons. Exons 2–4 encode the intracellular and transmembrane domain. Exons 5–8 encode the ligand-binding, membrane-bound domain which protrudes into extracellular space.

The NKG2D molecule is a member of a C-type lectin-like family receptor called CD94/NKG2. It is a transmembrane anchored receptor expressed as a disulphide-linked homodimer on the cell surface, with a molecular weight of ~42 kDa. Each NKG2D homodimer associates with two DAP10 homodimers to form a hexameric structure (DAP10 and DAP12 are signalling subunits which are highly conserved in evolution and associate with a large family of receptors in many cell types).

Signals triggered by the NKG2D receptor binding to MICA ligands are transmitted through the associated DAP10 dimer.

In the alloimmune response, CD56+ NK cells expressing granzyme have been shown to accumulate in kidney biopsies of patients undergoing acute rejection. One proposed mechanism of action is that DSAs are able to bind to the endothelium and to recruit NK cells that produce IFNγ and trigger antibody dependent cellular cytotoxicity (ADCC). Expression of NKG2D on NK cells and CD8+ T cells is modulated by cytokines. IL-2, IL-7, IL-12, IL-15 and IFN-α upregulate NKG2D expression, whereas TGFβ, IFNβ1, IL-21, IL-4, IL-12 and IFNγ downmodulate NKG2D. NKG2D has been shown to be upregulated as part of the alloimmune response.

MINOR HISTOCOMPATIBILITY ANTIGENS

HLA presents the major genetic barrier to stem cell transplantation. However, evidence that other genetic systems are involved includes GvHD and some degree of rejection even when transplanting with HLA identical siblings. A non-HLA system which is thought to contribute to this is the minor histocompatibility antigen (MiHA) system. Minor histocompatibility antigens comprise of peptides derived from proteins in which some degree of polymorphism exists such that there may be differences between the patient and donor repertoires. These peptides can be presented to the immune system by both HLA class I and II antigens.

The best characterised minor antigens are the Y chromosome derived HY peptide and the autosomal HA1 to HA5 peptides. Minor histocompatibility antigens such as HA1 and HA2 have restricted tissue distribution and are present normally only on haematopoietic cells. Others such as HY are more ubiquitously distributed, expressed for instance on gut epithelium. HA1 and HA2 are expressed on leukemic cells and some tumour cells, making them potential targets for cellular therapy. In mice, allogeneic stem cell transplantation donor CD8+ T cells specific for a MiHA found in the recipient has been shown to inhibit the division of leukemic cells. However, there is a risk in developing GVHD if the T cells are specific for MiHAs expressed ubiquitously on epithelial cells. Immune cell restricted MiHAs such as MiHA HB-1, are ideal targets for graft-versus- leukemia (GVL) since not all nucleated cells would be targeted by responding T cells.

COMPLEMENT

Complement was first discovered because of its activity in helping or complementing the action of antibodies in clearing pathogens from an organism. The Complement system consists of a number of small plasma proteins which normally circulate as inactive precursors capable of being activated upon cleavage by proteases. Several Complement proteins are themselves proteases which when activated by one of several possible mechanisms, can go on to activate other Complement components in a sequential cascade. The end result is a massively amplified response ultimately leading to the formation of the Membrane Attack Complex (MAC) which creates a pore in the cell membranes of certain pathogens causing their death.

There are three pathways to Complement activation, the Classical pathway, the Alternative pathway and the Mannose Binding Lectin (MBL) pathway which all lead to a common final pathway, forming of MAC. The Classical pathway is triggered by the binding of the first protein in the Complement cascade, C1q which is part of the C1 complex, to the Fc portion of immunoglobulin bound to antigen or by C1q binding directly to target antigens on the pathogen surface. This leads to activation of C1s, which in turn leads to cleavage of C4 into C4a and C4b. This in turn leads to cleavage of C2 into C2a and C2b. A complex of C4b2a then leads to cleavage of C3 into C3a and C3b. C3b can act as an opsonising agent for phagocytes and/or can initiates the common final pathway to the formation of MAC.

The Alternative pathway, which is antibody independent, depends on C3 undergoing spontaneous cleavage and assembly with other factors to form C3 Convertase which is a highly efficient C3 cleaving enzyme. Cleavage of C3 into C3a and C3b and is part of the common final pathway to the formation of MAC.

The Mannose Binding Lectin pathway is similar to the Classical pathway and activates many of the components of the Classical pathway. MBL binds to mannose on bacterial cell surfaces leading to activation of serine proteases which lead to the cleavage steps of C4 and C2 of the classical pathway leading to cleavage of C3 into C3a and C3b is part of the common final pathway to the formation of MAC.

Multiple regulatory molecules act on various stages of these complement pathways either enhancing or inhibiting Complement activity. These include Complement Receptors which can bind C3 and inhibit its action and Decay Accelerating Factor (DAF or CD55) which accelerates the disassembly of C3 Convertase, thus blocking the formation of MAC.

T AND B CELL DEVELOPMENT DEFECTS

B and T cell development are complex and tightly regulated processes. Failure at any stage can lead to defective and/or missing immunity. Severe Combined Immune Deficiency (SCID) is the prototypic example of defect in B and T cell development.

B cells develop initially in the bone marrow before migrating to secondary lymphoid organs where they undergo class switching and somatic hypermutation. Failures can occur at any stage. Types of failures include failure to create immunoglobulin (hypogammaglobulinemia and agammaglobulinemia), failure to create the B cell receptor (BCR), failure of a created BCR to transmit signals, alterations to the BCR, failure of T cell to B cell follicular interaction, IgM class switching failure and failure of B cell survival signals.

T cell development defects and be partial or total. A total T cell defects which arise as a result of the thymus being absent, small or defective are known as DiGeorge syndrome. One treatment modality for DiGeorge syndrome is thymus transplantation.

Well-known partial deficiencies in T-cell function include chromosomal breakage syndromes, B-cell and T-cell combined disorders such as SCID and Wiskott-Aldrich Syndrome (an X-linked recessive disorder characterized recurrent infections, eczema and bleeding). Partial T cell defects predispose patients to more frequent or extensive infections.

GENOMICS AND PERSONALISED MEDICINE

Since the full sequencing of the first full human genome, other projects such as the 100,000 genomes project have emerged which have brought genomics and personalised medicine into a new era. Genomics is transforming our understanding of disease and our ability to deliver healthcare in a way that is personal to each individual.

The sequencing of an individual's genome makes it possible to identify mutations or variants which are specific to that person. This makes it possible to identify where such mutations or variants may cause disease, predispose to disease or affect the benefits or side effects that parson might experience in response to a particular medication or treatment.

In H&I, disease association studies are the obvious place where genomics and personalised medicine interact. Many autoimmune diseases have HLA associations though other genetic loci are often involved.

In addition, H&I labs are increasingly testing for HLA types associated with drug reactions such as Abacavir with HLA-B*57:01, Carbamazepine and HLA-B*15:01 in Chinese and potentially A*31:01 in Caucasian populations, Allopurinol and B*58:01,

the NSAID feprazone is associated with Fixed-drug eruptions (FDE) in patients with HLA-B22.

In drug metabolism, mutations in the gene for cytochrome P450 are associated with high or low metabolism of calcineurin inhibitors. A mutation in the gene for the enzyme thiopurine methyltransferase (TPMT) is associated with high or low metabolism of Azathioprine. Patients who certain mutations of the TPMT gene may therefore be at higher risk of adverse side effects from azathioprine treatment.

Another area where personalised medicine is making it's make on H&I service provision is the rapidly evolving field of chimeric antigen receptor (CAR) T cell therapy. This is being used in the UK in clinical trials for the treatment of B cell ALL.

TRANSPLANTATION IMMUNE REACTIONS

DIRECT AND INDIRECT ALLORECOGNITION

One of the reasons why the HLA system acts as such as efficient immunological barrier to transplantation and transfusion is the fact that HLA molecules can be recognized directly or indirectly by the cells of the immune system.

In the Direct Pathway, following kidney transplantation, donor derived dendritic cells, triggered into maturation by the transplant Ischemic and Reperfusion Injury (IRI) as well as locally released pro-inflammatory cytokines, migrate from the kidney to the recipient lymphoid organs where they stimulate the host immune response by presenting host and donor MHC derived peptides loaded into intact donor dendritic cell MHC, to host T cells. This Direct Pathway of allorecognition is the main route for stimulating of the acute phase of the alloimmune response. In the normal immune response, only a small proportion (less than 0.1%) of host T cells will be specific for a given MHC-peptide complex, however in the alloimmune response, a high number of T cells (approximately 10%) react to the allogeneic MHC-peptide complex leading to a strong alloimmune response. Indeed, depletion from the graft of donor dendritic cells prior to transplantation has been shown to lead to graft tolerance or at least a reduction in acute rejection.

In the Indirect Pathway, following kidney transplantation, host dendritic cells which infiltrate the graft as part of the post-transplant inflammatory process have their MHC loaded with donor peptides derived from the take up and processing of donor MHC. Following migration to the lymphoid organs, these MHC-peptide complexes are presented to host T cells. This indirect pathway is similar to the process by which antigen is presented to T cells in the normal immune response and involves a smaller number of T cells than the direct response.

A number of studies have indicated that, in solid organ transplantation, T cell alloimmune response elicited by the Direct Pathway decreases with time post-transplant whilst the T cell alloimmune response elicited via the Indirect Pathway which can also be initiated in the acute phase, sustains and contributes to chronic rejection.

A third pathway the Semi-Direct Pathway of allorecognition has been described in which host antigen presenting cells acquire intact MHC molecules from donor cells thus allowing direct presentation to host T cells. This pathway may contribute to sustaining the alloimmune response.

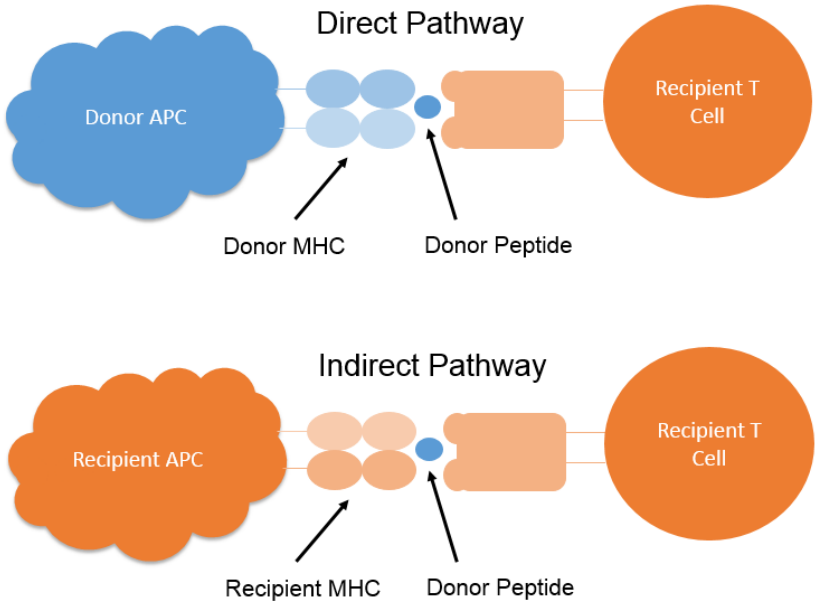


Figure 3 - Allorecognition

STAGES OF ALLOGRAFT REJECTION

Rejection of the renal allograft has been classified into four key stages related to the timing of the rejection – hyperacute rejection, accelerated rejection, acute rejection and chronic. Hyperacute rejection occurs within minutes to hours of transplant. Accelerated rejection occurs within days, acute rejection occurs within days to weeks, while Chronic rejection occurs months to years post-transplant.

Hyperacute rejection occurs almost immediately after the organ is transplanted and is usually due to the presence of pre-formed donor specific anti-HLA antibodies. The mechanism of hyperacute rejection involves deposition of antibodies against HLA antigens expressed on the endothelium (of the glomeruli in the case of kidneys) and the graft microvasculature. This leads to activation of the classical complement cascade causing endothelial necrosis, platelet deposition, local coagulation causing massive thrombosis in the capillaries, which prevents the vascularization of the graft.

Hyperacute rejection is usually accompanied by C4d deposition, though this may be negative early on. Hyperacute rejection is not typically reversible and requires the immediate removal of the graft. Accelerated rejection usually occurs within days and

is due to a memory or anamnestic response to pre-formed antibodies directed against mismatched donor antigen which are absent at the time of transplantation. Improved antibody screening and identification techniques as well as improvements in crossmatch techniques have significantly reduced the incidence of hyperacute and accelerated rejection.

The kidney is most susceptible organ to hyperacute rejection. The liver is relatively resistant.

Acute rejection occurs days to weeks (or months in the case of late acute rejection) post-transplant and may contain a cellular (Acute Cellular Rejection) as well as a humoral (Acute Humoral Rejection) component.

Acute cellular rejection, which is the most common form of rejection, is mediated by recipient T lymphocytes that have been activated directly or indirectly against donor antigens, primarily in the lymphoid tissues of the recipient. The donor dendritic cells enter the circulation and function as antigen-presenting cells (APCs) in the direct pathway. As donor APCs die out or are destroyed, recipient dendritic cells process and present alloantigens to recipient T-cell in the indirect pathway. The alloactivated T cells mediate cellular rejection with release of cytokines.

Compared to other solid organs, the lungs appear to be at a particularly high risk for Acute cellular rejection though the reasons for this are not entirely clear.

Acute humoral rejection is caused by pre-existing donor specific anti-HLA antibodies which are negative at the time of the pre-transplant crossmatch and/or de-novo donor specific antibodies. Diagnosis of Acute antibody mediated rejection involves identification of rapid graft dysfunction, accompanied by the presence of circulating anti-donor HLA antibodies and biopsy evidence of C4d deposition in the peritubular capillaries. In kidney grafts, C4d may also be deposited in the glomeruli though this is variable. Acute antibody mediated rejection is reversible with treatment such as plasmapheresis and intravenous immunoglobulin plus increased immunosuppression (with for example tacrolimus and mycophenolate mofetil). The Anti-CD20 antibody Rituximab is also used in some cases.

Chronic rejection appears as fibrosis and scarring in all transplanted organs. The type of injury is organ specific. In heart transplants, chronic rejection manifests as accelerated coronary artery atherosclerosis. In lungs, it manifests as bronchiolitis obliterans (BOS). In liver, it is characterized by the vanishing bile duct syndrome, the progressive destruction and disappearance of intra-hepatic bile ducts leading to cholestasis in which where bile cannot flow from the liver to the duodenum. In kidney recipients, chronic rejection is called chronic allograft nephropathy and manifests as fibrosis and glomerulopathy.

Some drugs used as part of the immunosuppressive regime, such as cyclosporin and tacrolimus, are known to have a nephrotoxic effect and contribute to chronic rejection.

The role of alloantibodies in chronic rejection is increasingly being recognised. Recent data from the 14th international histocompatibility workshop demonstrated that four year deceased donor kidney allograft survival was 20% less in patients with donor specific antibodies compared to donors with no HLA antibodies. MICA antibodies were also demonstrated in these patients. In kidneys, chronic rejection is characterised by slow progressive loss of renal function with endothelial antibody deposition leading to endothelial injury and glomerular basement membrane duplication characteristic of transplant glomerulopathy.

ACCOMMODATION

Immunological reactions of interest in the transplant setting include Tolerance, Accommodation and Rejection.

Tolerance refers to a state of sustained non-immune responsiveness to alloantigens. Tolerance is different from Accommodation, which refers to a state of resistance to immune damage. A state of Tolerance remains a goal for solid organ transplantation as it would allow for the withdrawal of immunosuppressive regimes and their associated toxic effect to grafts.

Accommodation permits patients to maintain grafts even in the presence of Donor Specific Antibodies (DSA). Accommodation was originally identified in ABO blood group incompatible renal transplantation in which the graft survived and functioned normally despite the presence in the patient of high titre ABO blood group antibodies. Proposed mechanisms for Accommodation include the expression in the graft of several protective genes which block the activation of the transcription factor NF-KB, thereby suppressing induction of proinflammatory genes and inhibition of the membrane attack complex thereby disrupting the action of complement.

Immune mechanisms which contribute to graft rejection include acute and chronic alloantibody mediated rejection (AMR) as well as acute and chronic cellular rejection.

TOLERANCE

The clonal selection theory explains how the adaptive immune system is able to respond to an almost infinite diversity of antigen. One potential option for achieving such diversity would have been for the cells of the adaptive immune system to bear an almost infinite number of different receptors, each capable of recognizing a different feature shared by pathogen. This would however have been limiting in terms of the level of diversity that could be achieved given the finite amount of space on the cell surface. Instead, each cell expresses a single specific receptor, generated by a process of somatic recombination. On binding antigen, the cell is activated and produces progeny, all with the same specificity. This generates a clone of cells and is the basis of the clonal selection theory. The clonal selection mechanism allows each individual to expand clones specific for the antigens to which they have been exposed.

The clonal selection theory not only explains how the adaptive immune system is able to respond to an almost infinite diversity of antigen, it also provides a basis for the mechanisms that are involved in developing self-tolerance. These mechanisms include central tolerance by clonal deletion, peripheral tolerance by deletion and inactivation (anergy) and the action of Suppressor T cells.

Central tolerance by clonal deletion refers to mechanisms of tolerance acting during lymphocyte development in the thymus or bone marrow. Experimental studies show

that central tolerance is mostly due to the elimination or inactivation of those T and B cells that strongly recognise self-antigens. These cells are destroyed or inactivated after they have expressed receptors for self-antigens and before they develop into fully immunocompetent lymphocytes.

Peripheral tolerance by deletion and inactivation (anergy) refers to mechanisms acting on mature lymphocytes after they have left the primary lymphoid organs. Not all genes are expressed in the thymus so developing T cells cannot be exposed to all self-antigens. Therefore, additional mechanisms for tolerising self-reactive mature T cells are necessary. Mechanisms of peripheral B cell self-tolerance are also necessary because after stimulation with antigen B cells expand and undergo somatic mutation, generating a population of B cells with new antigen specificities. Some of these cells may be specific for self-antigens.

Sometimes T cells are not deleted but become specifically unresponsive to antigen stimulation (i.e. they do not proliferate). This is called clonal anergy. One of the molecular mechanisms responsible for inducing anergy is signalling via CTLA4, a molecule expressed by activated T cells.

While the principal mechanisms of tolerance to self-antigens are clonal deletion and anergy, suppressor T cells have been proposed as a backup mechanism. Suppressor T cells specific for a given antigen are thought to be able to inactivate other lymphocytes specific for the same antigen. For example, under some circumstances it is possible to induce specific tolerance to allografts which appears to be maintained by suppressor T cells.

APOPTOSIS

Apoptosis is programmed cell death, a process in which cells die in a controlled way as opposed to cells that die as a result of acute injury. Cells that die as a result of acute injury typically swell and burst. This is known as necrosis. Necrosis results in cells that die spilling their contents over adjacent cells, causing a potentially damaging inflammatory response.

In apoptosis, cells die neatly, without damaging their neighbours. The cells shrink and condense. Their cytoskeleton collapses, their nuclear envelopes disassemble and their nuclear DNA break up into fragments. The cell surfaces are altered, displaying properties that cause the dying cells to be rapidly phagocytosed by other cells or by macrophages before any leakage of their contents occurs. This not only avoids the damaging consequences of cell necrosis but also allows the organic components of the dead cell to be recycled by the cell that ingests it. The average adult human loses between 50 and 70 billion cells each day due to apoptosis

The intracellular machinery responsible for apoptosis depends on a family of caspases that have a cysteine at their active site and cleave their target proteins at specific aspartic acids. Caspases are synthesized in the cell as inactive precursors (Procaspases), which are usually activated by cleavage at aspartic acids by other caspases. Once activated, caspases cleave and activate other procaspases resulting in an amplified proteolytic cascade. Some of the activated caspases then cleave other key proteins in the cell. Some caspases cleave the fibrous meshwork of proteins on the inner surface of the inner nuclear membrane, causing the irreversible breakdown of the nuclear membrane. Other caspases cleave a protein that normally holds a DNA-degrading enzyme (a DNAse) in an inactive form, freeing the DNAse to cut up the DNA in the cell nucleus. In this way, the cell dismantles itself quickly and neatly and its corpse is rapidly taken up and digested by other cells.

Apoptosis of T cells plays a central role in developmental, physiologic and pathologic processes including deletion of T cell clones expressing self-antigens in the thymus, elimination of T cells which are infected with viruses and homeostasis of T cell populations that have expanded following high dose antigen exposures.

Transplantation tolerance can be achieved through several mechanisms, including the action of suppressor cells, the induction of anergy or the deletion of graft-reactive donor T cells. Apoptosis may also represent a mechanism of induction of transplantation tolerance.

In the acute phase of rejection, allografts consistently show evidence of massive apoptosis both of the transplanted organ and graft-infiltrating T-cells. Ischaemic and reperfusion injury lead to the production of reactive oxygen species which are directly toxic to cells inducing apoptosis and/or necrosis. In liver transplantation,

apoptosis contributes to death of hepatocytes and biliary duct cells. In renal transplantation, apoptosis has been detected in the acute and chronic rejection states. Apoptosis has also been detected in pancreatic transplantation. Apoptosis has been observed in intestinal transplantation. Apoptosis has also been shown to potentially play a role in cardiothoracic transplantation.

Apoptosis contributes to the outcome after organ transplantation, being involved both in graft rejection and in transplantation tolerance.

ROLE OF DENDRITIC CELLS IN THE ALLOIMMUNE RESPONSE

Dendritic cells are the most potent of the Antigen Presenting Cells (APC) responsible for priming the immune response. Distributed throughout the tissues of the body, they possess surface pattern recognition receptors that recognize pathogen associated molecular patterns making them capable of initiating an immune response to infection. Two main subsets of dendritic cells have been described, the myeloid dendritic cells (mDC) and plasmacytoid dendritic cells (pDC) with the mDC being the more abundant. These are traditionally believed to derive from myeloid and lymphoid precursors respectively, though more recently both cells lines have been proposed to derive from both cell types of precursors. Dendritic cells are capable of stimulating naive T cells and are therefore known as professional APC's. More recently, an additional role for dendritic cells as important mediators of peripheral immune tolerance and maintenance of immune homeostasis has been described.

Dendritic cells play a central role in the alloimmune response. After kidney transplantation, donor derived dendritic cells, triggered into maturation by the transplant Ischemic and Reperfusion Injury (IRI) as well as locally released pro-inflammatory cytokines, migrate from the kidney to the recipient lymphoid organs where they stimulate the host immune response through the direct, indirect and semi direct routes of allorecognition. These functions of Dendritic cells set in train the rejection response. Transplanted organs are eventually depleted of donor derived dendritic cells but it has been shown that recipient dendritic cells that infiltrate transplanted organs sustain the alloimmune response after T-cell activation has already occurred.

Dendritic cells are also considered to play a role in tolerance induction though the mechanism is yet to be fully elucidated. Potential mechanisms include promotion of clonal deletion, the induction of T Regs and inhibition of memory T cell responses.

In stem cell transplantation, the number of circulating plasmacytoid and myeloid dendritic cells and their origin, donor or recipient, have been shown to be associated with the initiation of acute Graft versus Host Disease (aGvHD), relapse and graft failure. The recognition of alloantigen presented by residual host dendritic cells to donor T cells in the direct pathway of allorecognition initiates GvHD. Ongoing antigen presentation involves donor derived dendritic cells presenting host antigen to donor T cells in the indirect pathway of allorecognition. Some studies have shown that the absolute numbers of circulating dendritic cells post stem cell transplantation is an independent predictor of aGvHD. Patients with aGvHD after stem cell transplantation have lower numbers of circulating mDC and pDC compared to healthy individuals but do have a higher number of dendritic cells in affected areas such as the skin.

UNDERLYING MECHANISMS OF THE INFLAMMATORY RESPONSE

Inflammation is the body's response to insults or injury such as would result from infection, trauma, burns, hypersensitivity to toxins, irritants and allergens etc. The inflammatory response involves a variety of mechanisms to defend against pathogens and to repair tissue damage.

During inflammation, numerous types of inflammatory cells are activated. Each inflammatory cell releases cytokines and mediators to modify activities of other inflammatory cells. Orchestration of these cells and molecules leads to progression of inflammation and the five classical signs of inflammation i.e. heat, pain, redness, swelling and loss of function.

The inflammatory response proceeds as follows:

1. The activation of resident cells at the site of infection or injury (mast cells, resident macrophages and dendrite cells) and rapid entry of granulocytes (neutrophils, basophils and eosinophils) in response to injury in the innate immune response

2. The innate immune system responds rapidly to infection or injury with macrophages, NK cells, CD8+ T cells and neutrophils providing an early response
3. Infiltration of effectors immune cells (lymphocytes) to enable specific immune responses in the adaptive immune response follows
4. The adaptive immune response is mediated primarily by CD4+ T cells which are primed by dendritic cells. Dendritic cells and macrophages are antigen-presenting cells, which stimulate naïve T cell proliferation. Originating in bone marrow, dendritic cells reach tissues through blood circulation. Once the dendritic cell identifies, ingests and processes an antigen, it migrates to the lymph nodes and presents the antigen to resident T cells, inducing the immune response
5. Recruitment and activation of mesenchymal cells such as endothelial cells and fibroblasts to form new blood vessels and a collagenous matrix
6. Tissue remodelling

Inflammation is characterized by the release of chemical mediators leading to vascular changes, primarily vasodilatation and cellular changes.

Amongst the chemical mediators released are histamine, prostaglandin and complement factors. Histamine triggers vasodilation and increases vascular permeability allowing cells to enter tissue at the site of injury. Prostaglandins increase the effects of other substances that promote vascular permeability or affect the aggregation of platelets, which is a part of the clotting process. Activated complement proteins serve as chemotactic factors for neutrophils, increase vascular permeability and stimulate the release of histamine from mast cells.

The initial vascular change is vasoconstriction. This is transient and is followed by vasodilation, under the influence of regulatory molecules on endothelial cells of blood vessels. When blood vessels dilate the walls of the blood vessels become more permeable and allow protein-rich fluid along with water and salts into the tissues of damaged area which is what causes the swelling.

Cellular changes then follow. In less than an hour after injury or infection, a large number of neutrophils reach the site of injury, under the direction of a concentration gradient of chemical mediators. Within 24 to 28 hours of the initiation

of the inflammatory process, monocytes which have matured into cell-eating macrophages, reach the site of infection. However, if the inflammation is caused by parasitic worms, eosinophils predominate in the inflammatory response rather than neutrophils.

While the acute inflammation is fundamentally beneficial, severe inflammation can lead to systemic inflammatory response syndrome, which is characterized by hyperinflammation and can cause organ injury, shock and death in its most severe forms.

TRANSPLANTATION INDUCTION AND IMMUNOSUPPRESSIVE DRUGS

ANTI-LYMPHOCYTE GLOBULIN (ALG)

Anti-Lymphocyte Globulin (ALG) is an anti-human T cell antibody immunosuppressive used in the prevention and treatment of acute rejection in kidney transplantation. It has also been used to induce remission in Aplastic Anaemia. ALG use is less common than ATG use.

ANTI-THYMOCYTE GLOBULIN (ATG)

Anti-Thymocyte Globulin (ATG) is an anti-human T cell antibody immunosuppressive used for solid organ transplant induction in highly sensitised patients and in the prevention and treatment of acute rejection in kidney and cardiac transplantation and in the prevention and treatment of GvHD in stem cell transplantation. It is also used to induce remission in Aplastic Anaemia.

ATG depletes T cells by direct complement action and by cell mediate cytotoxicity. Side effects include activation of the T cells and release of IL-2 before the T cells are destroyed thus potentially causing a cytokine storm. For this reason, anti-IL-2 receptor antibodies such as Basiliximab and Daclizumab are increasingly being used in place of ATG.

AZATHIOPRINE

Azathioprine is an immunosuppressive antimetabolite. It inhibits purine synthesis. Azathioprine is used in rheumatoid arthritis, granulomatosis with polyangiitis, severe Crohn's disease, severe ulcerative colitis and in kidney transplants to prevent rejection.

BASILIXIMAB

Basiliximab is a chimeric mouse-human monoclonal immunosuppressive antibody to the α chain (CD25) of the IL-2 receptor of T cells. Basiliximab binds to CD25 thus blocking IL-2 from binding to activated lymphocytes.

Basiliximab is used for induction in solid organ transplantation as an alternative to the more potent ATG. Basiliximab can also be used in the prevention and treatment of acute rejection in kidney transplantation in combination with ciclosporin and corticosteroid-containing immunosuppression regimens.

BORTEZOMIB

Bortezomib is proteasome inhibitor used for the treatment of relapsed multiple myeloma and mantle cell lymphoma. Proteasomes are thought to support the immortal phenotype of multiple myeloma by rapidly degrading pro-apoptotic factors. Bortezomib binds to the catalytic site of proteasome with high affinity and specificity, inhibiting its action and permitting the activation of programmed cell death.

Bortezomib is one of the options for post-transplant treatment of patients with DSA, often secondary to plasma exchange, IVIg and Rituximab.

BUSULFAN

Busulfan is a cell cycle nonspecific alkylating antineoplastic agent which causes profound myelosuppression in patients. Alkylating agents are compounds which are capable of adding an alkyl group (CH_4) to the guanine nucleotide bases of DNA

molecules, causing damage by cross linking the guanine bases. As cancer cells have less error correction of their DNA during replication, they are disproportionately affected when compared to normal cells.

Busulfan is often used together with Cyclophosphamide (Bu/Cy) as a conditioning regimen prior to allogeneic hematopoietic progenitor cell transplantation.

CAMPATH

Campath (also known as Alemtuzumab) is an anti-CD52 recombinant humanized monoclonal antibody used for the treatment of chronic CLL and T cell Lymphoma. It binds to CD52, which is present on all mature lymphocytes but not on stem cells. Binding targets these cells for destruction.

Campath is indicated for the treatment of B-cell chronic lymphocytic leukaemia (B-CLL) in patients who are refractory to first line treatment with Fludarabine. It is also used as an anti-rejection agent in some conditioning regimens for bone marrow transplantation, kidney transplantation and islet cell transplantation.

CARMUSTINE

Carmustine (also known as BCNU) is a mustard gas related compound with an alkylating mechanism of action. Alkylating agents are compounds which are capable of adding an alkyl group (CH_4) to the guanine nucleotide bases of DNA molecules, causing damage by cross linking the guanine bases. As cancer cells have less error correction of their DNA during replication, they are disproportionately affected when compared to normal cells.

Carmustine is used in the treatment of several types of brain cancer, for treatment of Multiple Myeloma and for treatment of Hodgkins and non-Hodgkins Lymphoma. It is often used as a conditioning regime prior to allogeneic hematopoietic progenitor cell transplantation for lymphoma as part of the BEAM cocktail which includes BCNU (Carmustine), Etoposide, Arabinoside-C (also known as Cytarabine) and Melphalan.

CYCLOPHOSPHAMIDE

Cyclophosphamide, an alkylating agent, is one of the most efficacious immunosuppressive drugs available. Alkylating agents are compounds which are capable of adding an alkyl group (CH_4) to the guanine nucleotide bases of DNA molecules, causing damage by cross linking the guanine bases. As cancer cells have less error correction of their DNA during replication, they are disproportionately affected when compared to normal cells.

Cyclophosphamide is used for treatment of autoimmune disorders such as SLE and multiple sclerosis and as a broad-spectrum anti-cancer drug often given together with other drugs for treatment of non-Hodgkin's lymphoma, leukaemia's, multiple myeloma, breast and ovarian cancer. Cyclophosphamide is often used together with Busulfan (Bu/Cy) as a conditioning regimen prior to HSCT.

CYCLOSPORIN

Cyclosporin-A is a Calcineurin inhibitor. Calcineurin is a protein phosphatase also known as protein phosphatase 3. In T-cells, activation of the T-cell receptor normally increases intracellular calcium, which acts via calmodulin to activate Calcineurin. Calcineurin then activates NFAT (Nuclear Factor of Activated T-cells) by dephosphorylating it. Upon activation, NFAT is translocated into the nucleus where it upregulates the expression of IL-2 which, in turn, stimulates the growth and differentiation of T cell response. Cyclosporin binds to the cytosolic protein Ciclophilin and the resulting complex binds to and inhibits Calcineurin.

Cyclosporin was used in solid organ transplantation though its use has mostly been overtaken by the use of Tacrolimus.

Cyclosporine and methotrexate has historically been used as GvHD prophylaxis in HSCT.

CYTARABINE

Cytarabine (also known as Arabinoside-C/Ara-C) is an anti-metabolite that competes with dCTP for incorporation into DNA, thereby inhibiting DNA synthesis and is used most often to treat acute leukaemia's and non-Hodgkins lymphomas.

When used as a conditioning regime prior to allogeneic hematopoietic progenitor cell transplantation for lymphoma, it is often part of the BEAM cocktail which includes BCNU (Carmustine), Etoposide, Arabinoside-C (also known as Cytarabine) and Melphalan.

DACLIZUMAB

Daclizumab is, like Basiliximab, a monoclonal immunosuppressive antibody to the α chain (CD25) of the IL-2 receptor of T cells. Daclizumab binds to CD25 thus blocking IL-2 from binding to activated lymphocytes. Daclizumab is used in the prevention and treatment of acute rejection in kidney transplantation. Daclizumab is often used in combination with Glucocorticoids and Cyclosporine.

DACTINOMYCIN

Dactinomycin is a chemotherapy medication that inhibits transcription by binding DNA at the transcription initiation complex and preventing elongation of RNA chain by RNA polymerase.

It is used to treat a number of types of cancer, including Wilms tumour, Childhood rhabdomyosarcoma and other soft-tissue sarcomas, Ewing's sarcoma, trophoblastic neoplasm, testicular cancer and certain types of ovarian cancer.

DEXAMETHASONE

Dexamethasone is a Glucocorticoids, a steroid hormone that bind to and activate the glucocorticoid receptor, which is expressed on almost every cell and regulates inflammatory and allergic responses. Activation of the glucocorticoid receptor up-

regulates the expression of anti-inflammatory proteins and down-regulate the expression of pro-inflammatory proteins.

Dexamethasone is used to treat many inflammatory conditions such as allergic disorders and skin conditions, to treat ulcerative colitis, arthritis, lupus, psoriasis, and breathing disorders. Dexamethasone is also used in induction for HSCT for patient with multiple myeloma and some other haematological malignancies.

ECULIZUMAB

Eculizumab (aka Soliris) is a monoclonal antibody directed against the complement component C5 which is a component on the final common Complement pathway leading to the formation of Membrane Attack Complex (MAC). Eculizumab blocks the cleavage of C5 and thus halts the process of Complement mediated cell destruction.

Eculizumab is licensed in the UK for treating adults and children with atypical haemolytic uraemic syndrome (aHUS) or paroxysmal nocturnal haemoglobinuria (PNH). Transplant centres often wish to use Eculizumab for preventing recurrence of glomerulopathy post-transplant, however it is a very expensive drug and is not licenced by NICHE for this use.

ETOPOSIDE

Etoposide is an alkaloid anticancer that exerts its effect by disrupting the cell cycle. It is used in the treatment of solid tumours, including testicular, breast and small cell lung cancers and some lymphomas. Etoposide acts by inhibiting the enzyme topoisomerase II, which normally unwinds DNA, causing the DNA double strand to break. Cancer cells are less able to repair this damage compared to normal cells and are therefore disproportionately affected.

When used as a conditioning regime prior to allogeneic hematopoietic progenitor cell transplantation for lymphoma, Etoposide is often part of the BEAM cocktail

which includes BCNU (Carmustine), Etoposide, Arabinoside-C (also known as Cytarabine) and Melphalan.

EVEROLIMUS

Everolimus is a derivative of Sirolimus (aka Rapamycin) and is an mTOR (mammalian Target of Rapamycin) inhibitor. mTOR is a serine-threonine protein kinase that regulates cell growth, proliferation, motility, survival, protein synthesis and transcription. mTOR also functions as a tyrosine protein kinase that promotes the activation of insulin receptors and insulin-like growth factor 1 receptors.

Unlike the Calcineurin inhibitors which block the synthesis of IL-2, the mTOR inhibitors block cell responses to IL-2. Inhibition of mTOR blocks cell responses to IL-2, thereby preventing the differentiation of T cells into effector T cells and into memory T cells.

Everolimus is used as an immunosuppressant to prevent rejection in heart and kidney transplants and for treatment of advanced kidney cancer.

FLUDARABINE

Fludarabine is a purine analog that inhibits DNA synthesis by interfering with ribonucleotide reductase and DNA polymerase. Fludarabine is highly effective in the treatment of chronic lymphocytic leukaemia (CLL), producing higher response rates than alkylating agents.

Fludarabine is often used in Reduced Intensity Regimes (RIC) prior to stem cell transplantation either alongside reduced intensity TBI or together with Busulfan with or without ATG.

IFOSFAMIDE

Ifosfamide is an alkylating agent often used in the treatment of solid tumours, including testicular, breast and lung cancers and some lymphomas. Alkylating agents are compounds which are capable of adding an alkyl group (CH_4) to the guanine nucleotide bases of DNA molecules, causing damage by cross linking the guanine bases. As cancer cells have less error correction of their DNA during replication, they are disproportionately affected when compared to normal cells.

When used for lymphoma treatment, Ifosfamide is often administered together with Cisplatin and Etoposide as part of the ICE cocktail or with Rituximab and ICE as part of the R-ICE cocktail.

IMATINIB

Imatinib is a Tyrosine Kinase Inhibitor used to treat CML. It selectively inhibits BRC-ABL, a constitutively active tyrosine kinase typical of CML. Tyrosine Kinase Inhibitors (TKIs) act by inhibiting tyrosine phosphorylation, which is a key step in the signalling pathways which lead to various cellular processes including growth and differentiation. Inhibition disrupts the cell signalling pathway and reduces proliferation.

Use of Imatinib has significantly reduced the need for stem cell transplantation in CML patients.

INFLIXIMAB

Infliximab is an anti TNFα monoclonal antibody used in the treatment of many autoimmune diseases including Psoriasis, Crohn's disease, Ankylosing Spondylitis, Psoriatic Arthritis, Rheumatoid Arthritis and Ulcerative Colitis.

LENALIDOMIDE

Lenalidomide is a derivative of thalidomide. It is an immunomodulatory agent, the exact mechanism of action of which is not known. It has anti-neoplastic, anti-angiogenic and pro-erythropoietic properties.

In the UK, Lenalidomide is licenced in combination with dexamethasone as a second line treatment for multiple myeloma when first line treatment has failed.

LOMUSTINE

Lomustine (also known as CCNU) is a lipid soluble alkylating agent capable of crossing the blood brain barrier and is used most often to treat brain tumours. Alkylating agents are compounds which are capable of adding an alkyl group (CH_4) to the guanine nucleotide bases of DNA molecules, causing damage by cross linking the guanine bases. As cancer cells have less error correction of their DNA during replication, they are disproportionately affected when compared to normal cells.

Lomustine is also used to treat Hodgkins and non-Hodgkins Lymphomas.

MELPHALAN

Melphalan is a nitrogen mustard alkylating agent. Alkylating agents are compounds which are capable of adding an alkyl group (CH_4) to the guanine nucleotide bases of DNA molecules, causing damage by cross linking the guanine bases. As cancer cells have less error correction of their DNA during replication, they are disproportionately affected when compared to normal cells.

Melphalan is mainly used in the treatment of multiple myeloma and for ovarian cancer. It is also used as a conditioning regime prior to HSCT for lymphoma as part of the BEAM cocktail which includes BCNU (Carmustine), Etoposide, Arabinoside-C (also known as Cytarabine) and Melphalan.

METHOTREXATE

Methotrexate (MXT) is a folic acid antagonist that inhibits dihydrofolate reductase, a key enzyme in the synthesis of the amino acids serine and methionine, thereby interfering with the formation of DNA.

Methotrexate is used in the treatment of some autoimmune diseases, including Psoriasis and Rheumatoid Arthritis as well as treatment of many types of cancer including ALL.

METHYLPREDNISOLONE

Methylprednisolone is a Glucocorticoids, a steroid hormone that bind to and activate the glucocorticoid receptor, which is expressed on almost every cell and regulates inflammatory and allergic responses. Activation of the glucocorticoid receptor up-regulates the expression of anti-inflammatory proteins and down-regulate the expression of pro-inflammatory proteins.

Methylprednisolone is used to treat many different inflammatory conditions such as arthritis, lupus, psoriasis, ulcerative colitis, allergic disorders, gland (endocrine) disorders, and conditions that affect the skin, eyes, lungs, stomach, nervous system or blood cells.

MYCOPHENOLIC ACID / MYCOPHENOLATE MOFETIL (MMF)

Mycophenolic acid is the active derivative of Mycophenolate mofetil or MMF. It is an antiproliferative immunosuppressant which inhibits purine synthesis thus suppressing T and B cell responses. MMF inhibits primary antibody responses more efficiently than secondary responses.

MMF is indicated for the prophylaxis of acute rejection in kidney and cardiac transplant patients. It is increasingly utilized as a steroid sparing treatment. MMF can be used concomitantly with cyclosporine and corticosteroids.

PREDNISOLONE

Prednisolone is a corticosteroid it is used to treat many different inflammatory conditions such as arthritis, lupus, psoriasis, ulcerative colitis, allergic disorders, gland

(endocrine) disorders, and conditions that affect the skin, eyes, lungs, stomach, nervous system or blood cells.

RITUXIMAB

Rituximab is an anti-CD20 recombinant chimeric murine-human monoclonal antibody. It is often used to treat non-Hodgkins lymphoma and CLL. It binds to CD-20 which is expressed on all B cells (but not on plasma cells). Binding triggers a series of cytotoxic immune response resulting in the elimination of B cells. The mechanism of action includes complement-mediated lysis, antibody-dependent cellular cytotoxicity and induction of apoptosis in the malignant lymphoma cells.

Rituximab is used as part of the induction for ABOi kidney transplantation. It is also often one of the options for post-transplant treatment of patients with DSA. Under expert guidance, Rituximab is also indicated for post-transplantation lymphoproliferative disease, Non-Hodgkin's lymphoma, Hodgkin's lymphoma, Severe cases of resistant immune modulated disease including idiopathic thrombocytopenia purpura, haemolytic anaemia and systemic lupus erythematosus.

SIROLIMUS (AKA RAPAMYCIN)

Sirolimus is an mTOR (mammalian Target of Rapamycin) inhibitor used in the prevention of rejection in heart and kidney transplants. Unlike the Calcineurin inhibitors which block the synthesis of IL-2, the mTOR inhibitors block cell responses to IL-2. For this reason, Calcineurin nephrotoxicity is often reduced by use of Sirolimus, allowing a reduction in the dose of Cyclosporin/Tacrolimus.

TACROLIMUS

Tacrolimus is a Calcineurin inhibitor which is similar in action to cyclosporin. Tacrolimus prevents NFAT dephosphorylation by complexing with the immunophilin FK binding protein 12 (FKB12) creating a new complex which interacts with and inhibits Calcineurin, thereby inhibiting the expression of IL-2 which, in turn, inhibits the growth and differentiation of T cell response.

Tacrolimus is indicated for prophylaxis of graft rejection following liver transplantation, kidney and heart transplantation.

CHAPTER 2 KEY H&I LABORATORY TECHNIQUES

HLA TYPING

LUMINEX TYPING

The Luminex technology is based on the use of 5.6 micron polystyrene microspheres (beads) each internally dyed with a unique combination of red and infrared dye. The combination of different intensities of the two dyes allows for the identification of each bead by its unique signature when excited by a laser beam. This permits multiplexing of up to 100 reactions in a single tube.

The surface chemistry of the beads allows them to be chemically coated with a number of different targets, including HLA sequences and antigens. The beads can therefore be used to interrogate samples for the presence or absence of specific analytes. The Luminex platform uses the principles of flowcytometry to stream beads in single file past a pair of lasers. A red laser is used to excite and therefore identify the specific bead and a green laser is used to excite and therefore identify any reporter dyes captured on the beads during the assay. As both the bead identification and reporter dye readings are made on each individual bead, a multiplex system can be developed with, typically, up to 100 beads. The probes used to coat the 100 beads are carefully selected such that individual analytes of interest can be identified by unique reaction patterns of the beads.

THE APPLICATION LUMINEX TO HLA TYPING

HLA typing using Luminex is a reverse polymerase chain reaction sequence specific oligonucleotide (PCR-SSO) system which involves PCR amplification of targeted regions within the MHC class I or II regions with group specific primers, followed by a process of probing the amplicon with Luminex beads, each coated with sequence specific oligonucleotide probes to identify the presence or absence of specific alleles. The assignment of HLA type is then based on the reaction pattern observed, compared to patterns associated with published sequences.

Primers used for the amplification are biotinilated. Amplification can then be either symmetrical, which therefore requires a denaturation step to create single strands or can be asymmetrical to generate an excess of a single strand. The single stranded product is then hybridised with a multiplex of up to 100 beads, all of which can be uniquely identified by their internal dyes and all of which are selectively coated with specific oligonucleotide sequences. The amplified DNA hybridise to complementary DNA probed on the beads. A washing stage may then be required depending on the Luminex typing kit used. Bound amplicon is detected by labelling with a Streptavidin – Phycoerytherin (SAPE) conjugate, with Streptavidin binding to the biotin used to label the primers and phycoerytherin serving as the reporter dye for the presence of bound amplicon. Again a wash step may be required depending on the kit in use.

The Luminex platform is used to identify any SAPE bound to the beads. The observed reaction patterns are used to assign HLA type. Positive and genitive control beads are used to quality control the typing test.

The current Luminex kits on the market tend to yield mostly medium resolution HLA types, though high resolution results are occasionally obtained. Suppliers are experimenting with high definition beads and increased numbers of beads in the multiplex in order to develop systems for high resolution HLA typing.

THE ADVANTAGES AND DISADVANTAGES OF LUMINEX FOR HLA TYPING

The Luminex methodology for HLA typing combines some of the speed typically associated with a PCR-SSP technique, with the high sample throughput of an SSO technique. This gives the ability to rapidly type a large number of samples with high reproducibility. The combinations of speed and reproducibility, together with the removal of the need to maintain and validate in-house methods, form the main advantages of Luminex as used for HLA typing. The technique is fairly robust and requires very little DNA. The methodology also makes better use of laboratory staff with fewer staff required to test the same number of samples when compared to SSP or other traditional SSO techniques.

The Luminex technique as used by most H&I laboratories is semi-automated but can be fully automated, contributing to the speed with which results can be obtained.

There does however remain a need to experienced scientists to check, confirm and if required modify, the software proposed HLA types.

Another advantage of Luminex compared to PCR-SSP is the reduction in use of or even elimination of the use of agarose gel electrophoresis and its associated use of ethidium bromide and the H&S risks associated with that.

A potential disadvantage of the Luminex methodology is that even though it is rapid, it is still not as rapid as PCR-SSP and may therefore be unsuitable for use in an on call situation where a rapid turnaround of results is needed. In addition, the system may be better suited to batch testing of samples rather than the single sample testing typical of the on-call situation. In this situation the Luminex system may have a disadvantage both in terms of cost and speed. However, some laboratories do use it as a backup technique.

Another current disadvantage of the Luminex methodology is that results are low to medium resolution and therefore require further testing to obtain high resolution results where required. Luminex suppliers have developed sets of beads capable of yielding higher resolution results. One potential problem is the current limit of 100 beads used in a multiplex and ways of increasing this number are being examined.

A further limitation of the Luminex technology is that is does produce a small number of heterozygous ambiguities, though suppliers claim this is less than traditional SSO techniques. Where heterozygous ambiguities are identified, specific probes can be developed to help resolve these. This however points to another disadvantage of the Luminex methodology for HLA typing. With traditional in house SSO techniques or with SSP techniques, new probes could be rapidly added to help identify new alleles or resolve ambiguities. Use of Luminex does rely on the suppliers rapidly updating their kits.

Given the rapid turnaround of batched samples and the high associated high throughput, the advantages of the Luminex methodology do perhaps outweigh the disadvantages. The Luminex kits are however relatively expensive compared to in house techniques though staff time for these in house techniques needs to be taken into account.

RELEVANCE OF LUMINEX TYPING

Molecular techniques have long replaced serological typing as a means of doing HLA typing, especially as more and more alleles are being discovered. Over time, there have been a number of molecular techniques for low to intermediate resolution HLA typing including Sequence Specific Primers (SSP), Restriction Fragment Length Polymorphism (RFLP), Single Strand Conformational Polymorphism (SSCP) and various SSO and reverse SSO techniques, with hybridisation on cards or strips. These techniques have varying degrees of automation and manual input required. Luminex HLA typing is a reverse SSO technique which involves PCR amplification of targeted regions within the MHC class I and/or II with group specific primers, followed by a process of probing the amplicon with Luminex beads, each coated with sequence specific oligonucleotide probes to identify the presence or absence of specific alleles. The assignment of HLA type is then based on the reaction pattern observed, compared to patterns associated with published sequences.

The Luminex HLA typing kits on the market today give a resolution of results that means that for solid organ, platelets and most disease association typing, no further testing is required to get the resolution of results required. Testing can be undertaken at all the relevant loci including HLA-A, B, C for platelets plus DRB1, DRB3, 4, 5, DQA, DQB1, DPA1 and DPB1 for solid organs. For stem cell services, Luminex typing gives a resolution of results that allows the potentially matched related donors to be identified and further high-resolution testing undertaken for suitable donors. Most laboratories are however switching to Next Generation Sequencing (NGS) which means that Luminex typing may be less relevant to stem cell transplantation in the future.

In terms of cost, the Luminex systems does compare well with other rapid high throughput systems. Luminex can be fully or semi-automated and allows for much easier batching of samples compared to some of the other techniques. This means that a large number of samples can be rapidly HLA typed in a single run. On the other hand, each run does take several hours (4-6hrs) which means that Luminex is not necessarily suitable for single sample HLA typing as would be required for deceased donor typing for instance where a turnaround time of 4 hours is required. It may potentially be suitable as a backup technique. Overall, the ability to rapidly type a large number of samples to a good level of resolution at a reasonable cost means that Luminex typing remains highly relevant to H&I service provision today.

QPCR

One qPCR system used for HLA typing is LinkSeq, supplied by Linkage Bioscience, the other being QType, supplied by CareDX. These are real-time allele specific PCR SSP methods. Both are set up on a plate like traditional SSP, however the samples are read on a real-time platform instead of gel electrophoresis.

LinkSeq uses a DNA intercalating dye SYBR-Green which fluoresces brightly when it intercalates with double stranded DNA. The real-time PCR platform takes fluorescent readings of each well on the plate at different temperatures. The double stranded DNA melts during each cycle, releasing the SYBR-Green and reducing the fluorescence. These changes in fluorescence over the temperature range generates a melt curve that can be used to detect the presence or absence of allele specific amplification. QType on the other works by incorporating a hydrolysis probe which binds ton the sequence somewhere in between the primer binding sites. The hydrolysis probe includes a fluorescent dye on one end and a quencher on the other. The close proximity of these prevents the dye from fluorescing when excited. During amplification, the hydrolysis probe is degraded and the dye and quencher are no longer in close enough proximity to prevent excitation which can be detected.

The RCPath college guidelines set out an 8-hour turnaround time from a donor becoming identified to the tissue typing being available for a matching run. As much of this time is taken up with the processes before the sample arrives in the H&I laboratory, a lab typically only has a four-hour window in which to complete a HLA type for a deceased donor. Traditionally, deceased donor typing has been performed by various PCR-SSP techniques. These have a four-hour turnaround time meaning that it was not always possible to obtain a result with the target time. qPCR has found a niche in decease donor typing. The process is mostly automated, does not require gel electrophoresis and result transfer can be automated so that no manual transcription is involved. It also has a 2-2.5hr turnaround time. This has advantages not just in speed and quality of results but also in the time of on call staff and is now highly relevant to H&I service provision.

SBT

PRINCIPLES OF SEQUENCE BASED TYPING (SBT)

DNA sequencing techniques were first described in the 1970's by Gilbert & Maxam using a chemical sequencing approach and by Sanger using a chain termination method. Both lead investigators were awarded the Nobel Prize for chemistry for their work. The Sanger sequencing method is the simpler of the two and is widely used for Sequence Based Typing (SBT).

The sequencing step in sequence based typing is preceded by locus specific PCR amplification to generate templates for the sequencing step. The sequencing step requires single stranded DNA templates, DNA primers, a DNA polymerising enzyme, deoxynucleotidephosphates (dNTPs) and fluorescently labelled di-deoxynucleotidephosphates (ddNTP**Error! Bookmark not defined.**). At a minimum, HLA class I requires sequencing at exons 2 and 3 and HLA class II requires sequencing at exon 2. Other exons are often also sequenced to help increase the resolution. The amplified PCR products are divided into four separate sequencing reactions (eight if sequencing in the forward and reverse directions), each containing all four dNTPs (dATP, dCTP, dGTP & dTTP) and the DNA polymerase. To each reaction, one of the ddNTPs is added. The ddNPT will terminate chain elongation once incorporated into the growing sequence as they lack the 3'-OH group required for the formation of a phosphodiester bond between themselves and an incoming nucleotide. The products of the sequencing reaction are analysed using automated high-throughput DNA sequence analyzers. Modern DNA sequencers use capillary electrophoresis for size separation, detection of dye fluorescence and data output as peak traces on a chromatogram.

Up until very recently, SBT has been critical to Haematopoietic Stem Cell transplantation where HLA typing at high resolution may be needed in the related setting and is critical in the unrelated setting. Most laboratories are now however introducing NGS which is reducing the reliance on SBT.

In stem cell transplantation, Petersdorf et. al., and others have shown that the risk of GvHD increases with increasing numbers of mismatches in the Graft versus Host direction (i.e. HLA genes present in the patient but absent in the donor). Similar studies have also shown that the risk of graft failure increases with increasing numbers of mismatches in the Host versus Graft direction (i.e. HLA genes present in the donor but absent in the patient). SBT can be used to obtain high resolution

second field level for HLA-A, B, C, DR and DQ and in some cases for HLA DP for such transplants.

Sequence based typing is also an option in some disease association and drug sensitivity cases where a high resolution HLA type is required. Examples include HLA-B*57:01 typing for Abacavir drug hypersensitivity in HIV positive patients and HLA-DRB1*06:02 typing in Narcolepsy cases.

RELEVANCE OF SANGER SBT

SBT is carried out using the Sanger sequencing method in which the sequencing step is preceded by locus specific PCR amplification to generate target templates. The sequencing step is carried out using labelled ddNTP's which cause chain termination. The products of the sequencing reaction are analysed using automated high-throughput capillary electrophoresis DNA sequence analysers.

Up until very recently, SBT has been highly relevant to Haematopoietic Stem Cell transplantation though most laboratories are now switching to NGS. Whichever technique is used, high resolution HLA class I and II typing has been shown to be relevant in the related HSCT setting. High resolution HLA-A, B, C, and DR have been shown to be relevant in unrelated HSCT and in cord blood transplantation. In the UK HLA-DQ and HLA-DP high resolution typing and matching are often carried out.

SBT is also an option in some disease association and drug sensitivity cases where a high-resolution HLA type is required. Examples include HLA-B*57:01 typing for Abacavir drug hypersensitivity in HIV positive patients and HLA-DRB1*06:02 typing in Narcolepsy cases.

The long-term relevance of SBT is in question with the emergence of NGS. One of the disadvantages of SBT is that it often requires further exons to be amplified to resolve ambiguities. These are resolved at first pass with NGS. NGS is already making an important contribution to registry donor HLA typing. As newer, faster

NGS techniques emerge, they are being applied to patient and family donor typing and are set to replace SBT.

NGS

PRINCIPLES OF NGS

There are multiple NGS systems but they all follow the same key stages of target generation, library prep, clonal amplification and sequencing on the platform. The GenDx kit on the Illumina MiSeq system is described here:

- Long range PCR for target generation
    - Starts with HLA locus specific amplification for target generation
    - Currently, the whole of class I but not all exons in all class II are amplified. There are plans to expand to all class II exons
    - The amplification can be verified on a gel
    - The amplicon concentration can be determined using a DNA quantification system such as a Qubit
- Library Prep
    - Two enzymes are used to generate fragments
    - The fragments are then end repaired to generate blunt ends, followed by dA tailing to generate fragments with an average size of 400bp
    - The dA tailing generates binding sites for the illumina adaptors
    - The next step is the addition of the adaptors
    - After adaptor ligation, the DNA is size selected (>400bp) and cleaned up using magnetic beads
    - The fragments + adaptors are then indexed with a pair of indexes, one on each side which allows amplicons from different HLA genes and different samples to be pooled
    - This early pooling is one of the advantages of the GenDx system
    - The indices contain sequences required for cluster formation
    - After indexing, a second size selection and DNA clean-up is required
    - After indexing the libraries can be pooled to generate a single tube with all amplified loci for all samples
    - The concentration of the pooled library is then determined to generate an optimum sample for the flowcell
    - The library quantification can be carried out on a qPCR platform
- Clonal Amplification

    - The library is then set to be sequenced on the MiSeq
    - The library is flowed over the flowcell and the indices anneal to complimentary targets on the flowcell
    - The fragments are amplified into clonal clusters
- Sequencing reaction
    - The clonally amplified clusters are then sequenced in a 'sequencing by synthesis' method
    - Just one base is added in each cycle of the 'sequencing by synthesis' reaction
    - The fluorescence of each incorporated base is captured in each cycle and the incorporated base recorded
    - Sequencing takes place in a 'paired-end' approach, with sequencing from each end, rather than a single direction
- Analysis and Reporting
    - NGS Engine is used for data analysis
    - No additional GSSP's etc. required
    - Gives 'unambiguous' allele level HLA types

NGS has several advantages over traditional SBT.

- NGS allows multiple loci from multiple samples to be sequenced in parallel and is therefore allows for high throughput compared to SBT
- SBT sequences single strands while NGS has a depth of read often greater than 30, resulting in fewer ambiguities
- The paired end sequencing approach of NGS makes it straight forward to phase-resolve
- NGS generates allele level HLA typing without requiring further amplification and sequencing as often required for SBT
- The cost of NGS has come down in recent years and is now comparable to, if not cheaper than SBT when other factors such as staff time are taken into account

HLA ANTIBODY TESTING

CDC SCREENING

Many H&I laboratories have significantly reduced or even stopped doing CDC antibody screening but it is potentially a very useful technique for helping the lab to understand the clinical relevance of high MFI Luminex antibodies.

CDC screening helps predict donor mismatches which could potentially lead to a positive CDC crossmatch. CDC screening allows IgM to be separated out form IgG antibodies. All IgG CDC Pos. specificities must be listed as unacceptable mismatches with ODT.

In some patients, some Luminex class I positive specificities, especially where the MFI is less than 5,000-10,000, can turn out to be repeatedly negative by CDC screening (unseparated cells). Although CDC Neg, Luminex Pos. specificities can cause acute and chronic rejection, in carefully selected patients who can tolerate enhanced immunosuppression, use of CDC can potentially allow such specificities not to be listed as unacceptable mismatches, widening the pool of potential donors available to such patients and ensuring that highly sensitised patients do not accumulate on the waiting list. However, in the UK, this approach needs to be considered carefully in light of the new kidney and pancreas allocation scheme introduced in 2019.

CDC screening can also prove useful in addition to acid treatment of Luminex beads, in identifying Luminex reactivity which may be due to denatured antigens on beads.

LUMINEX ANTIBODY TESTING

There are three types of Luminex systems for HLA antibody detection and definition. These are a pooled antigen system, a phenotype panel system and a single antigen bead (SAB) system. The pooled antigen system serves as a screen for determining the presence or absence of HLA antibody though limited specificity information may be gleaned in some cases. Specificity testing can then be performed either by the phenotype panel approach or by the more detailed single antigen method.

The pooled antigen and phenotype panel methods use affinity purified HLA antigens to coat Luminex beads whilst the SAB method uses recombinant HLA proteins. The

actual test procedures in all three systems follow the same basic steps. Sample serum is incubated with Luminex beads. Up to 100 different beads, each uniquely internally labelled, can be used. Any HLA antibodies present will bind to their complementary antigens on the beads. A series of wash steps remove unbound antibody. Bound antibody is then labelled with an anti-human IgG – Phycoerytherin conjugate. A washing stage may then be required to remove unbound conjugate depending on the Luminex typing kit used. The Luminex platform is then used to identify each bead and any phycoerytherin and therefore HLA antibody bound to each bead. The reaction pattern is then compared to a predefined reactivity pattern for each batch of beads used. Negative and positive control beads and a negative control sample are used to quality control the tests.

The results may be interpreted using assistance from software supplied with the kits. This must however be done with due consideration of the clinical context. The supplier recommended cut off values for positive reaction may be adjusted up or down depending on locally validated procedures.

THE ADVANTAGES AND DISADVANTAGES OF LUMINEX FOR ANTIBODY DETECTION

The two main advantages of the Luminex methodology for antibody detection when compared to other techniques, particularly Complement Dependent Cytotoxicity (CDC), are the speed with which tests can be turned around and the sensitivity, specificity and semiquantitative nature of the results obtained.

The Luminex methodology has completely changed the speed with which it is possible to obtain HLA antibody identification on a sample and the detailed specificity of HLA antibodies that can now be identified. With Luminex it is possible to look at reactivity against single HLA antigens which means that the interpretation of results is no longer complicated by issues such as linkage disequilibrium as is the case with CDC. In addition, with Luminex we are able to detect antibodies directed against HLA-DPA and HLA-DQA which are increasingly being shown to be relevant in some solid organ transplantation. In kidney transplantation, the specificity of the single antigen beads means that most Centres can now list a detailed set of unacceptable mismatches without any residual reactivity.

This and the rapid turnaround time has influenced clinical practice in many areas, including in the post solid organ transplant setting. For post-transplant patients, Luminex gives rapid results of a HLA antibody investigation when a suspected rejection or reduced graft function due to donor specific antibodies (DSA) is suspected. Another area where this speed and specificity proves clinically useful is in HLA antibody incompatible transplantation (HLAi), where the rapid turnaround of samples during both the desensitisation and immediate post-transplant phases allow the residual DSA levels to be rapidly determined (though paired/pooled exchange has reduced the need for HLAi transplantation).

The Luminex technique only requires a small amount of serum and yields a significant amount of information, especially when single antigen beads are used, not just on the specificity of the HLA antibodies present but also on their relative strengths in the form of median fluorescence intensity (MFI). Information on the MFI of antibodies present allows for a risk based approach to the management of highly sensitised patients where it is no longer a case of an antibody being present or absent but if present, using it's MFI to tailor clinical response such as changes in immunosuppression.

This leads though to a question of the clinical significance of the reported MFI's and maybe a disadvantage of the Luminex methodologies. The sensitivity of the Luminex methodology means it is possible to obtain Luminex positive, flow and CDC crossmatch negative results. Transplant units need to work with their laboratories to determine the clinically significant MFI cut off for their various transplant programs - kidney/pancreas, heart, lung, liver etc, taking into account any national guidelines.

One potential disadvantage of the Luminex platform is that its use limits the laboratory to being able to identify antibodies only if its cogent antigen has been included on the beads. In CDC, this situation is overcome by adding more cells to the panel. Also, standard Luminex kits give no information of whether or not detected antibodies are complement fixing. On the other hand, the Luminex methodology does allow for identification of some allele specific antibodies. A disadvantage is the role denatured antigens on beads play in complicating the interpretation of results.

Given the speed, sensitivity and specificity of the Luminex methodology, the advantages do therefore outweigh the disadvantages. Due care is required when considering the clinical significance of Luminex positive results however, especially if negative by other techniques. HLA antibody testing now perhaps is in need of a new next generation antibody detection, identification and quantification technology.

RELEVANCE OF LUMINEX ANTIBODY TESTING

Luminex antibody testing remains relevant to the assessment of immunological risk in transplantation today. Luminex is a solid phase technique which uses microspheres coated with antigen. The single antigen bead in particular has made the identification of antibodies to individual specificities much clearer and faster to obtain than was possible with cell-based techniques such as CDC or even with ELISA.

The use of Luminex, which is semi quantitative, allows risk levels to be assigned in the form of MFI's to any antibodies present. This has enables virtual crossmatching which has revolutionised the way crossmatching is now done, allowing for transplantation without a pre transplant wet crossmatch to be followed later with a retrospective crossmatch in carefully selected patients.

Use of Luminex also allows the crossmatch results, particularly flowcytometric but also CDC crossmatches, to be put in the clinical context of antibodies present. This allows the interpretation of crossmatch results, taking into acclount reactivity that may potentially not be due to HLA.

HLA incompatibly transplantation (HLAi) would probably not be possible or would be very difficult without Luminex antibody testing. Use of Luminex allow us to predict the potential number of plasma exchanges that may be required to bring a DSA down to a level that would result in a negative crossmatch. We can do pre and post plasma exchange Luminex tests for each round of plasma exchange to assess the level of DSA.

Luminex testing is also vital when assessing the patient post-transplant for potential signs of AMR. In these circumstances, a Luminex result can be turned around the

same day to detect donor specific antibodies (DSA) to help inform the clinical response of a poorly functioning graft.

However, caution is required with the use of Luminex as the MFI values are only semi quantitative and do not always correlate in a completely linear manner with crossmatch MCS's/RMF's. There are also low-level MFI's which, if they are DSA, are not necessarily a contraindication to transplant. Also, some Luminex positive reactions can be due to denatured antigens on the beads and may not be true DSA's.

Overall however, Luminex antibody testing remains very relevant to transplantation today.

CROSSMATCHING

FLOWCYTOMETRIC CROSSMATCHING

The Flowcytometric crossmatch (FCXM) is highly relevant to transplantation and for many H&I labs is the only technique used for crossmatching. It is semi-quantitative, giving Mean Channel Shift (MCS) and Relative Mean Fluorescence (RMF) values that can help inform clinical risk assessment. When used in association with Luminex, it can potentially help the transplant team to take a risk-based approach to transplantation rather than a blanket yes or no to the presence or absence of DSA.

The FCXM is much more sensitive than a CDC XM and can pick up reactivity at MFI's that would typically be negative by CDC. A negative FCXM can therefore give confidence to the renal team to proceed with a transplant, taking all other clinical information into account. A positive FCXM on the other hand can be used as part of an overall risk assessment, taking into account the nature of any DSA present, i.e. class I or II or both, the MFI levels, the CDC crossmatch result and the patient history. BSHI/BTS guidelines state that a positive T and B cell FCXM in the presence of Class I DSA or a positive B cell FCXM in the presence of class II DSA are intermediate risk and transplant should be avoided if reasonably possible. Transplantation in these circumstances may however proceed as part of a high-risk protocol. The decision to proceed or not will also be influenced by the CDC crossmatch results if available and the actual FCXM MCS/RMF value as well as the DSA MFI.

In settings where a prospective crossmatch is undertaken, such as the live donor transplant or transplantation in sensitised patients with potential DSA's, the FCXM is relevant for assessing risk and determining whether or not to proceed as part of a standard or high-risk protocol.

In the retrospective transplant setting, such as in cardiothoracic transplants or transplantation in minimally sensitised renal patients following a virtual prospective crossmatch, the FCXM is useful for informing the post-transplant clinical response.

In HLAi, rounds of desensitisation are typically continued until the post plasma exchange FCXM is negative or a decision is made that desensitisation is not a viable option for the given patient.

The FCXM needs to be interpreted in light of patient history. Patients with autoimmune diseases for instance often have auto antibodies which result in a positive Allo FCXM. For such patients and Auto FCXM is required. A Pos. Allo FCXM which is mirrored in the Auto and where DSA is Neg is not a contraindication to transplantation.

Patient treatment can also affect the FCXM. A patient treated with Rituximab for instance prior to and ABO incompatible transplant will have a Pos. B cell FCXM. In the live donor transplant setting, such patients must have final crossmatch sample taken pri0r to commencing Rituximab. Even donor treatment can affect the outcome of the FCXM. Where a donor is treated with Cyclophosphamide for example, will have little or no B cells, making a B cell FCXM impossible.

CDC CROSSMATCHING

Many H&I laboratories have significantly reduced or even stopped doing CDC crossmatching but it remains a true predictor of hyperacute rejection. The BSHI/BTS guidelines state that a T and B cell positive CDC crossmatch in the current sample in the presence of class I DSA has a high risk of hyperacute rejection and is a transplant veto. A B cell CDC positive crossmatch (XM) in the current sample in the presence

of class II DSA is high risk and should be avoided if possible. A T and B cell positive CDC XM in the historic sample in the presence of class I DSA or a B cell positive CDC XM in the historic sample in the presence of class II DSA both carry a high risk of an anamnestic reaction and should only be carried out as part of a high-risk immunosuppression strategy.

In highly sensitised patients who would otherwise accumulate on the waiting list, use of CDC crossmatching alongside FCXM is highly effective for patients who can tolerate an enhanced immunosuppression regime. In such patients, a FCXM Pos, CDC Neg XM can potentially be undertaken, taking into account all other factors such as antibody priming source, repeat mismatches, MCS/RMF, DSA MFI, potential for the patient to get better deceased donor offer and live donor options including paired/pooled exchange.

In settings where a prospective XM is undertaken, such as the live donor transplant, the CDC XM is useful, alongside the FCXM, for assessing risk and determining where or not to proceed as part of a standard or high-risk protocol.

In some retrospective transplant settings, such as in minimally sensitised renal patients following a virtual prospective crossmatch, the CDC XM is often not undertaken.

In HLAi transplantation, rounds of desensitisation are typically continued until the post plasma exchange FCXM is negative. In some very highly sensitised patients where IvIG is used as the desensitisation protocol, it often proves useful to also perform CDC crossmatching as part of the immunological risk assessment.

VIRTUAL CROSSMATCHING

A Virtual Crossmatch (vXM) is a crossmatch that involves a determination of the presence or absence of donor HLA specific antibodies (DSA) in a patient by a comparison of the patients' HLA antibody specificity profile with the HLA type of the proposed donor without carrying out a 'wet' crossmatch such as a Complement Dependent Cytotoxic (CDC) or flowcytometric crossmatch.

Patel and Terasaki demonstrated in 1969 that hyperacute rejection can result from allograft injury caused by preformed donor specific anti-HLA antibodies (DSA) and since then a pre transplant crossmatch became a mandatory requirement for renal transplantation with a positive cytotoxic HLA crossmatch a contraindication to transplant. This pre transplant crossmatch almost completely eliminated hyperacute antibody mediated rejection. The single most important technological advance which has allowed this wet crossmatch to be replaced with a vXM in carefully selected patients is the development of solid phase assays such as Luminex for HLA antibody screening and identification. Use of Luminex assays has revolutionised HLA antibody investigation. One of the main advantages is the sensitivity and specificity of the results obtained. When using single antigen bead assays, for each antibody detected, a Mean Fluorescence Intensity (MFI) value is obtained which provides a measure of the strength of the antibody. Studies have found good correlation (>85%) between the vXM as predicted based on MFI values obtained in Luminex and flowcytometric crossmatch results, with MFI figures of greater than 5000 shown to correlate with positive flowcytometric crossmatch. There is however some inter laboratory variation which makes it important for each Transplant unit and their laboratory to establish the MFI values that correlate with positive crossmatch in their hands. The correlation indicated in published literature between a vXM and CDC results has not been as strong.

The concordance between a vXM and a FCXM is not 100% with rare cases of vXM Neg, FCXM Pos., as well as cases of vXM Pos., FCXM Neg. The cases of vXM Neg, FCXM Pos. are possible explained by non-HLA antibodies such as antibodies to HNA or by the presence of auto antibodies. The cases of vXM Pos., FCXM Neg. are possibly explained by denatured epitopes in the solid phase giving false positive.

The current British Transplant Society/British Society for Histocompatibility and Immunogenetics (BTS/BSHI) guidelines permit the use of Virtual Crossmatching for renal transplantation in place of the pre-transplant wet crossmatch provided the laboratory has validated procedures in place. When a Virtual Crossmatch is used, an early post-transplant retrospective wet crossmatch is currently required by for EFI accreditation.

CHIMERISM

RELEVANCE OF CHIMERISM TESTING

Chimerism is a vital part of post-transplant testing after Hematopoietic Stem Cell Transplantation (HSCT). Chimerism monitoring can be used to obtain accurate information on a patient's' engraftment status post stem cell transplant by quantitatively determining the proportion of donor and recipient derived cells in the patient post-transplant. This requires the use of DNA markers that are informative enough to distinguish donor derived DNA from recipient derived DNA. Most H&I laboratories use Short Tandem Repeats (STR's) for chimerism monitoring. STR's are short sequences of DNA, distributed throughout the genome, which are repeated in tandem a variable number of times. The number of repeats of different STR markers varies between individuals, from 4 to 50 repeats for some STRs, giving a highly polymorphic system that can be used to uniquely identify donor derived DNA from patient derived DNA. With the exception of monozygotic twins, careful selection of a number of STR markers will enable most individuals to be uniquely identified and this technique has been in use in forensic science for just such a purpose. Examples of STR markers include TH01, a tetra nucleotide repeat of AATG located on chromosome 11 and FES/FPS a tetra nucleotide repeat of AAAT located on chromosome 15.

Chimerism is important clinically as it can highlight when engraftment has taken place and also when it may have started to fail. Treatment decisions can be based on the results of chimerism to try to rescue a failing graft. In the case of rising patient chimerism after stem cell transplant the clinicians may decide to lower the immunosuppression of the patient to try and promote engraftment. Other investigations which could be undertaken would be a post-transplant antibody screening of the patient in case the patient has produced HLA antibodies against any mismatches as may be the case with a cord blood or mismatched adult stem cell transplant. If this is the case the patient could undergo plasma exchange to reduce the antibody titre.

PRINCIPLES OF CHIMERISM TESTING BY SHORT TANDEM REPEAT (STR)

Chimerism monitoring can be used to obtain accurate information on a patient's engraftment status post stem cell transplant by quantitatively determining the proportion of donor and recipient derived cells in the patient post-transplant. H&I laboratories typically report chimerism results as % donor chimerism. A 100% donor chimera implies complete engraftment. A 0% donor chimerism implies no donor

engraftment with all other percentages reported as mixed chimerism showing the proportion of donor engraftment. A longitudinal study of chimerism is of more value than a single static result and the H&I laboratory would typically test at agreed intervals and report a history of the chimeric status of the patient since transplant rather than a single test report. Relative changes in the magnitude of donor chimerism provides key information which helps clinicians to intervene and to monitor the patients' response to such intervention. Intervention options include changes in immunosuppression regimes and donor lymphocyte infusion (DLI). Many H&I laboratories offer lineage specific chimerism monitoring, separating T and B cells from myeloid cells. This approach proves useful in some cases of mixed chimerism, where one cell line may dominate and mask clinically significant changes in other cell subsets.

The use of chimerism monitoring has also proved useful in the case of double cord transplants where it is possible to see a mixed chimera consisting of patient and one or both cords early in the post-transplant period before one cord eventually expands to 100% present in the patient.

IN SILICO ASSAYS

CRF

The Calculated Reaction Frequency (CRF) was introduced to overcome problems with the previous Panel Reactivity (PRA) system which relied on the composition of the testing panel to give the percentage reactivity. This was however flawed as the panel composition changes between tests and may have an antigen frequency that does not reflect the donor population. Developed first in the USA and adopted by UNOS in 2006 and later in the UK, the CRF is based on a calculation of the reaction frequency of a listed set of unacceptable mismatches for a patient, against a panel of 10,000 recently added deceased donors. The CRF therefore gives a measure of the chances of a patient finding a compatible donor in the donor pool.

The use of CRF based on a set of listed unacceptable mismatches for each patient is made much easier by the wide adoption of solid phase assays such as Luminex. Luminex assays, especially those involving the use of single antigen beads (SABs)

allow fine specificity definition and allows the strength of the reactions (MFI) to be used to assess immunological risk and help decide whether or not a specificity should be listed.

In the UK, patients with a CRF of 100% are prioritised into Tier A for kidney and pancreas allocation in the newly updated organ matching scheme. All other patients in Tier B are allocated points for high matchability scores which helps improve their chances of getting a offer.

MATCHABILITY

Matchability is a measure of how easy or difficult a patient is to match for an organ. In the UK it is calculated based on blood group, HLA type and HLA antibodies. It gives an estimate of the number of ABO identical, HLA compatible donors within a 10,000 donor pool a given recipient is matched with.

Matchability is scored on a scale from 1 -10, where 1-3 represent easy to match, 4-6 are moderate and 7 -9 are difficult. A matchability score of 10 denotes very difficult to match. In the UK, patients with a matchability score of 10 are prioritised into Tier A when deceased donor pancreas and kidneys become available.

HLA MATCHMAKER

It has long been known that antibodies do not bind to the whole of the HLA antigen but rather to specific epitopes on the antigens. Each HLA antigen potentially has many sites or epitopes that can bind antibody. These epitopes may be private to a given HLA antigen or they may be shared by more than one HLA antigen, i.e. they may be public. In recent years it has become possible to identify the polymorphic amino acid residues that define many of these public and private HLA epitopes. This has enabled the development of laboratory based and in-silico strategies for assessing HLA compatibility at the epitope level.

One laboratory based strategy for defining epitopes has been described by El-Awar and Terasaki et al. They have used single antigen beads (SAB's) to define a set of 103

epitopes on HLA class I antigens. Their assays were performed with mouse monoclonal antibodies directed against HLA as well as with anti-HLA from sensitized patients and multiparous women. Where alloantisera was used, is was first absorbed out using SAB's or cell lines before being eluted for testing in a Luminex assay.

An example of an in-silico assay is that proposed by Kosmoliaptsiset et. al. They have described an epitope definition system based on interlocus and intralocus comparison of patients and donors to identify amino acid differences but also crucially, they have carried out an assessment of the physiochemical properties of the amino acid mismatches and the role these may play in clinical outcome. They have applied this scheme to kidney transplantation in the UK.

The most widely known in-silico strategy in the field of HLA is however the HLA Matchmaker algorithm (http://www.hlamatchmaker.net) as proposed by Duquesnoy. Two versions of HLAMatchmaker have been described, the 'triplet' and 'eplet' versions. In the triplet version, epitopes are defined by linear sequences of triplets of amino acid residues in alloantibody-accessible positions of the HLA molecules (the α-helices and β-loops of the protein chain). The eplet based version of HLAMatchmaker defines epitopes as those residues located within a diameter of about 3-3.5 Å around a non self residue. Although some of these eplets consist of amino acids in linear sequences as described in the triplet model, many consist of amino acids from discontinues regions of the sequence, brought together by the folding of the molecule. In HLAMatchmaker, Donor-Recipient HLA compatibility is based same intralocus and interlocus comparisons of triplets or eplets.

CHAPTER 3 IMMUNOGENETICS AND DISEASE

DISEASE ASSOCIATIONS

ACTINIC PRURIGO

Actinic Prurigo is a sunlight induced itchy, pimply, idiopathic photodermatoses. It is uncommon in Caucasian populations with preponderance in native populations in North, Central and South America. It is more frequent in females by a factor of 2 – 4 and has a childhood onset, with a mean age at presentation of less than 10 years.

Actinic Prurigo is triggered by UV radiation in genetically susceptible individuals. Some researchers have proposed that it is an autoimmune disorder, the putative antigen being an epidermal protein which is transformed by UV exposure. Family studies have indicated that the disorder has a strong association with the HLA class II gene DRB1*04:07, which is found in 60 – 70% of patients compared to 4 – 8% of DR4 controls. HLA-DRB1*04:01 is the next most prevalent gene associated with Actinic Prurigo, found in approximately 20% of patients.

Actinic Prurigo presents with symptoms very similar to polymorphous light eruption (PLE), which is another sunlight induced skin irritation. Genetic testing helps to distinguish the two as PLE has no HLA association. Care is needed in interpreting the results though as a small number of patients do present with both Actinic Prurigo and PLE.

ADDISON'S DISEASE

Addison's disease is a disease of the Adrenal Cortex caused by autoimmune destruction of the Adrenal gland with fibrosis and mononuclear cell infiltration. Symptoms become evident when more than 90% of the Adrenal gland is destroyed. Addison's disease is characterised by increased production of adrenocorticotropic hormone (ACTH) and adrenal insufficiency with reduced production of corticosteroids and androgens. Patients present with hypotension, weakness, fatigue, light headedness when standing up, anorexia, nausea, salt craving and increased

melanin pigmentation of the skin. Autoantibodies to 21-hydroxylase are present in 90% of cases.

Family studies have shown that Addison's disease has a genetic component with HLA class II the most strongly associated genetic region. Addison's disease is associated with DRB1*03:01-DQB1*02 (DR17, DQ2) and DRB1*04-DQB1*03:02 (DR4, DQ8). The most strongly associated DRB1*04 allele is DRB1*04:04. The major histocompatibility complex class I related chain - A (MICA) is an additional risk factor. MICA genes are highly polymorphic with over 107 alleles described. MICA is a ligand for the Natural Killer (NK) cell receptor NKG2D which is important for thymic maturation of T cells.

Autoantibody testing for anti-21-hydroxylase is more diagnostic in Addison's disease than genetic testing. Genetic testing does however contribute to a better understanding of the aetiology of the disease.

ANKYLOSING SPONDYLITIS

Ankylosing Spondylitis (AS) is one of the major forms of chronic inflammatory arthritis and is the prototypical example of the spondyloarthropathies, a group of chronic autoimmune joint diseases. Ankylosing Spondylitis has a global distribution, though rarer in Africans. It is characterised by arthritis affecting the spine and pelvis, specifically the sacroiliac joint, initially causing pain and reversible stiffness (stiffness in the mornings that goes away later in the day with exercise) but in a proportion of cases leading to progressive joint fusion and irreversible stiffness and deformity.

Twin studies have confirmed that susceptibility to AS is genetically determined. There is a strong association with the HLA class I molecule HLA-B27, found in upwards of 90% of patients. Association of AS to B27 is amongst the strongest genetic associations with a common disease, although the mechanism of action remains uncertain. Ankylosing Spondylitis is thought to be triggered by exposure to a common environmental pathogen. Proposed mechanisms include the 'arthritogenic peptide', 'molecular mimicry' and endoplasmic reticulum stress due to B27 misfolding and accumulation. The arthritogenic peptide theory proposes that disease results from an HLA-B27 restricted cytotoxic T cell response to a peptide or peptides found only in joints and other affected tissues. The molecular mimicry theory

proposes that some invading pathogens share antigenic determinants with native cell surface antigens in the joint resulting in an autoimmune response.

Different B27 alleles have different strengths of association with AS, making genetic testing useful over and above serological testing. HLA-B*27:02 and B*27:05 are strongly associated. Until recently B*27:06 and B*27:09 were thought to be protective but a number of AS cases have now been reported in patients carrying these alleles, making these alleles protective only relative to the strongly associated alleles.

Family studies suggest that less than 50% of the overall genetic risk is due to HLA-B27. HLA-B27 is found in 8 – 10% of the population with only a minority of carriers going on to develop the disease. It is likely that other genes both within and outside the MHC are involved. Other MHC genes which have shown some association in studies include HLA-B*40:01, B*52 and B*38. One haplotype study looking at the HLA-B - DRB1 haplotype has suggested the existence of non B27 genes in AS carried by both B27 positive and B27 negative individuals though the actual association was not identified. A number of non HLA genes have been shown, through genome wide association studies, to be associated with AS. These include the Interleukin-23 Receptor (IL23R) and the protein cleaving enzyme Endoplasmic Reticulum Aminopeptidase 1 (ERAP1).

The strong association of HLA-B27 with AS makes B27 testing a useful component of the diagnostic work up which includes a physical examination, use of X rays MRI and a check for family history of AS. Waiting for a patient to fulfil all the classification criteria for a diagnosis of AS may been too late in that the damage to joints would already have happened. The value of B27 testing is that it allows a presumptive diagnosis and early treatment in patients showing some of the symptoms.

BEHCETS

Behcet's disease is a systemic inflammatory vasculitis, characterised by multiple courses of remissions and relapse of oral ulcers, genital ulcers, skin lesions and ocular lesions. It can affect the arteries and veins of almost any system and has been shown to involve the gastrointestinal and neurological systems. It was first described by the Turkish dermatologist Dr Behcet in the 1930 and is endemic in Turkey and

other parts of the old 'Silk Route' through Europe and Asia. The cause of Behcet's is unknown but it is believed to be triggered by an infectious or environmental agent in a genetically predisposed individual. The most generally accepted theory is that the pathogen derived antigen 'Heat Shock Protein 65 (HSP65)', which has a high sequence homology with the human HSP60 results in cross-reactivity which leads to an autoimmune response.

The HLA class I molecule HLA-B51 is the most strongly associated risk factor and has been shown to be associated with Behcet's in Turkish and Asian patients though the association in Caucasian patients is much weaker. This has lead to the search for other genes which may be the true disease markers. One study has found a potential association with HLA-B*57:01. Other MHC genes which may be involved include MICA, though this is in linkage disequilibrium with HLA-B51 and the TNF genes. Non MHC genetic systems which have been proposed to be involved in Behcet's include the interleukin-1 (IL-1) gene and mutations in the Mediterranean fever gene (MEFV).

Testing for HLA-B51 is a useful, though not a diagnostic tool for Behcet's as the vast majority of HLAB51 carriers do not develop Behcet's. Diagnosis is instead based on the recurrence of symptoms including oral ulcers, genital ulcers, skin lesions and ocular lesions. Treatment options depend on organs involved. Available treatments include corticosteroids, azathioprine, Cyclophosphamide, cyclosporine A and anti-TNF agents.

BIRDSHOT CHORIORETINOPATHY

Birdshot Chorioretinopathy is a rare form of bilateral posterior Uveitis, accounting for between 1 - 3% of all Uveitis cases. A slight female predominance has been reported. The disease is uncommon in children. Birdshot Chorioretinopathy causes severe, progressive inflammation of both the choroid and the retina. The name of the disease stems from the hypopigmentation pattern of the lesion on the retina, which resembles the impacts from a shotgun. Symptoms of Birdshot Chorioretinopathy include retinal vasculitis, particulate matter in the vitreous or vitreous inflammation, macular oedema, flashing lights in the eyes, night blindness and loss of colour vision. A frequent prognosis is complete loss of visual acuity.

Birdshot Chorioretinopathy is the disease with the strongest association to a HLA class I antigen, with more than 95% of patients carrying the HLA-A29 antigen. The relative risk of HLA-A29 carriers developing Birdshot Chorioretinopathy has been estimated to be between 50 and 250. HLA-A*29:02, which is the most frequent A29 allele in the Caucasian population is also the allele most frequently associated with Birdshot Chorioretinopathy in Caucasians. The disease has however been observed in HLA*29:01 Caucasian patients. Birdshot Chorioretinopathy is extremely rare in Asia where the most frequent HLA-A29 allele is A*29:01. The mechanism by which HLA-A29 confers susceptibility to Birdshot Chorioretinopathy is unknown. Proposals include antigen specific molecular mimicry following an infection or a role for retinal S-Antigen.

The presence of HLA-A*29 alone is not sufficient for a diagnosis of Birdshot Chorioretinopathy, as there are many cases of patients who do not carry HLA-A*29. Indeed the prevalence of HLA-A29 in the Caucasian population (around 7%) is far higher than the disease frequency and it is likely, as with many HLA disease associations, that other factors are involved. Nonetheless the strong association suggests that genetic testing for HLA-A*29 and A*29 alleles, over serological testing, is useful as a supportive finding as part of the diagnosis.

COELIAC DISEASE

COELIAC OVERVIEW

Coeliac disease is an inflammatory disorder of the small intestine with an autoimmune component and strong heritability. It is characterised by diarrhoea, abdominal distension, poor weight gain and short stature. Coeliac disease is a lifelong condition, with the only effective treatment being complete exclusion of gluten from diet. This is effective in over 95% of cases.

Gluten is a dietary protein found in wheat, barley and rye. Gluten peptides pass through the epithelial barrier of the small intestine into the lamina propria, where they are deaminated by the enzyme transglutaminase to give negatively charged gluten peptides. In genetically predisposed individual, the deaminated gluten peptides trigger a cascade of innate and adaptive immune responses including lymphocyte infiltration into the proximal part of the small intestines leading to destruction of the intestinal epithelium and mucosa including villous atrophy and crypt hyperplasia.

The HLA class II antigens DQ2 and DQ8 are the major risk factors predisposing individuals to Coeliac Disease and account for over 35% of the genetic risk. Close to 90% of patients with Coeliac Disease express the HLA-DQ2 molecules with most of the remainder expressing the HLA-DQ8 molecule. There is a gene dosage effect so that those patients homozygous for the DQ2 or DQ8 genes or who are heterozygous for the DQ2 and DQ8 genes have a higher disease susceptibility. Deaminated gluten peptides bind strongly to HLA-DQ2 and DQ8 presenting a HLA-gluten peptide complex that activates CD4+ T cells which produce proinflammatory cytokines, including interferon gamma, which leads directly to tissue remodelling and flattening of the intestinal mucosa. The immune response also includes the development of antibodies against gluten and auto-antibodies to endogenous tissue transglutaminase. The best HLA genetic test for Coeliac Disease is now recognised to include HLA-DQA1 testing alongside the traditional HLA-DQB1 testing. The HLA-DQA1*05:01 - DQB1*02:01 encoded molecule is the DQ2 most associated with Coeliac Disease. A small percentage of Coeliac patient express the DQA1*02:01 – DQB1*02:02 genotype. The DQ8 alleles associated with Coeliac Disease are DQA1*03 – DQB1*03:02.

Presence of HLA-DQ2 and DQ8 on their own are not predictive of coeliac disease. Genome wide association studies have indicated a large number of non HLA genes which segregate with Coeliac Disease and may potentially be associated with it. Coeliac disease is therefore a complex disorder, requiring environmental trigger. Diagnosis of Coeliac Disease is based mainly on histology, though this is not always reliable. Genetic testing for HLA-DQ2 and DQ8 as a complement to histology has proved to be important for helping to confirm the diagnosis. Typing for HLA-DQ2 and DQ8 does provide good negative predictive value. However, HLA-DQ2 and DQ8 typing does not provide any additional diagnostic benefit in patients already known to be tissue transglutaminase antibody positive.

COELIAC REPORTING

Presence of HLA-DQ2 and DQ8 on their own are not predictive of coeliac disease. Genome wide association studies have indicated a large number of non-HLA genes which segregate with Coeliac Disease and may potentially also be associated with it. Coeliac disease is therefore a complex disorder, requiring environmental trigger. Diagnosis of Coeliac Disease is based mainly on histology, though this is not always reliable. Genetic testing for HLA-DQ2 and DQ8 as a complement to histology has

proved to be important for helping to confirm the diagnosis. Typing for HLA-DQ2 and DQ8 does provide good negative predictive value. However HLA-DQ2 and DQ8 typing does not provide any additional diagnostic benefit in patients already known to be tissue transglutaminase antibody positive.

HLA-DQA1*01:01, *02:01; DQB1*05:01, *03:03

This patient is NEGATIVE for HLA-DQ2 (and is DQA1*05 NEGATIVE) and NEGATIVE for HLA-DQ8 (DQA1*03, DQB1*03:02). Patients with this genotype have a **VERY LOW RISK** of predisposition to Coeliac disease

HLA-DQA1*05:01, *01:02; DQB1*01:01, *05:01

This patient is NEGATIVE for HLA-DQ2 (but is DQA1*05 POSITIVE) and NEGATIVE for HLA-DQ8 (DQA1*03, DQB1*03:02). Patients with this genotype have a **VERY LOW RISK** of predisposition to Coeliac disease

HLA-DQA1*03:02, *02:01; DQB1*02:01, *03:01

This patient is Heterozygous POSITIVE for HLA-DQ2 (but is DQA1*05 NEGATIVE) and NEGATIVE for HLA-DQ8 (DQA1*03, DQB1*03:02). Patients with this genotype have a **LOW RISK** of predisposition to Coeliac disease

HLA-DQA1*03:01, *03:02; DQB1*03:02, *03:01

This patient is NEGATIVE for HLA-DQ2 (DQA1*05, DQB1*02) and POSITIVE for HLA-DQ8 (DQA1*03, DQB1*03:02). Patients with this genotype have a **HIGH RISK** of predisposition to Coeliac disease though other factors are likely involved

HLA-DQA1*03:01, *01:02; DQB1*02:01, *03:02

This patient is POSITIVE for HLA-DQ2 (but is DQA1*05 NEGATIVE) and POSITIVE for HLA-DQ8 (DQA1*03, DQB1*03:02). Patients with this genotype have a **HIGH RISK** of predisposition to Coeliac disease though other factors are likely involved

HLA-DQA1*03:02, *05:01; DQB1*02:01, *03:01

This patient is POSITIVE for HLA-DQ2 (DQA1*05, DQB1*02) and NEGATIVE for HLA-DQ8 (DQA1*03, DQB1*03:02). Patients with this genotype have a **HIGH RISK** of predisposition to Coeliac disease though other factors are likely involved

HLA-DQA1*02:02, *02:02; DQB1*02:01, *02:01

This patient is Homozygous POSITIVE for HLA-DQ2 (but is DQA1*05 NEGATIVE) and NEGATIVE for HLA-DQ8 (DQA1*03, DQB1*03:02). Patients with this genotype have a **HIGH RISK** of predisposition to Coeliac disease though other factors are likely involved

HLA-DQA1*03:01, *03:01; DQB1*03:02, *03:02

This patient is Homozygous POSITIVE for HLA-DQ8 (DQA1*03, DQB1*03:02). Patients with this genotype have a **HIGH RISK** of predisposition to Coeliac disease though other factors are likely involved

HLA-DQA1*03:01, *05:01; DQB1*03:02, *03:01

This patient is NEGATIVE for HLA-DQ2 (but is DQA1*05 POSITIVE) and POSITIVE for HLA-DQ8 (DQA1*03, DQB1*03:02). Patients with this genotype have a **HIGH RISK** of predisposition to Coeliac disease though other factors are likely involved

HLA-DQA1*03:01, *05:01; DQB1*02:01, *03:02

This patient is Heterozygous POSITIVE for HLA-DQ2 (DQA1*05, DQB1*02) and POSITIVE for HLA-DQ8 (DQA1*03, DQB1*03:02). Patients with this genotype have a **VERY HIGH RISK** of predisposition to Coeliac disease though other factors are likely involved

HLA-DQA1*05:01, *05:01; DQB1*02:01, *02:01

This patient is Homozygous POSITIVE for HLA-DQ2 (DQA1*05, DQB1*02). Patients with this genotype have a **VERY HIGH RISK** of predisposition to Coeliac disease though other factors are likely involved

COLITIS

Ulcerative Colitis is one of two major forms of Inflammatory Bowel Disease, the other being Crohn's disease. Colitis literally means inflammation of the colon and ulcerative refers to the presence of ulcers. Colitis is primarily a disease of the large intestine (colon and rectum). The inflammation and ulcers are the cause of the common symptoms of diarrhoea mixed with blood and mucus seen with Ulcerative Colitis. Other symptoms include crampy pain in the abdomen, pain when passing stool and inflammation of the rectum. If flare ups persist then Ulcerative Colitis may be accompanied by fever, weight loss and feeling sick. Onset of Ulcerative Colitis is typically between the ages of 15 and 35 with a population prevalence of around 200 per 100,000 persons in Western populations.

The most consistently replicated association of Ulcerative Colitis is with the HLA class II allele HLADRB1*01:03. This association is particularly strong in patients with severe disease, as defined by a need to colectomy. Among patients who do require colectomy, HLA-DRB1*01:03 may be associated with a shorter mean time to surgery. HLA-DRB1*15:02 but not HLA-DRB1*15:01, has also been shown to be associated with Ulcerative Colitis in some populations though the strength of the association varies in different populations. In some populations HLA-DRB1*04 has some protective effect. HLA risk alleles however only contribute a minor part of the overall genetic picture. The advent of genome wide association studies have identified a large number of potential candidate genes which are being studied for association with Ulcerative Colitis, including the Interleukin-23 Receptor (IL23R).

The association between HLA and Ulcerative Colitis is of low specificity and sensitivity, limiting the value of genetic testing for diagnostic purposes. However HLA-DRB1*01:03 typing may be of value in predicting patients who may potentially require colectomy as potential candidates for more aggressive treatment in a bid to spare the colon.

CHRON'S

Crohn's is one of two major forms of Inflammatory Bowel Disease, the other being Ulcerative Colitis. Crohn's differs from ulcerative colitis in that Crohn's can affect any part of the gastrointestinal tract from the mouth to the anus, whereas ulcerative colitis mainly affects the large intestine. Crohn's disease is characterised by abdominal pain, diarrhoea, which may be bloody, vomiting and weight loss. Crohn's is often associated with complications outside of the gastrointestinal tract such as skin rash, arthritis, Uveitis, tiredness and lack of concentration. Onset of Crohn's during childhood can have a significant effect on development.

The specific cause of Crohn's is unknown but epidemiological studies do suggest a dysregulation of the immune response against the gut flora in a genetically susceptible individual, perhaps as a result of infection of the gut with an atypical microbe. Other environmental factors do perhaps play a part with some studies showing that smoking and appendectomy may act as triggers.

The most consistently replicated association of Crohn's disease is with the HLA class II allele HLADRB1*07. The association is specifically in patients with ileal involvement. HLA-DRB1*01:03 has also been shown more recently to be associated with Crohn's. As with ulcerative colitis, HLA-DRB1*01:03 may be associated with more severe disease, as defined by patients who require colectomy. HLADRB1*15:01 appears to confer protection against Crohn's.

The advent of genome wide association studies have identified a large number of potential candidate genes which are being studied for association with Crohn's. Of particular interest are the innate pattern recognition receptors 'Nucleotide-binding Oligomerisation Domain containing 2' / 'Caspase Recruitment Domain family, member 15' (NOD2/CARD15). NOD2/CARD15 is a member of the family of pattern recognition receptors that recognise microbial components. NOD2/CARD15 expression is high in the intestinal crypts.

The association between HLA and Crohn's is of low specificity and sensitivity, limiting the value of genetic testing for diagnostic purposes. In addition, as Crohn's shares some HLA gene susceptibility with ulcerative colitis, e.g. HLA-DRB1*01:03, it is not possible to use HLA typing to distinguish Crohn's from ulcerative colitis.

Knowledge of the HLA type of an already diagnosed Crohn's patient may be of value in helping to predict disease course and may indicate treatment options. This may for example include the use of Infliximab as an anti-TNF agent for certain stages of Crohn's disease.

GRAVES DISEASE

Graves Disease is an autoimmune disease of the thyroid gland. It is characterised by hyperthyroidism associated with goitre, palpitations, bulging eyes, sweating, heat intolerance, tremor, anxiety and weight loss. The immunological response in Graves Disease comprises diffuse lymphocyte infiltration into the thyroid gland with thyroid stimulating immunoglobulin (TSI) autoantibody production. The hyperthyroidism is caused by activation of the thyroid stimulating hormone receptor (TSHR) by binding of autoantibodies.

Monozygotic and dizygotic twin studies have shown a much higher concordance in monozygotic twins (20%) than dizygotic, indicating a genetic component to Graves Disease. The most strongly associated gene is the HLA class II gene. Implicated haplotypes include DRB1*03:01-DQA1*05:01DQB1*02:01 (DR17, DQ2) and DRB1*04:01-DQA1*03:01-DQB1*03:02 (DR4, DQ8). The highest risk is associated with DR17, DQ2. HLA-DRB1*07 (DR7) is protective for Graves Disease.

Diagnosis is based on thyroid function tests and biochemical and clinical manifestations of hyperthyroidism rather than on genetic testing. Genetic testing does however contribute to a better understanding of the aaetiology of the disease.

HEMOCHROMATOSIS (HFE)

HFE OVERVIEW

Haemochromatosis is an adult onset disorder, characterised by inappropriately high iron absorption resulting in progressive iron overload. Increases in the body's iron levels may be acquired by for example, blood transfusion or may be due to inherited disturbances to the mechanisms regulating intestinal iron absorption. Hereditary Haemochromatosis is an autosomal recessive disease with estimated prevalence of 2

in 1,000 in the Caucasian population, with lower incidence in other races. It is characterised by cirrhosis of the liver, diabetes, cardiomyopathy, arthritis, testicular failure and tanning or bronzing of the skin.

Haemochromatosis is usually related to the deficient synthesis or reduced activity of the peptide hormone Hepcidin (coded for by the 'Hepcidin Anti-Microbial Peptide' gene - HAMP), which is produced in the liver. Hepcidin normally down-regulates the entry of iron into the blood stream and therefore regulates iron homeostasis. Mutations in the gene for Hepcidin or in the genes which regulate Hepcidin synthesis such as the Human Haemochromatosis gene (HFE) and 'Transferrin Receptor 2' gene - (TFR2) can affect Hepcidin activity. More than 80% of Hereditary Haemochromatosis is due to mutations in such regulatory genes. In addition, mutations in HFE can affect the ability of HFE to bind the Transferrin receptor, thereby affecting ferritin levels.

Hereditary Haemochromatosis caused by mutations in the HFE gene is the most common and is termed Type 1 haemochromatosis. Type 2 haemochromatosis is caused by mutations to HAMP and Type 3 by mutations to TFR2. Other types exist.

The HFE gene is located on the short arm of chromosome 6. It codes for a HFE protein which is homologous to MHC class I and is composed of alpha 1 and 2 domains and an immunoglobulin like alpha 3 domain which associates with beta-2 microglobulin. HFE does not however present peptide to T-cells. Several mutations in the HFE gene have been described. The cysteine to tyrosine substitution at position 282 (C282Y) is present in the homozygous state in over 80–93% of Hereditary Haemochromatosis cases. In a further 5% of cases, the C282Y mutation is present as a compound heterozygous with a histidine to aspartic acid substitution at position 63 (H63D). These mutations result in excessive, chronic iron absorption in the gut, as well as a relative inability to store iron in macrophages. However, a significant proportion of people with these genotypes do not develop iron overload. Other genetic mutations as well as environmental factors are likely to contribute to iron overload in patient.

Genetic testing for HFE, while useful, is not completely diagnostic and other clinical factors such as iron levels and familial history need to be taken into account.

HFE REPORTING

HHCC

This individual is **HOMOZYGOUS** for the **NORMAL** HFE gene at position **282** (C/C).

This individual is **HOMOZYGOUS** for the **NORMAL** HFE gene at position **63** (H/H).

This genotype makes a diagnosis of Type-1 (HFE-related) hereditary haemochromatosis **VERY UNLIKELY**.

If iron levels (transferrin saturation and serum ferritin) are currently **RAISED,** then **ALTERNATIVE REASONS** (e.g. alcohol consumption, fatty liver disease and/or metabolic syndrome) should be considered. In individuals with severe iron overload, other rare forms of hereditary haemochromatosis cannot be excluded. If iron levels are currently **NORMAL**, this individual is **UNLIKELY** to develop Type-1 hereditary haemochromatosis.

CARRIER TESTING or **GENETIC COUNSELLING** of relatives of an individual with this genotype is **NOT RECOMMENDED**.

HDCC

This individual is **HOMOZYGOUS** for the **NORMAL** HFE gene at position **282** (C/C).

This individual is **A CARRIER** for the **MUTATION** in the HFE gene at position **63** (H/D).

This genotype makes a diagnosis of Type-1 (HFE-related) hereditary haemochromatosis **VERY UNLIKELY.**

If iron levels (transferrin saturation and serum ferritin) are currently **RAISED,** then **ALTERNATIVE REASONS** (e.g. alcohol consumption, fatty liver disease and/or metabolic syndrome) should be considered. In individuals with severe iron overload, other rare forms of hereditary haemochromatosis cannot be excluded**.** If iron levels

are currently **NORMAL**, this individual is at **NO INCREASED RISK** of developing Type-1 hereditary haemochromatosis.

CARRIER TESTING or **GENETIC COUNSELLING** of relatives of an individual with this genotype is **NOT RECOMMENDED**.

DDCC

This individual is **HOMOZYGOUS** for the **NORMAL** HFE gene at position **282** (C/C).

This individual is **HOMOZYGOUS** for the **MUTATION** in the HFE gene at position **63** (D/D).

This genotype makes a diagnosis of Type-1 (HFE-related) hereditary haemochromatosis **UNLIKELY** but may be **ASSOCIATED WITH** a slight **INCREASE** in iron levels (transferrin saturation and serum ferritin).

If iron levels are currently **RAISED,** then **ALTERNATIVE REASONS** (e.g. alcohol consumption, fatty liver disease and/or metabolic syndrome) should be considered. In individuals with severe iron overload, other rare forms of hereditary haemochromatosis cannot be excluded**.** If iron levels are currently **NORMAL**, this individual is at **NO INCREASED RISK** of developing Type-1 hereditary haemochromatosis.

CARRIER TESTING or **GENETIC COUNSELLING** of relatives of an individual with this genotype is **NOT RECOMMENDED**.

HHCY

This individual is **A CARRIER** for the **MUTATION** in the HFE gene at position **282** (C/Y).

This individual is **HOMOZYGOUS** for the **NORMAL** HFE gene at position **63** (H/H).

This genotype makes a diagnosis of the Type-1 (HFE-related) hereditary haemochromatosis **UNLIKELY.** If iron levels (transferrin saturation and serum ferritin) are currently **RAISED,** then **ALTERNATIVE REASONS** (e.g. alcohol consumption, fatty liver disease and/or metabolic syndrome) should be considered. In individuals with severe iron overload, other rare forms of hereditary haemochromatosis cannot be excluded. If iron levels are currently **NORMAL**, this individual is at **NO INCREASED RISK** of developing Type-1 hereditary haemochromatosis.

CARRIER TESTING of relatives of an individual with this genotype is NOT RECOMMENDED but GENETIC COUNSELLING MAY BE CONSIDERED.

HHYY

This individual is **HOMOZYGOUS** for the **MUTATION** in the HFE gene at position **282** (Y/Y).

This individual is **HOMOZYGOUS** for the **NORMAL** HFE gene at position **63** (H/H).

If iron levels (transferrin saturation and serum ferritin) are currently **RAISED,** this genotype is **COMPATIBLE WITH** a diagnosis of Type-1 (HFE-related) hereditary haemochromatosis.

If iron levels are currently **NORMAL**, then this individual is **AT RISK** of developing Type-1 hereditary haemochromatosis and it is recommended that the indices of iron overload be **MONITORED EVERY YEAR**.

CARRIER TESTING and **GENETIC COUNSELLING** of adult first-degree relatives of an individual with this genotype **SHOULD BE CONSIDERED**.

HDCY

This individual is **A CARRIER** for the **MUTATION** in the HFE gene at position **282** (C/Y).

This individual is **A CARRIER** for the **MUTATION** in the HFE gene at position **63** (H/D).

This genotype makes a diagnosis of the Type-1 (HFE-related) hereditary haemochromatosis **UNLIKELY** but may **PREDISPOSE** to **MILD/MODERATE IRON OVERLOAD**.

If iron levels (transferrin saturation and serum ferritin) are currently **RAISED,** then **ALTERNATIVE REASONS** (e.g. alcohol consumption, fatty liver disease and/or metabolic syndrome) should be considered. In individuals with severe iron overload, other rare forms of hereditary haemochromatosis cannot be excluded. If iron levels are currently **NORMAL**, then this individual may be **AT RISK** of developing **MILD-TO-MODERATE** iron overload and it is recommended that the indices of iron overload be monitored **EVERY 3 YEARS**.

CARRIER TESTING of relatives of an individual with this genotype is **NOT RECOMMENDED** but genetic counselling **MAY BE CONSIDERED.**

HDYY

This individual is **HOMOZYGOUS** for the **MUTATION** in the HFE gene at position **282** (Y/Y).

This individual is **A CARRIER** for the **MUTATION** in the HFE gene at position **63** (H/D).

If iron levels (transferrin saturation and serum ferritin) are currently **RAISED** this genotype is **COMPATIBLE WITH A DIAGNOSIS** of Type-1 (HFE-related) hereditary haemochromatosis.

If iron levels are currently **NORMAL**, then this individual is **AT RISK** of developing Type-1 hereditary haemochromatosis and it is recommended that the indices of iron overload be **MONITORED EVERY YEAR**.

CARRIER TESTING and **GENETIC COUNSELLING** of adult first-degree relatives of an individual with this genotype **SHOULD BE CONSIDERED**.

DDCY

This individual is **A CARRIER** for the **MUTATION** in the HFE gene at position **282** (C/Y).

This individual is **HOMOZYGOUS** for the **MUTATION** in the HFE gene at position **63** (D/D).

This genotype makes a diagnosis of Type-1 (HFE-related) hereditary haemochromatosis **UNLIKELY** but may be **ASSOCIATED WITH** a slight **INCREASE** in iron levels (transferrin saturation and serum ferritin).

If iron levels are currently **RAISED,** then **ALTERNATIVE REASONS** (e.g. alcohol consumption, fatty liver disease and/or metabolic syndrome) should be considered. In individuals with severe iron overload, other rare forms of hereditary haemochromatosis cannot be excluded**.**

If iron levels are currently **NORMAL**, this individual is at **NO INCREASED RISK** of to developing Type-1 hereditary haemochromatosis.

CARRIER TESTING of relatives of an individual with this genotype is NOT RECOMMENDED but GENETIC COUNSELLING MAY BE CONSIDERED.

DDYY

This individual is **HOMOZYGOUS** for the **MUTATION** in the HFE gene at position **282** (Y/Y).

This individual is **HOMOZYGOUS** for the **MUTATION** in the HFE gene at position **63** (D/D).

If iron levels (transferrin saturation and serum ferritin) are currently **RAISED,** this genotype is **COMPATIBLE WITH A DIAGNOSIS** of Type-1 (HFE-related) hereditary haemochromatosis.

If iron levels are currently **NORMAL**, then this individual is **AT RISK** of developing Type-1 hereditary haemochromatosis and it is recommended that the indices of iron overload be **MONITORED EVERY YEAR**.

CARRIER TESTING and **GENETIC COUNSELLING** of adult first-degree relatives of an individual with this genotype **SHOULD BE CONSIDERED**.

INSULIN DEPENDENT DIABETES MELLITUS

Diabetes Mellitus is a group of diseases in which the body fails to maintain normal glucose levels either because the body does not produce enough insulin (type 1) or because the cells of the body do not respond to the insulin that is produced (type 2). Type 1 diabetes is also known as Insulin Dependent Diabetes Mellitus (IDDM) as it requires patients to inject insulin.

IDDM is characterised by progressive infiltration of immune cells into the Islets of the pancreas and autoantibody production leading to autoimmune destruction of the insulin producing pancreatic Islet cells. Primary damage in IDDM is caused by a cellular immune response in which CD4+ Th1 helper T cells activate in situ CD8+ cytotoxic T cells directed against the beta cells. Clinical symptoms of diabetes occur when over 90% of an individual's beta cells are destroyed. Autoantibody production pre dates the development of clinical symptoms. Antibodies are developed against most components of beta cells, including Insulin, Glutamic Acid Decarboxylase (GAD) and Islet-cell Antigen2 (IA-2).

IDDM is a polygenic disease where the concordance rate in twins is 30 – 50%. An as yet unknown trigger is required for genetically susceptible individuals to develop the condition. Environmental factors such as diet and viral infection have been shown to be associated with an increased incidence of IDDM. The HLA system contributes approximately 50% of the heritable risk of IDDM and is the most important susceptibility genetic region and has been named IDDM1. The next most important genetic region is the Insulin gene region on chromosome 11, which is thought to contribute approximately 10% of the heritable risk. This region has been named IDDM2. An additional 15 genes located on different chromosomes, with variable but small effects have been described and named IDDM3 – IDDM17.

More than 90% of Caucasian patients with IDDM carry the haplotypes DRB1*03:01, DQA1*05:01, DQB1*02:01 (DR17, DQ2) or DRB1*04:01, DQA1*03:01, DQB1*03:02 (DR4, DQ8), particularly where the age of onset is less than 15. Patients heterozygous for these haplotypes carry a greater susceptibility risk. The critical residues are thought to be DQ alpha Arg-52, DQ beta Asp-57 and DQ beta Arg-74. Absence of DQ beta Asp-57 and DQ beta Arg-74 is strongly associated with

IDDM. Resistance to IDDM is conferred by some DQ6 alleles. DQA1*01:02, DQB1*06:02 confers protection in Caucasian populations.

Genetic testing is useful as an aid to diagnosis of IDDM. In addition, as IDDM is characterised by a variable length silent period before overt disease, genetic testing for disease susceptibility genes in siblings of IDDM patients may be a useful measure.

JUVENILE IDIOPATHIC ARTHRITIS

Juvenile Idiopathic Arthritis (JIA) is a broad disease which comprises a set of clinically heterogeneous chronic arthropathies of unknown aaetiology which develop before the age of 16. It was previously called Juvenile Chronic Arthritis in Europe and Juvenile Rheumatoid Arthritis in the USA. Several subtypes of Juvenile Idiopathic Arthritis have been defined, including Systemic Juvenile Arthritis, Oligoarthritis, Polyarthritis, Enthesitis-related arthritis and Psoriatic arthritis. Each of these conditions has distinct methods of presentation and clinical symptoms and in some cases, different genetic background.

Systemic Juvenile Arthritis is a form of JIA which affects more than 5 joints and is typically accompanied by or preceded by fever and rash. Oligoarthritis affects 4 or fewer joints during the first 6 months of disease. Polyarthritis affects 5 or more joints. Patients may be rheumatoid factor pos or neg. Enthesitis related arthritis most commonly affects sites such as cranial insertion points and the Achilles tendon. Psoriatic arthritis is diagnosed by the presence of arthritis and a psoriatic rash as well as swelling of one or more fingers.

Both genetics and non genetic factors are believed to play a role in susceptibility to JIA. HLA class I and II genes are the most commonly linked genes in association studies, though non HLA genes have also been implicated. Systemic Juvenile Arthritis has been associated with a single nucleotide polymorphism in the regulatory region of the interleukin-6 gene. Oligoarthritis has been associated most frequently with HLA-A2, DRB1*08 and DRB1*11. HLA-DRB1*04 and HLA-DRB1*07 are thought to be protective for Oligoarthritis. Polyarthritis is associated in most studies with HLA-DRB1*04. Enthesitis related arthritis is associated with HLA-B*27 and Psoriatic arthritis is associated with HLAC*06:02 and with polymorphisms in the IL-23 receptor gene.

There is no cure for JIA and treatment is based mainly on early diagnosis and a combination of drug and physical therapy aimed at relieving symptoms. In this regard genetic testing is clinically relevant as a contributor to early diagnosis.

MULTIPLE SCLEROSIS

Multiple Sclerosis (MS) is an idiopathic autoimmune neurodegenerative disease in which dysregulation of the immune system causes myelin sheath degradation. Multiple sclerosis is characterised by plaques or lesions in the brain and spinal cord. The location and size of the lesions is unpredictable. Symptoms of Multiple Sclerosis include affected coordination, balance and vision and disturbances in the bowel, bladder and sexual organs. Disease onset is typically between the ages of 20 and 40. Women have a 2 -3 fold higher incidence of MS than men. The distribution of MS inversely parallels the global distribution of UV light, suggesting a role for vitamin D in the disease.

First degree relatives of patients with MS are generally at 20 – 40 times greater risk of developing the disease themselves, compared to the general population, showing that the disease has a large genetic component. Family studies, including monozygotic and dizygotic twin studies have shown that HLA-DRB1*15:01 on the HLA-DRB1*15:01, DQA1*01:02, DQB1*06:02 haplotype, is a disease susceptibility gene, accounting for up to 35% of the heritability of the disease. Homozygosity for this haplotype increases disease risk six fold. Linkage disequilibrium initially made it difficult to confirm disease association with HLA-DR*15:01 rather than HLA-DQB1*06:02 but studies of MS in Black populations have confirmed HLA-DR*15:01 as the susceptibility gene. A role for other HLA class I and II alleles has been indicated by a number of studies but the overall effect of these remain small compared to HLA-DRB1*15.

The exact mechanism by which HLA increases the susceptibility to MS is unknown. A recent finding of a vitamin D response element in the promoter region of HLA-DRB1, which is completely conserved in HLA-DRB1*15:01 together with evidence showing that vitamin D is significantly lower in MS patients is suggestive.

A number of genome wide association studies have indentified other candidate genes other than HLA, including IL-7 Receptor alpha and the IL-2 Receptor alpha genes as MS disease susceptibility loci.

The main value of genetic testing in MS is to provide insights into the mechanism of the disease, thereby potentially suggesting strategies for prevention and treatment, rather than as a diagnostic tool.

A small number of Auto-HSCTs have been performed for patients with MS with promising results.

MYASTHENIA GRAVIS

Myasthenia Gravis (MG) is a rare autoimmune neuromuscular disease, aimed at the neuromuscular junction. It is characterised by fluctuating muscle weakness and fatigability. It is a potentially life-threatening condition when respiratory muscles are involved. In 85% of cases, autoantibodies directed against nicotinic acetylcholine receptors (AChR's) in the postsynaptic membrane of the neuromuscular junction have been found. Autoantibodies directed against the Muscle Specific tyrosine Kinase (MuSK) also on the postsynaptic membrane have been found in 70% of cases. Together, autoantibodies to AChR and/or MuSK are present on over 90% of MG cases. Other autoantibodies are also often present.

The most frequent form of Myasthenia Gravis presents with thymus follicular hyperplasia and has an early onset. It has been shown to be associated with the 'ancestral HLA haplotype' A1 - B8 - Cw7 - DR17 - DQ2. The strong linkage disequilibrium had made it difficult to determine precisely which locus on this haplotype is most strongly associated. Early studies implicated HLA-B8 but this was later replaced with a stronger associated with HLA class II, specifically HLA-DR17. One consistent finding is that HLA-DR3 has a positive association with early onset Myasthenia Gravis and a negative association with late onset Myasthenia Gravis, whilst HLA-DR7 has the opposite, i.e. a negative association with early onset Myasthenia Gravis and a positive association with late onset Myasthenia Gravis. Another study using SNPs in a large group of patients found the strongest association to be with HLA-Cw7. The real disease association locus could even be another locus on the A1 - B8 - Cw7 - DR17 - DQ2 haplotype. One recent study has

identified a region that overlaps the MHC class III and class I regions of the A1 - B8 - Cw7 - DR17 - DQ2 haplotype, which they termed MYAS1, that associates strongly with Myasthenia gravis.

Diagnosis of Myasthenia gravis does not depend on genetic testing. Tests to measure concentrations of serum autoantibodies are a more effective diagnostic tool. Genetic testing may however be an aid to diagnosis and is of value in providing insights into the mechanism of the disease, thereby potentially suggesting strategies for prevention and treatment.

NARCOLEPSY

Narcolepsy is a chronic, debilitating sleep disorder first described in the late 19th century which is characterized by excessive daytime sleepiness, cataplexy i.e. sudden transient muscular weakness, disturbed night sleep and sleep paralysis. Narcolepsy is caused by deficiencies in hypothalamic hypocretin (also known as orexin) neurotransmission, most likely through autoimmune-mediated postnatal cell death of hypocretin producing neurons. Hypocretin is a neuropeptide hormone that is responsible for controlling appetite and sleep patterns.

Prevalence studies have shown that genetic factors play a role on narcolepsy but are neither necessary nor sufficient to cause narcolepsy. Twin studies show that only 25 – 30% of twins are concordant. Family studies show that the risk of developing narcolepsy for a first degree relative of a patient is only 1 – 2%. This is however 30 – 40 times higher than the estimated prevalence of narcolepsy in the general population, showing that genetic heritability does play a role. One of the most important predisposing genetic factors is the HLA-DQB1*06:02 allele on the DRB1*15:01 - DQA1*01:02 - DQB1*06:02 haplotype. Between 85 and 95% of narcolepsy patients with cataplexy carry this haplotype. Persons homozygous for HLA-DQB1*06:02 carry a greater risk than heterozygous persons, though persons heterozygous for HLA-DQB1*03:01 and HLA-DQB1*06:02 are also at increased risk. HLA-DQB1*05:01 and DQB1*06:01 are thought to be protective for narcolepsy. HLA-DQB1*06:02 has a larger P4 pocket which would facilitate binding of larger hydrophobic residues compared to HLA-DQB1*06:01 and this may help explain the opposite effects these two allele have on narcolepsy susceptibility.

The genetic risk of developing narcolepsy is not however fully explained by HLA-DQB1*06:02 as there are many patients with narcolepsy who do not carry HLA-DQB1*06:02, pointing to the possibility of non HLA gene involvement. Candidate genes include the different hypocretin system genes, though initial studies have not shown an association and TNF alpha gene polymorphisms.

Genetic testing for narcolepsy, particularly, HLA-DQB1*06:02 typing is useful as an aid to diagnosis in patients with cataplexy but the association is not specific as there are many narcolepsy patients with HLA-DQB1*06:02 and many HLA-DQB1*06:02 persons who do not have narcolepsy.

OSTEOARTHRITIS

Osteoarthritis is the most common form of arthritis and is characterised by joint pain accompanied by varying degrees of functional limitation and reduced quality of life. Pain is usually in the large weight bearing joints such as the knees and hips. It also affects the joints of the thumb and fingers. Hand osteoarthritis has a good prognosis with most cases becoming asymptomatic after a few years. Involvement of the thumb base may have a worse prognosis. Knee osteoarthritis is very variable in its outcome. Over a period of several years about a third of cases improve, a third stay much the same and the remaining third develop progressive symptomatic disease. Hip osteoarthritis probably has the worst overall outcome of the three major sites though some hips heal spontaneously.

Osteoarthritis is a complex disorder caused by a combination of genetic predisposition and environmental factors. Environmental factors include lifestyle factors such as being overweight, a sedentary occupation, repetitive use of joints and history of trauma to affected joints.

The genes that predispose to Osteoarthritis remain to be clarified. Many studies have pointed to different HLA class I and II associations, perhaps indicating the heterogeneity of the condition. Several studies on generalized Osteoarthritis have revealed an association with HLA-B8. Linkage of HLA-B8 on the HLA-A1, B8, DR17 haplotype makes it difficult to be certain the association is actually with HLA-B8 and not with some other gene on the haplotype. A Japanese study showed an association with HLA-Cw4. Other studies have indicated associations with HLA-B35, B40, DR2

and DQ1 but again linkage disequilibrium makes it difficult to be certain what the true disease susceptibility gene is.

Diagnosis of Osteoarthritis is on the basis of persistent joint pain that is worse with use, age 45 years old and over and morning stiffness lasting no more than half an hour, rather than on genetic testing. The main value of genetic testing in Osteoarthritis is to provide insights into the mechanism of the disease, thereby potentially suggesting strategies for prevention and treatment, rather than as a diagnostic tool.

POLYARTHRALGIA

Arthralgia literally means pain and Polyarthralgia means pain in several joints, anything from two upwards. Polyarthralgia encompasses Simple Arthralgia in which the main symptom is pain with no clinical features of inflammation in the joints or morning stiffness, Osteoarthritis in which pain is usually in the large weight bearing joints such as the knees and hips and in the joints of the thumb and fingers, Seronegative Arthritis and Rheumatoid Arthritis. Each of these has a distinct clinical presentations and genetic basis.

Simple Arthralgia refers to arthritis with no clinical manifestation of inflammation. It is often associated with fever or infection. It is thought to be associated with HLA-B*27 or HLA-DRB1*04.

Osteoarthritis is the most common form of arthritis and is characterised by pain in the large weight bearing joints such as the knees and hips as well s in the joints of the thumb and fingers. Both genetic and environmental factors contribute to the predisposition to Osteoarthritis. Environmental factors include lifestyle factors such as being overweight, a sedentary occupation, repetitive use of joints and history of trauma to affected joints. Genetic factors include HLA-B*08, though the strong linkage disequilibrium on the HLA-A1, B8, DR17 haplotype makes it difficult to be certain.

Seronegative arthritis refers to arthritis without the presence of Rheumatoid factor. It typically affects multiple joints. It is thought to be associated with HLA-DRB1*04.

Rheumatoid is a chronic inflammatory symmetrical arthritis (it affects both sides of the body equally) which affects multiple synovial lined joints. It is characterised by progressive articular damage leading to joint deformation and disability. Patients are Rheumatoid factor positive and up to 70% of patients develop autoimmunity to citrullinated protein. Rheumatoid Arthritis is associated with a set of shared epitopes at positions 70 – 74 in the genetic sequence of the HLA-DRB1 gene. These include the sequences QKRAA, QRRAA, RKRAA and RRRAA. HLA-DRB1 alleles which share these sequences include HLA-DRB1*01:01, HLA-DRB1*01:02, HLA-DRB1*04:01, HLA-DRB1*04:04, HLA-DRB1*04:05, HLA-DRB1*04:08, HLA-DRB1*10:01, HLA-DRB1*13:03, HLA-DRB1*14:02 and HLA-DRB1*14:06.

Genetic testing for Polyarthralgia is an aid to diagnosis rather than a strong diagnostic tool.

POLYARTHRITIS

Polyarthritis is a form of arthritis which affects 5 or more joints simultaneously. It is usually associated with other autoimmune conditions such as psoriasis, Colitis and Crohns. Subcategories include Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), Seronegative Arthritis and Psoriatic Arthritis.

RA is a symmetrical arthritis that affects multiple synovial lined joints. It is characterised by progressive articular damage leading eventually to joint deformation. RA was initially thought to be associated with HLA-DR4 but is now seen to be more associated with a set of shared epitopes in positions 70-74 of the HLA-DRB1 locus. The epitopes include the amino acid sequences QKRAA, QRRAA, RKRAA AND RRRAA. HLA class II alleles which share these epitopes include HLA-DRB1*01:01, *01:02, *04:01, *04:04 and *04:05. Alleles which do not share these epitopes and which may be protective for RA include HLA-DRB1*03 and HLA-DRB1*04:03.

SLE is an arthritic condition characterised be repeated bouts of relapse and remission of symptoms including pain. It is associated with production of anti-nuclear autoantibodies, including anti-Sm, anti-Ro/SSA and anti-La/SSB. Presence of anti-Ro/SSA and anti-La/SSB autoantibodies is associated with the HLA class II DRB1*03:01 allele. Presence of anti-Sm autoantibodies is associated with the HLA class II DRB1*15:01 allele.

Seronegative Arthritis describes a set of arthritic conditions all of which are characterised by absence of rheumatoid factor (rheumatoid factor is an autoantibody against the Fc portion of IgG). These include the seronegative spondyloarthropathies and seronegative Rheumatoid Arthritis. Seronegative Polyarthritis is in some cases associated with other systemic autoimmune diseases including psoriasis, bowel and bladder symptoms and anterior Uveitis all of which have different HLA associations. The seronegative spondyloarthropathies are associated with HLA-B27. Uveitis is also associated with HLA-B27, while Behcet's is associated with HLA-B51. Seronegative Rheumatoid Arthritis may be associated with HLA-DR4.

Psoriatic Arthritis is an inflammatory joint disease which is associated with a psoriatic rash and swelling of the fingers (Dactylitis). Symptoms include spinal pain and enthesitis. Anterior Uveitis is seen in about a third of cases, though symptoms are often temporary. Nail involvement is common. HLA types implicated in susceptibility to Psoriatic Arthritis include HLA-B*27, HLA-B*39 and HLAC*06:02.

PSORIASIS VULGARIS

Psoriasis Vulgaris is the most common form (vulgaris means common) of Psoriasis. Psoriasis Vulgaris is a chronic, immune-mediated inflammatory skin disease which manifests as elevations or plaques of the skin that do not contain pus. The plaques are red or pink in colour and are covered by white or silvery scales. They may be thick or thin, large or small. Plaques appear most often on the elbows and knees and on the scalp. Psoriasis Vulgaris is associated with systemic disorders, including Crohn's and IDDM. It is rarely life threatening but it does cause considerable psychological stress for patients.

Psoriasis Vulgaris presents with inflammatory infiltrate of leukocytes predominantly into the dermis and involves a cell mediated immune response. However the auto-antigen to which the immune response is directed has not been identified.

Population studies have shown that Psoriasis Vulgaris has a genetic component with relatives of patients at far higher risk of developing the disease than the general population. The risk of Psoriasis Vulgaris is up to three fold higher in monozygotic twins than in dizygotic twins.

The gene found by association studies to be most closely linked to Psoriasis Vulgaris and probably accounting for 35 – 50% of the heritable risk is HLA-C*06. Approximately 87% of Psoriasis Vulgaris patients carry this gene. Other non HLA genes which have shown association are functional polymorphisms in genes for factors that control inflammation such as TNFα.

Genetic testing, especially for HLA-Cw6 is a useful aid to diagnosis, although most diagnosis is done quite easily on the basis of the skin presentation.

REACTIVE ARTHRITIS

Reactive Arthritis, formally known as Reiters syndrome, is an autoimmune condition that develops in response to an infection in another part of the body, generally the gastrointestinal tract but also the genitourinary tract. Reactive Arthritis shares some clinical characteristics with other spondyloarthropathies such as Ankylosing Spondylitis and Psoriatic Arthritis. Reactive Arthritis is characterised by predominantly lower limb arthritis and enthesitis (inflammation of tendon insertion sites) as well as inflammatory back pain, inflammation of the eye and in some cases genitourinary inflammation. A number of bacteria have been identified as the possible sources of infection leading to Reactive Arthritis. These include Campylobacter, Salmonella and Chlamydia species.

Reactive Arthritis affects both males and females equally and has an age on onset of between 20 and 50. The condition is usually self limiting within a few days though it has been known to go on for up to 4 months in some patients. Non steroidal anti-

inflammatory drugs and physical therapy may be used for the pain. The bacterial infection may need to be treated with antibiotics.

Population studies have shown the condition to be one of the most strongly associated with HLAB27 with over 80% of patients carrying the HLA-B*27 gene. The exact mechanism of action is unknown but may include the arthritogenic peptide theory in which a peptide either from B27 itself or from the site of attack such as the joint is presented to T cells by HLA-B27 leading to an immune response. Other theories include activation of auto reactive T cells which escaped thymic selection, molecular mimicry with bacterial peptide mimicking B27 derived self peptide and aberrant B27 heavy chain homodimers on the cell surface in the absence of beta 2 microglobulin acting as targets for inflammation.

HLA-B27 typing in Reactive Arthritis is a useful aid to diagnosis.

RHEMATOID ARHTRITIS

Rheumatoid Arthritis (RA) is a chronic inflammatory arthritis that affects multiple synovial lined joints. The natural history of the condition is one of progressive articular damage leading to joint deformation and disabilities. Rheumatoid Arthritis is associated with a number of co-morbidities particularly in the cardiovascular system. The onset of RA is preceded by a prearticular autoimmune phase, the presence of antibodies against citrullinated (deaminated) protein and the presence of rheumatoid factor. Autoimmunity to citrullinated protein appears to be highly specific for Rheumatoid Arthritis with anti - citrullinated protein antibodies detected in approximately 70% of Rheumatoid Arthritis patients.

The prevalence of RA seems to be relatively uniform around the world though prevalence is low in sub Saharan Africa. This might reflect underreporting. The risk of developing RA does however vary between ethnic populations in the same global region, demonstrating a genetic component to this disease. The prevalence of Rheumatoid Arthritis in the general population is less than 1% but rises to between 2 - 4% in siblings of RA patients. This is confirmed by studies in monozygotic twins.

Multiple genetic loci have been shown to contribute to the risk of developing RA. Of these, the HLA class II DRB1 is the most important and contributes 30 – 50% of the overall genetic susceptibility risk. The association was initially thought to be with HLA-DR4 but subsequent studies have shown an association between RA and multiple HLA-DRB1 alleles not all of them DR4. These alleles did however share common sequences at positions 70 – 74 in the sequence. This has lead to the shared epitope hypothesis. Amino acids in these positions influence both peptide binding and contact between MHC and T cell receptor. HLA-DRB1 alleles associated with RA, more specifically with anti - citrullinated protein antibodies RA, were shown to have the sequence Arg-Ala-Ala (RAA) at positions 72–74. Gln (Q) or Arg (R) at position 70 carries a higher risk than Asp (D) whilst Lys (K) or Arg (R) at position 71 carry a higher risk than Ala (A) or Glu (Q). The shared epitopes that carry an increased risk are therefore QKRAA, QRRAA, RKRAA and RRRAA. HLA-DRB1 alleles with one of these sequences include HLA-DRB1*01:01, HLA-DRB1*01:02, HLA-DRB1*04:01, HLA-DRB1*04:04, HLA-DRB1*04:05, HLA-DRB1*04:08, HLA-DRB1*10:01, HLA-DRB1*13:03, HLA-DRB1*14:02 and HLA-DRB1*14:06. There is a gene additive effect such that patient's homozygous or compound heterozygosity for the disease susceptibility alleles are at increased relative risk. Alleles which do not share any of these sequences and may be protective for RA are HLA-DRB1*03, HLA-DRB1*04:03, HLA-DRB1*04:07, HLA-DRB1*07, HLA-DRB1*08 and HLA-DRB1*09.

The shared HLA-DRB1 epitope does not explain the full RA genetic heritability. Genome wide association studies have led to recognition of an association between RA and the Arg620 to Trp polymorphism in the Protein tyrosine Phosphatase, non-receptor type-22 (PTPN22) gene, which codes for a powerful inhibitor of T-cell activation. A number of other candidate genes which have a much smaller effect than HLA-DRB1 and PTPN22 have also been described.

SCLERODERMA

Scleroderma, also known as systemic sclerosis, is a chronic systemic autoimmune disease. It is characterised by fibrosis or hardening of the skin, lungs, gastrointestinal tract and/or heart with endothelial dysfunction and a proliferative vasculopathy primarily affecting small blood vessels and capillaries. Patients often present with multiple autoantibodies, including antitopoisomerase I, anticentromere and anti-RNA polymerase III antibodies. In addition, patients have elevated levels of Th2 cytokines.

Skin biopsies of scleroderma patients show perivascular infiltrates of mononuclear inflammatory cells.

Scleroderma occurs in genetically susceptible individuals who have encountered specific environmental triggers. Genome wide associated studies have implicated a number of candidate genes in the pathogenesis of the disease. Principle amongst these are HLA class II genes. Strong correlations have been demonstrated between certain HLA-class II alleles and each of the Scleroderma specific autoantibodies.

Scleroderma with anticentromere autoantibodies has been shown to be associated with HLADQB1*05:01 and other DQB1 alleles encoding non-leucine residues at position 26 in the peptide binding groove.

Scleroderma with antitopoisomerase I autoantibodies has been shown to be associated with HLADRB1*11, especially the DRB1*11:04 and DQB1*03:01 in Caucasian subjects and DRB1*15:02 and DQB1*06:01 in Japanese and Korean subjects.

Scleroderma with anti-RNA polymerase III antibodies has been shown to be associated with HLADRB1*13:02 and DQB1*06:04.

A number of other candidate genes outside of HLA, such as connective tissue growth factor, have also been tested for association with scleroderma.

Genetic testing in Scleroderma may be of value in providing insights into the mechanism of the disease, thereby potentially suggesting strategies for prevention and treatment.

SJOGRENS

Sjogrens is a late onset chronic autoimmune disease which affects the exocrine glands, mainly the salivary and tears glands, resulting in insufficient secretion by those

glands. It is characterized by a progressive lymphoid and plasma cell infiltration of the salivary and tear glands, accompanied by the production of autoantibodies (such as the anti nuclear antibodies anti-Ro/SSA and anti-La/SSB) leading to mucosal (dry mouth) and conjunctival dryness (dry eyes). Hallmarks of Sjogrens are B cell hyperactivity as manifested by hypergammaglobulinemia, circulating autoantibodies and/or immune complexes. Sjogrens may occur alone (primary Sjogrens) or secondary to other autoimmune disorders such as SLE and rheumatoid arthritis. Primary Sjogrens generally affects females (9/10 patients are female) and has an onset at age of about 40. The reasons for the female bias are unknown though hormones such as prolactin have been implicated.

Multiple genetic loci have been shown to contribute to the risk of developing Sjogrens. Of these, the HLA class II DR and DQ are the most important. An association has been demonstrated between Sjogrens and multiple HLA-DRB1, DQA1 and DQB1 alleles. Two HLA types in particular have been implicated, the DRB1*03 - DQB1*02 - DQA1*05:01 and the DRB1*15 - DQB1*06 - DQA1*01:02 haplotypes. The DRB1*03 - DQB1*02 - DQA1*05:01 haplotype has been linked to Sjogrens patients with anti-Sjogrens A and B autoantibodies while the DRB1*15 - DQB1*06 - DQA1*01:02 haplotype has been linked to Sjogrens patients with anti-Sjogrens A autoantibodies only.

Two other non HLA genes have been shown to be significantly associated with genetic susceptibility to primary Sjogrens disease, Signal Transducer and Activator of Transcription 4 (STAT4) and Interferon Regulatory Factor 5 (IRF5).

Sjogrens is diagnosed on the basis of screening questions, eye tests and salivary flow rates tests rather than on genetic testing. Genetic testing in Sjogrens may be of value in providing insights into the mechanism of the disease, thereby potentially suggesting strategies for prevention and treatment.

SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic Lupus Erythematosus (SLE) is a systemic inflammatory autoimmune disease characterised by the production of autoantibodies (such as the anti-nuclear antibodies anti-Sm, anti-Ro/SSA and anti-La/SSB) with patients presenting with a diverse array of different clinical manifestations including skin, joint, haematologic,

neurologic, renal and other organ involvement. SLE is predominately a female disease, affecting females and males in a ratio of 9:1. Onset is usually between puberty and menopause with onset outside this range less common. Patients often undergo repeated cycles of flare ups and remissions. Complications of the disease seen in nearly half of all patients include glomerulonephritis and impaired renal function and/or neurological symptoms such as seizures, psychiatric symptoms, peripheral neuropathies or stroke.

The exact aaetiology of SLE is unknown, though it is thought to have a genetic component as well as environmental and hormonal components. The genetic component of SLE is supported by twin and family studies. The concordance of SLE in monozygotic twins in approximately 25 – 50% compared to only 5% in dizygotic twins. Population studies have revealed that susceptibility to SLE is associated with HLA class II, in particular with haplotypes bearing the DRB1*03:01, DQB1*02:01 and the DRB1*15:01, DQB1*06:02 alleles. These alleles were present in over 65% of SLE cases. The DRB1*03:01 allele carries a higher risk than the DRB1*15:01. Individuals homozygous for DRB1*03:01 or compound heterozygous for DRB1*03:01 and DRB1*15:01 carry the highest risk. The DRB1*03:01 allele is associated with production of anti-Ro/SSA and anti-La/SSB autoantibodies while the DRB1*15:01 allele is associated with production of anti-Sm autoantibodies.

Genome wide association studies have also identified a number of non-HLA genes that are risk factors for SLE. These include genes for the Fc receptors for immunoglobulin G which mediate clearance of immune complexes. The Arg620 to Trp polymorphism in the Protein tyrosine Phosphatase, non-receptor type-22 (PTPN22) gene (which codes for a powerful inhibitor of T-cell activation) may also have a role in SLE.

Genetic testing for SLE has significant clinical relevance for efforts to more fully characterise the aaetiology and pathway of development of SLE and may lead to improved diagnostic and prognostic tools. Developments in genetic testing for SLE may also lead to the development of more specific therapies for SLE and related conditions.

Uveitis is the most common form of inflammatory eye disease and is a significant cause of visual impairment and blindness. Uveitis specifically refers to inflammation of the layer of the eye known as the 'Uvea' (the iris, ciliary body, and choroid) but is commonly used to describe any inflammatory process involving the interior of the eye. Uveitis is classified anatomically into Anterior, Intermediate, Posterior and Pan-Uveitis. Anterior Uveitis involves inflammation of the anterior chamber and the iris and accounts for more than 90% of Uveitis referrals. Intermediate Uveitis involves inflammation of the vitreous cavity and Posterior Uveitis involves inflammation of the retina and choroid. Pan-Uveitis involves inflammation of all layers of the Uvea.

Uveitis onset is usually between the ages of 20 to 50 and is relatively uncommon in the very young (< 10 years of age) and in the elderly (> 70 years of age). Uveitis is often associated with a number of other seronegative spondyloarthropathies such as Ankylosing Spondylitis, Reactive Arthritis, Psoriatic Arthritis and Inflammatory Bowel Disease.

Twin and family studies have shown a strong association between susceptibility to Uveitis and the HLA-B*27 gene. HLA-B27 positive relatives of Uveitis patients are at higher risk of developing the disease than B27 positive individuals without an affected relative. However not all B27 positive individuals develop Uveitis, showing that HLA-B27 is often necessary but is not a sufficient genetic predisposing factor for the disease. Other non HLA genes may also be involved.

Studies of the frequencies of different HLA-B*27 alleles in HLA-B27 associated Uveitis in patients with other systemic diseases have revealed no significant difference in the frequencies of the alleles. In a recent study, the HLA*27:04 allele was found at a significantly lower frequency in Uveitis patients without other systemic diseases.

The association between HLA-B27 and Uveitis is not 100% which slightly limits its value as a diagnostic tool. However HLA-B27 testing may be of value in identifying a previously undiagnosed or misdiagnosed spondyloarthropathy among patients with recurrent Uveitis.

HLA – PHARMACOGENETICS AND INFECTION

IMMUNOSUPPRESSIVE DRUG METABOLISM

Many immunosuppressive drugs are characterised by a narrow therapeutic index. That is, the difference between a therapeutic dose and a toxic dose is small, often requiring patients on immunosuppressive regimes to have their drug dosage adjusted according to actual levels detected in the blood. Too little of an immunosuppressive drug can contribute to rejection whilst too much can be toxic. A classic example is the seen in the nephrotoxicity of the calcineurin inhibitors cyclosporin and tacrolimus.

Getting the right balance is complicated by the fact that individuals differ significantly their bioavailability, i.e. the fraction of a fixed administered dose of drug that reaches systemic circulation. This variability in drug response has been shown to have a heritable genetic component.

Immunosuppressive drugs are transported across the cellular membrane by the transmembrane pump P-glycoprotein in the liver and gut, where they are metabolised by Cytochrome P450 (CYP) enzymes, a large diverse group of enzymes which account to more than 75% of all drug metabolism. Polymorphisms in the genes for Cytochrome P450 enzymes have been associated with differences in immunosuppressive drug metabolism, particularly metabolism of tacrolimus.

The majority of Cytochrome P450 drug metabolic activity is accounted for by the CYP3A sub family. The genes encoding the CYP3A sub family are located on chromosome 7. The main isoforms encoded are CYP3A4 and CYP3A5. Both isoforms carry a number of single nucleotide polymorphisms (SNPs) resulting in a number of alleles. CYP3A4 polymorphisms include CYP3A4*1 and CYP3A4*1B. CYP3A5 polymorphisms include CYP3A5*1 and CYP3A5*3. Most studies have demonstrated that patients with the CYP3A5*3 allelic variant show higher blood concentrations of tacrolimus therefore allowing lower maintenance doses of the immunosuppressant compared with patients with the CYP3A5*1 allele. In one study, the CYP3A4*1/CYP3A5*1 and CYP3A4*1B/CYP3A5*1 genotypes were significantly more frequently associated with the development of biopsy-proven calcineurin related nephrotoxicity compared to the CYP3A4*1/CYP3A5*3 genotype.

Genetics variants also affect drug pharmocodynimics, i.e. the pharmacologic effect resulting from the interaction between the drug and the biologic system. Mutations in the ACE and Angiotensinogen genes for instance lead to differences in blood pressure reduction for the same dose of ACE inhibitors.

Mutations in the N-acetyltransferase (NAT) is genes (NAT1 and NAT2) lead to differences in the rates of acetylation of common drugs such as caffeine, isoniazid, nitrazepam and sulphonamides. Acetylation is a key step in the metabolism of these drugs.

DRUG HYPERSENSITIVITY

Drug hypersensitivity is a syndrome characterised by a combination of fever, skin rash and internal organ involvement, generally occurring more than 1 week after exposure to a drug. The most common internal organ manifestation is hepatitis. Other less common manifestations include pancreatitis, myocarditis, nephritis, intestinal lung disease and muscle inflammation. Adverse drug reactions can be classified into those, type A, which are predictable based on the pharmacogenetics of the drug and those, type B, which are not.

Many type B drug reactions are now thought to be immunologically mediated. The discovery of associations between drug hypersensitivity and specific HLA alleles has been a recent major advance and has led to the possibility that type B drug hypersensitivity reactions may be predictable and preventable. Common drugs associated with immunologically mediated drug hypersensitivity include the anticonvulsant Carbamazepine and the antiretroviral agents Nevirapine and Abacavir. Carbamazepine has been shown in one study to be associated with the HLA class I allele HLA-B*15:02. Different HLA class I and II associations have been described for the antiretroviral agent Nevirapine in different populations, including HLA-DRB1*01:01, HLA-B*35:05 and HLA-Cw8. The antiretroviral agent Abacavir has been shown to be associated with the HLA class I allele HLA-B*57:01 in all populations.

Abacavir is a guanosine analogue which works by competitive inhibition of the reverse transcriptase enzyme of HIV. It is effective as an antiretroviral agent when used in combination with other anti-retrovirals. Before its association with

HLA*57:01 was discovered, use of Abacavir was associated with drug hypersensitivity in about 8% of patients. Abacavir binds in the cleft of the class I molecule and interacts with residues that form the F-pocket of HLA-B*57:01, altering the amino acid that B*57:01 would normally favour from Tryptophan to Isoleucine or Leucine instead. This the repertoire of peptides presented by B*57:01 is altered by Abacavir leading to the hypersensitivity response. Other B57 alleles are not similarly affected by Abacavir.

Symptoms of Abacavir hypersensitivity have a 9-day post drug initiation onset and typically include fever, malaise, gastrointestinal symptoms and internal organ involvement. A mild to moderate rash occurs in approximately 70% of drug hypersensitivity cases. The characteristic feature of Abacavir drug hypersensitivity is that symptoms completely disappear within 72hours of drug discontinuation. However, in susceptible individuals, re-exposure to Abacavir can result in a severe reaction and even death.

Two recent large scale Abacavir drug studies which incorporated skin patch tests have shown that 100% of patients who develop Abacavir drug hypersensitivity carry the HLA-B*57:01 gene. This positive predictive value supports the use of HLA-B*57:01 genetic screening of patients prior to commencement of treatment with Abacavir even though a proportion of HLA-B57:01 patients do not develop hypersensitivity. Current guidelines in the UK recommend that HLA-B*57:01 patients be put on alternative regimes.

The clinical use of HLA-B*57:01 screening for Abacavir drug hypersensitivity prior to treatment may potentially serve as a blueprint for the application of genetic screening in other drug hypersensitivity scenarios.

DRUG REACTION WITH EOSINOPHILIA AND SYSTEMIC SYMPTOMS (DRESS)

Drug reaction with eosinophilia and systemic symptoms (DRESS syndrome) is a drug induced hypersensitivity syndrome which involves a severe, adverse reaction to a drug. The skin is one of the organs most often affected. Patients present with a widespread rash, fever, lymphadenopathy and haematological abnormalities. DRESS may present as a Severe Cutaneous Adverse Reaction (SCAR). Two of the most

well-defined SCARs include Stevens-Johnson Syndrome (SJS) and its more severe counterpart Toxic Epidermal Necrolysis (TEN).

SJS is characterised by severe cutaneous adverse reactions with epidermal detachment from the underlying dermis resulting in blisters and areas of denuded skin, particularly on the torso.

The implicated drugs in SJS and TEN include Carbamazepine and Allopurinol.

Carbamazepine is mainly used to treat neurological disorders, primarily seizures. It has been strongly associated with susceptibility to SJS and TEN in people of Han Chinese descent who have the HLA-B*15:02 allele. Some association has also been reported with HLA-A*31:01 but not HLA-B*15:02 in Japanese and Caucasoid populations.

Allopurinol is widely used to lower blood uric acid levels and prevent or treat its complications. When compared with the normal population, presence of HLA-B*58:01 is associated with a higher risk of susceptibility to SJS and TEN in patients treated with Allopurinol compared to the normal population.

It is recommended that patients are tested for the relevant HLA alleles before commencing treatment with these drugs and that if DRESS is suspected treatment is withdrawn immediately until symptoms resolve.

STEVENS JOHNSON SYNDROME

Stevens Johnson Syndrome (SJS) is a milder form of the Toxic Epidermal Necrosis (TEN) on the spectrum from SJS to SJS/TEN overlap to TEN. SJS is characterised by severe cutaneous adverse reactions with epidermal detachment from the underlying dermis resulting in blisters and areas of denuded skin, particularly on the torso.

SJS and TEN are assumed or identified as drug reactions though herpes has also been implicated. The implicated drugs include Carbamazepine and Allopurinol. Carbamazepine is mainly used to treat neurological disorders, primarily seizures. It has been strongly associated with susceptibility to SJS and TEN in people of Han Chinese descent who have the HLA-B*15:02 allele. Some association has also been reported with HLA-A*31:01 but not HLA-B*15:02 in Japanese and Caucasoid populations. The mechanism of action of Carbamazepine is thought to involve binding of Carbamazepine in the cleft of the class I molecule and interacts with residues of HLA-B*15:02, thus altering the repertoire of peptides presented by B*15:02, leading to a broad range of T cells being activated and a hypersensitive response. The range of T cells activated by Carbamazepine is not thought to be as large as the range activated by Abacavir in B*57:01 individuals (15% compared to 25% for Abacavir).

Allopurinol is widely used to lower blood uric acid levels and prevent or treat its complications. When compared with the normal population, presence of HLA-B*58:01 is associated with a higher risk of susceptibility to SJS and TEN in patients treated with Allopurinol compared to the normal population.

The H&I laboratory contribution would be to help pre-screen patients for HLA-B15:02 prior to commencing Carbamazepine treatment, especially if they have Chinese heritage and screen for B*58:01 prior to commencing Allopurinol treatment. There may also be some benefit to screening for HLA-A31:01 in non-Chinese patients prior to commencing Carbamazepine treatment. However, where a patient has already commenced Carbamazepine or Allopurinol treatment, typing for HLA could help with diagnosis if SJS and TEN are suspected.

HLA AND INFECTION

Human Immunodeficiency Virus (HIV) is a retrovirus, infection with which causes Acquired Immunodeficiency Syndrome (AIDS). HIV infection occurs via transfer with bodily fluids and is characterised by progressive failure of the immune system and life-threatening opportunistic infections. The virus primarily infects cells of the immune system including CD4+ T cells, macrophages and dendritic cells. The mechanism of action involves direct killing of infected cells, increased rates of apoptosis of infected cells and killing of infected CD4+ cells by CD8+ cytotoxic T

cells. The opportunistic infections are as a result of CD4+ T cells falling below critical levels for maintaining cellular immunity.

Genetic variations amongst humans have been shown to be associated with either protection against HIV infection, slow progression to AIDS once infected or rapid progression to AIDS. Several studies of highly exposed seronegative individuals, such as sex workers and the healthy new-borns of HIV infected mothers, have shown that the HLA molecules HLA-A2 and A68 are associated with reduced risk of seroconversion. Generally, concordance of HLA class I alleles between a HIV infected patient and their uninfected sexual partner results in increased likelihood of HIV transmission. Where HIV infection has taken place, a number of studies have shown that some patients do not progress on to full blown AIDS. A correlation has been shown to exist in these long term non progressers between the HLAB*57 and B*27 molecules and delayed AIDS onset. In general, patients heterozygous for HLA class I have delayed progression compared to homozygous individuals. The B*35 allele and the HLA-A1, B8, DR17 haplotypes have conversely been shown to be associated with a more rapid disease progression.

Non-HLA genes associated with delayed or rapid progression include the delta 32 variant of the CCR5 chemokine receptor which is associated with resistance to infection and delayed progression once infected and P1 variant of the CCR5 chemokine receptor which is associated with rapid progression.

HLA DISEASE ASSOCIATION MECHANISMS

Table 1 - Disease Association Mechanisms

Mechanism	Description	Example
1. Peptide Binding	Specificity of peptide binding associated with a particular disease in genetically susceptible individuals	• In Coeliac disease presentation of citrullinated gluten peptide by DQ2, DQ8 • In IDDM absence of specific residues in the bind grove of DR17, DQ2 and DR4, DQ8 affects the peptides that can be bound and therefore presented to T cells. Key residues include Asp57 and Arg75 on DQB1α chain and Arg52 on the DQB1α chain
2. Shared Epitope	Sequence of the HLA gene may affect not just the peptide that can be bound but also the interaction with T cells	• The shared epitopes associated with RA affect both the peptide that can be bound and also crucially, contact between MHC and T cells
3. Arthritogenic Peptide	Peptides derived from either the associated HLA antigen or from the affected site may cause activation of autoreactive T cells	• Proposed that B27 may present an arthritogenic peptide in AS

4. Molecular Mimicry	A molecule from a pathogen may be similar enough to self antigens to crossreactive with it and activate autoreactive T cells. The mimicry means the host does not recognise the pathogen as foreign and does not therefore mount an adequate immune response against it	• This is the proposed trigger for Behcet's disease with HSP65 crossreactive for human HSP60 in HLA-B51 individuals • This is the proposed mechanisms of action of A29 in Birdshot Chorioretinopathy, following an infection or a role for retinal S-Antigen • This is also one of the proposed mechanisms of action of B27 in Reactive Arthritis • And is also one of the proposed mechanisms for B27 in AS • This is also the proposed mechanisms of action of DR103 in Ulcerative Colitis
5. Thymic Involvement	Some autoreactive T cells escape thymic selection and are normally controlled by other post thymic selection mechanisms such as induction of anergy. This theory proposes that these T cell can, under certain circumstances, be activated. In addition defects in the thymus may be involved in disease initiation	• This is one of the proposed mechanisms of B27 susceptibility in Reactive Arthritis • In MG, the hyperplastic thymus is involved in the initiation of the anti-AChR immune responses
6. Unusual Biology	Some HLA antigens, particularly B27, proposed to have unusual biology relative to other HLA. B27 for instance is thought to be expressed stably at the cell	• This is one of the proposed mechanisms of B27 in AS and in Uveitis

	surface in some scenarios without binding peptide and can therefore bind and present extracellular peptides. In addition, B27 is able to bind unusually long peptides. HLA class I molecules normally present peptide to CD8+ T cells but B27 are thought capable of presenting to CD4+ T cells	
7. Altered Self	Some HLA antigens such as B27 can form heavy chain homodimers at the cell surface without engaging β2-microglobulin. These molecules on the cell surface can act as targets for inflammatory responses. Transgenic mice studies have shown that B27 homodimers can bind NK cell receptors leading to inflammation without antigen presentation	• This is one of the proposed mechanisms of B27 in AS and in Uveitis
8. Linkage to other disease associated gene	This theory proposes that the association may in fact be with another gene which is in linkage disequilibrium with the gene to which the disease is assigned	• Haemochromatosis was initially thought to be associated with HLAA3 before later being shown to be actually associated with HFE
9. Competition	Competition for receptor binding may affect the function of the receptor	• Mutations in HFE affect the ability of HFE to bind the Transferrin receptor thereby affecting ferritin levels
10. Epistasis	This describes a phenomenon in which the effects of one gene are modified by one or several other genes	• In MS presence of a conserved vitamin D response element in the promoter region of HLADRB115:01 may enhance the disease susceptibility of this gene In

		addition, Homozygosity for HLA-DRB1*15:01 significantly (x6) increases disease susceptibility
11. HLA as receptor	Some HLA antigens such as B27 are proposed to act receptors for bacterial ligands, without T cell involvement. There is however very little evidence supporting this theory	• This mechanisms may be involved in B27 susceptibility in Reactive Arthritis
12. Superantigen	Some HLA antigens such as B27 are proposed to bind directly to bacterial superantigens, without T cell involvement	• This mechanisms may be involved in B27 susceptibility in Reactive Arthritis

LINKAGE DISEQUILIBRIUM AND HARDY WEINBERGER EQUILIBRIUM (HWE)

LINKAGE DISEQUILIBRIUM

Linkage disequilibrium is the phenomenon in which certain alleles at different loci appear more frequently together in the population, than is suggested by their individual frequencies. Examples of linkage disequilibrium in HLA include HLA-B*07 being often found with HLA-C*07 and HLA-DRB1*03:01 being often found with HLA-DQB1*02:01.

In a randomly mating population, there is an assumption that given sufficient evolutionary time, random recombination events should result in an equilibrium distribution of alleles at each locus. The frequency of a particular allele at a given locus should be independent of alleles at other loci. When this is not the case, the alleles are said to be in linkage disequilibrium. Factors that can affect linkage disequilibrium include:

- **System of Mating –** A non-randomly mating population can cause certain alleles within the mating groups to be found more frequently together
- **Selection pressures** – Certain alleles may be protective for certain diseases and therefore carry an evolutionary advantage
- **Genetic recombination** – The generation of new traits in offspring that differ from those found in either parent
- **Mutation rates** – Certain alleles may be as a result of recent mutations and will not therefore not yet reached equilibrium
- **Population structure** - Systematic difference in allele frequencies between subpopulations in a population, possibly due to different ancestry
- **Genetic drift** - Changes in the frequency of an existing allele in a population due to random sampling of parents to form offspring can lead to two alleles being associated together more frequently
- **Admixture** - When two or more previously isolated populations begin interbreeding
- **Genetic linkage** - Two allele that are physically near to each other may be inherited together due to their physical closeness
- **Gene conversion** - The process by which one DNA sequence replaces a homologous sequence such that the sequences become identical after the conversion event

Study of HLA and disease - Linkage disequilibrium influences the study of HLA and disease as it can potentially mask the true genetic association. A disease which is thought to be in association with one HLA allele may in fact be in association with a different allele which happens to be in linkage disequilibrium. For example Iron overload was once thought to be associated with HLA-A3 until the association with HFE was discovered

Anthropological studies – Genetic anthropological meta-analyses can potentially allow for comprehensive evaluation of the relationships within and between given populations. Analysis of the HLA profiles of two adjacent tribes for instance can potentially yield insights into how those tribes have historically migrated from where they originated to where they find themselves today as well as give insights into historical intermingling of the tribes.

Stem cell donor selection – Stem cell donor identification can be tricky for patients who do not have a standard HLA-B Cw or HLA-DR DQ association. Many donors on the registry do not have HLA-Cw or HLA-DQ types. Calling such a donor for a patient who has an uncommon association is often futile.

Interpretation of HLA antibody results – HLA antibody screening by CDC is complicated by linkage disequilibrium which means that HLA-Cw7 for instance could not be readily identified in isolation from HLA-B8 as these are in LD. This problem is largely resolved by the use of SAB technology however wherever there is a clinical need for CDC screening the issue remains.

Interpretation of HLA typing results – Knowledge of the common HLA associations often serves as a valuable internal quality control check when reviewing HLA typing results.

HARDY WEINBERGER EQUILIBRIUM (HWE)

The Hardy Weinberg Equilibrium (HWE) states that in a randomly mating population, allele and genotype frequencies will remain constant from generation to generation unless there is an evolutionary force influencing change.

Factors that can disrupt HWE include:

- **Mutation**: The rate of gene mutation under normal conditions is usually very low (~$1x10^{-7}$/gamete/locus/generation)
- **Migration**: Migration means populations inter-mate and this leads to changes gene frequency of original group (gene flow)
- **Genetic heterogeneity**: A condition in which different mutations and therefore different genotypes in individuals however leads to similar phenotypes or identical clinical symptoms.
- **Random genetic drift**: The chance increases and/or decreases of gene frequency in small separated section of population over time
- **Founder effect**: This is a phenomenon in which there is the loss of genetic variation a new population if that population is established by a very small number of individuals from a larger population. The population of this new group may increase but its gene variation is small due to reduced intermating between this group and other populations
- **Selection**: This refers to either reproductive fitness or heterozygote dominance (in some recessive hereditary diseases and under certain conditions the heterozygote state may be more favourable to survival and progeny reproduction in contrast to homozygotes)

CHAPTER 4 TRANSFUSION

KEY ANTIGEN SYSTEMS

HUMAN PLATELET ANTIGENS

Platelet antigens are expressed on a number of glycoproteins (GP). Interactions of the platelet glycoproteins with the extracellular environment is important to platelet function:

- GPIIb/IIIa, a heterodimeric integrin made up of α and β subunits and encoded on chromosome 17, is a fibrinogen receptor
- GPIb/IX/V, made up of 4 transmembrane components, with subunits encoded on chromosome 17 and chromosome 22, is a von Willibrand factor receptor involved in platelet adhesion

- GPIa/IIa is a collagen receptor
- CD109 may be involved in cell-cell interactions

Single amino acid substitutions in the sequences of these glycoproteins give rise to different platelet specific antigens.

The platelet antigen system is made up of 24 alloantigens which have been defined by serology. Twelve of these antigens have had the thetical and antithetical antigens defined i.e. they are biallelic antigens - HPA-1a and -1b, -2a and -2b, -3a and -3b, -4a and -4b, -5a and -5b and -15a and -15b. The remaining 12 HPA antigens have had the thetical but not the antithetical antigens observed. Altogether, the molecular basis of 22 of the 24 HPA antigens have been defined. These include the biallelic as well as HPA-6w, -7w, -8w, -9w, -10w, -11w, -12w, -13w, -14w, -16w.

The distribution of HPA antigens on glycoproteins is as follows:

Table 2 - HPA to GP mapping

Antigen	Glycoprotein
HPA-1	GPIIIa
HPA-2	GPIbα
HPA-3	GPIIb
HPA-4	GPIIIa
HPA-5	GPIa
HPA-6w	GPIIIa
HPA-7w	GPIIIa
HPA-8w	GPIIIa
HPA-9w	GPIIb
HPA-10w	GPIIIa
HPA-11w	GPIIIa

HPA-12w	GPIbβ
HPA-13w	GPIa
HPA-14w	GPIIIa
HPA-15	CD109
HPA-16w	GPIIIa

Table 3 - GP to HPA Mapping

Glycoprotein	HPA
GPIa	HPA-5
GPIb	HPA-2 HPA-12w
GPIIa	
GPIIb	HPA-3 HPA-9w
GPIIIa	HPA-1 HPA-4 HPA-6w HPA-7w HPA-8w HPA-10w HPA-11w HPA-14w HPA-16w
CD109	HPA-15

Table 4 - GP Complex to Common HPA Mappings

GP Complex	Biallelic HPA
GPIIIa/IIb	HPA-1 HPA-3 HPA-4
GPIb/IX/V	HPA-2
GPIa/IIa	HPA-5
CD109	HPA-15

HUMAN NEUTROPHIL ANTIGENS

Human Neutrophil Antigens (HNAs) are expressed on five different glycoproteins (GP) on neutrophils. The HNA system comprises five antigen systems HNA-1 to HNA-5. HNA-1 is expressed on GP FcγRIIIb (CD16b) and comprises HNA-1a, -1b, -1c and -1d. HNA-2 is an iso-antigen on CD177, i.e. patients either express it or are null. HNA-3 is expressed on the choline-transporter-like protein 2 (CTL2) and is the only HNA antigen expressed on tissue other than neutrophils, including on kidney endothelium. HNA-3 comprises HNA-3a and -3b. HNA-4 is expressed on CD11b and comprises HNA-4a and -4b. HNA-5 is expressed on CD11a and comprises HNA-5a and -5b.

Table 5 - HNA TO GP MAPPING

Antigen	Glycoprotein
HNA-1 **-1a, -1b, -1c, -1d**	FcγRIIIb aka CD16b
HNA-2	CD177
HNA-3 **-3a, -3b**	CTL2

HNA-4 **-4a, -4b**	CD11b
HNA-5 **-5a, -5b**	CD11a

Antibodies directed against these antigens are implicated in a number of conditions, including:

- Transfusion-related acute lung injury (TRALI)
- Neonatal alloimmune neutropenia (NAIT)
- Febrile non-haemolytic transfusion reactions (FNHTR)
- Autoimmune neutropenia
- Immune neutropenia after bone marrow transplant

H&I laboratory tests used for the detection of HNA antibodies include the granulocyte agglutination test (GAT), the granulocyte immunofluorescence test (GIFT), Monoclonal antibody immobilisation of granulocyte antigens (MAIGA) and HNA typing.

TRANSFUSION REACTIONS

IMMUNOLOGICAL PLATELET REFRACTORINESS

PLATELET REFRACTORINESS OVERVIEW

Platelet refractoriness is defined as a failure to obtain a platelet count increment (PCI) of more than 10 x 10^9/L between 1 and 24 hours post transfusion with ABO compatible platelets on at least two separate occasions.

A patient who meets these criteria and who has had non-immune causes of platelet refractoriness excluded, needs to be tested for HLA antibodies to determine if HLA matched platelets are indicated. Note however that a patient may have immune as well as non-immune causes concomitantly. Non-immune causes include active bleeding, splenomegally, hepatomegaly, DIC, sepsis, fever and use of some anti-fungal agent.

Platelets are routinely transfused to patient with clinically significant haemorrhage and patients with severe thrombocytopenia. Normal platelet counts range from 150 – 450 x 10^9 cells/L and a patient is considered to be thrombocytopenic when platelet counts drop to below 150 x 10^9 cells/L. In a number of transfusion protocols, platelets are transfused prophylactically to prevent bleeding and this is in keeping with best practice guidelines.

Traditionally the main indication for prophylactic platelet transfusion is the platelet count, with haemorrhage considered likely when platelet count is 10–30 x 10^9 cells/L. Patients with various forms of bleeding may receive therapeutic platelet transfusions. Such patients include those with petechiae (pinpoint skin haemorrhages), ecchymoses (bruising), patients with bleeding in the gums and mucosa, patients with nose bleeds, haematuria and menorrhagia and sometimes intracranial haemorrhage. However, in England prophylactic platelet transfusion remains the predominate practice even in the autologous stem cell transplant setting. Guidelines recommend transfusion when platelet counts drop to 10 x 10^9 cells/L or 20 x 10^9 cells/L in the presence of sepsis, fever or for patients undergoing active treatment. These thresholds may vary depending on the clinical condition. A threshold as low as 5 x 10^9 cells/L in stable non-bleeding patients who are not in receipt of active treatment has been reported.

Platelet transfusions may also be given prophylactically as clinically required to prevent bleeding in patients with inherited thrombocytopathies such as Glanzmann's disease or Bernard Soulier Syndrome.

The main immune cause of platelet refractoriness is the presence of antibodies directed against HLA. Alloantibodies directed against HLA class I antigens account for up to 95% of immune refractoriness. The HLA antibodies bind to the donor platelets, making them susceptible to opsonisation and Fc receptor-mediated

phagocytosis primarily in the spleen by resident macrophages. Other immune factors such as antibodies directed against human platelet antigens (HPA) in combination with HLA antibodies account for < 10% of immune refractoriness, while anti-HPA antibodies alone account for <1% of immune refractoriness. In a small number of cases, high titre anti ABO antibodies, autoantibodies, some drug dependent antibodies and immune complexes have also been implicated.

HLA matched platelets are indicated for patients who have an immune cause of their platelet refractoriness. The patient should have a sample taken for platelet count pre and 15min – 1 hour (or no more than 24hours) post transfusion to determine the platelet count increment.

NON-RESPONSE TO MATCHED PLATELETS

Not all patients respond to HLA matched platelets with adequate platelet count increments. Where this is the case, if the last HLA antibody test was carried out a month or more prior to the transfusion, then a new sample may be requested to see if the patient has developed new HLA antibodies. If the patient has been receiving A grade, ABO matched, fresh platelets, then a test for HPA antibodies is indicated. If the patient has HPA antibodies, then platelets need to be matched for HPA and HLA where possible, prioritising HPA matching over HLA matching if there are limited options. The laboratory must discuss with the clinicians to ensure non-immune causes are definitely excluded. Even if evaluated before, new non-immune causes such as bleeding, sepsis, splenomegally, hepatomegaly, DIC and use of medication such as Amphotericin may now be present.

EPITOPE MATCHING IN PLATELET TRANSFUSION

An epitope is the part of an antigen that is recognized by the immune system, specifically by antibodies, B cells or T cells and is therefore also known as an antigenic determinant. Early attempts at epitope definition of HLA were based on direct analysis of amino differences between patients and donors. The HLAMatchmaker system was introduced by Rene Duquesnoy as an in silico system for assessing patient - donor compatibility. This original version of the HLAMatchmaker system treated HLA class I antigens as linear sequences of Triplets of amino acids in antibody accessible regions of the HLA molecule. A later, Eplet version of HLAMatchmaker was introduced which was based on an analysis of the

three-dimensional structure of the HLA molecule rather than on linear sequences of amino acids. Instead of epitopes being defined by three amino acids in a linear sequence, these new epitopes were defined as all the amino acids within a 3 to 3.5Å radius of each polymorphic residue position.

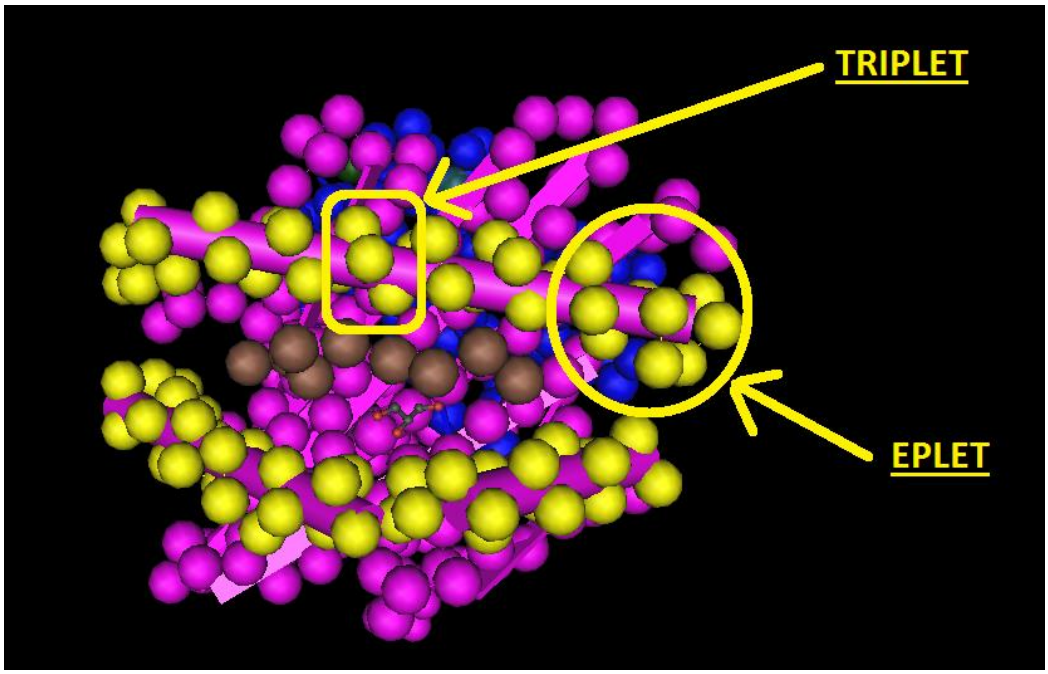


Figure 4 - Epitopes

In many instances, the Eplet epitopes are identical to Triplets. However, Eplets remove the artificial limitation to three amino acids. Eplet epitopes could consist of any number of amino acids that fall within the radius. In addition, Eplet epitopes extended the repertoire of epitopes to include epitopes formed by the folding of the molecule bringing together discontinuous amino acid sequences to form new epitopes.

Epitope matching has two key concepts:

1. Epitopes in a given location on a HLA antigen at one locus are equivalent to the same epitope in the same position on another HLA antigen on a different locus. The definition of epitopes requires that HLA typing be carried out at high resolution so that the exact sequence can be used to identify the epitopes present

2. A patient cannot make antibodies against their own Triplets

Epitope matching gives the potential of increasing the donor pool available to any given patient. A platelet unit which is poorly matched by standard HLA antigen matching for instance could prove to actually have only a few epitope mismatches and may be suitable for that patient. In addition, where there was a choice of equally matched donor platelets, there is some evidence that the one with the fewer epitope mismatches may result in a higher increment. There is also some evidence that Epitope matching may be associated with a lower level of de-novo HLA antibody formation.

TRANSFUSION ASSOCIATED GRAFT VERSUS HOST DISEASE (TA-GVHD)

Transfusion Associated Graft versus Host Disease (TA-GvHD) is a rare complication of blood transfusion which occurs when immunocompetent leucocytes, which are incompatible with the recipient in the graft versus host direction, are transfused into a recipient who is unable to mount an immune response against the leucocytes due to them being compatible in the host versus graft direction and/or the patient being immunocompromised. This allows engraftment of the transfused leucocytes which then proceed to reject the host. TA-GvHD risk is also high in directed donations from related donors as well as in transfusion with HLA matched blood products.

TA-GvHD presents with the same symptoms as bone marrow associated GvHD but typically has a much more rapid onset. The difference with BMT GvHD is that whereas following a bone marrow transplant, the recipient marrow is of donor origin and is not attacked by the GvHD, in TA-GvHD, the majority of the marrow is of recipient origin and is attacked by the TA-GvHD leading to a hypocellular bone marrow and ultimately to bone marrow failure. Prognosis for TA-GvHD is poor.

Where there is skin involvement, test for chimerism by STR using affected and unaffected skin biopsies to detect donor chimerism in the affected skin. Where there is no skin involvement then a blood sample may be tested to look for donor chimerism with a buccal sample providing the patient type.

Most treatment options have not proved very effective but options include support of haematopoiesis with cytokines and discontinuation of antibiotics or any drugs that might be myelosuppressive.

TRANSFUSION RELATED ACUTE LUNG INJURY (TRALI)

TRALI is defined by SHOT as acute dyspnoea (shortness of breath) with hypoxia and bilateral pulmonary infiltrates during or within 6 hours of transfusion, not due to circulatory overload or other likely causes. Blood components mainly implicated in TRALI are plasma rich products such as Fresh Frozen Plasma (FFP) though TRALI following transfusion with low plasma products such as red blood cells have been demonstrated.

The pathogenesis of TRALI is considered to be related to the transfusion of donor HLA and/or HNA antibodies that recognise matching antigens in the recipient or the infusion of lipids and other biological response modifiers that accumulate during storage of blood components, leading to pulmonary neutrophil activation.

The lungs contain the largest pool of neutrophils in the body where they fulfil an important sentinel role in maintaining sterility. The evidence suggests direct binding of antibodies to the neutrophils results in cellular activation leading to degranulation and respiratory burst responses, which in turn damage pulmonary endothelium. Degranulation involves the release of cytokines and other proinflammatory substances. Granule release from neutrophils depends on activation of intracellular signalling pathways, including β-arrestins, the Rho guanosine triphosphatase Rac2, soluble NSF attachment protein (SNAP) receptors, the src family of tyrosine kinases and the tyrosine phosphatase MEG2.

Antibodies in the recipient are not considered to be a likely cause of TRALI in the leucodepletion era except for granulocyte transfusion. A two event hypothesis has been put forward to explain the neutrophil activation. The first event is the underlying condition of the patients (trauma, infection, surgery etc.) resulting in priming of neutrophils. The second event is the transfusion of blood products resulting in activation of the primed neutrophils.

H&I laboratory investigations of suspected cases of TRALI usually start with HLA and HNA antibody testing of all implicated female donors and implicated transfused male donors. HLA antibody screening and identification would likely be carried out by Luminex and HNA antibodies investigations by the flowcytometric Granulocyte Immunofluorescence Test (GIFT) and/or the Monoclonal Antibody Specific Immobilisation of Granulocyte Antigens (MAIGA) test.

The patients would be HLA class I and II and HNA typed to establish the presence of matching antigens for any antibodies that may be present. Crossmatching is no longer considered a requirement.

Suspected cases of TRALI are treated by immediate cessation of transfusion and administration of Oxygen, followed by laboratory investigation.

NEONATAL ALLOIMMUNE THROMBOCYTOPAENIA (NAIT)

Neonatal alloimmune thrombocytopaenia (NAIT) (platelet count $<50x10^9/L$) results from maternal alloimmunisation against foetal platelet antigens inherited from the father, which are different from those present in the mother. This results in the destruction of foetal platelets by maternal IgG antibodies and presents as a severe isolated thrombocytopaenia in otherwise healthy new-borns. Approximately 20% of new-born NAIT cases present with intracranial haemorrhage which can be fatal if not treated promptly. Mothers are typically healthy with no previous history of thrombocytopaenia themselves or autoimmune disorders.

In the neonate, the major risk is intracranial bleeding due to NAIT. A platelet count at birth is required. A cranial ultrasound may be required if the platelet count < 50. Treatment involves rapid platelet transfusion, usually with HPA-1a5b Neg platelets ahead of platelet genotyping. Use of IvIG has been demonstrated to increase platelet count in some NAIT cases.

For ongoing support, HPA typing of donors is required to identify donors lacking the antigen against which maternal antibodies have been formed. Mothers with previous NAIT babies need to be closely monitored in subsequent pregnancies.

H&I laboratory investigations of suspected cases of NAIT would involve HPA antibody testing of the mother, typically by either the flowcytometric Platelet Immunofluorescence Test (PIFT) and/or the Monoclonal Antibody Specific Immobilisation of Platelet Antigens (MAIPA) test. The father or child may be HPA typed to determine if they express the cogent HPA antigen against which antibodies have been formed. As presence of the HLA type HLA-DRB3*01 in the mother has been implicated as the antigen presenting molecule, HLA typing of the mother may also be carried out.

NEONATAL ALLOIMMUNE NEUTROPAENIA (NAIN)

Neonatal Alloimmune Neutropaenia (NAIN) results most often from maternal alloimmunisation against foetal neutrophil antigens inherited from the father which are different from those present in the mother although the antibodies can also arise as a result of autoimmune diseases such as systemic lupus erythematosus (SLE) or rheumatoid arthritis. The presence of the maternal antibodies in the foetus results in destruction of mature neutrophils in the foetus. New-borns affected by NAIN are almost always neutropaenic at birth with neutrophil counts of less than 0.5×10^9/l for weeks or even months. However, NIAN is often asymptomatic until the children present later with fever, lethargy, skin infections, mucosal and respiratory infections and/or urinary infections. The presence of maternal anti-HNA antibodies in the child's circulation aids diagnosis of NAIN.

Symptomatic NAIN cases are treated with antibiotics to counter any infections, otherwise treatment is typically with granulocyte colony stimulating factor (G-CSF) to increase neutrophil counts, though many patients improve with no specific therapy.

H&I laboratory investigations of suspected cases of NAIN would involve maternal HNA antibody investigations most commonly by the flowcytometric Granulocyte Immunofluorescence Test (GIFT) and/or the Monoclonal Antibody Specific Immobilisation of Granulocyte Antigens (MAIGA) test. Antibodies against all the HNA antigens HNA-1a, -1b, -1c, -2a, -3a, -4a and -5a have all been implicated. The father or child may be HNA typed to determine if they express the cogent HNA antigen against which antibodies have been formed.

POST TRANSFUSION PURPURA (PTP)

Post Transfusion Purpura (PTP) is a rare but serious complication of transfusion in which thrombocytopaenia and purpura (bleeding underneath the skin) develops 5 – 10 days post transfusions as a result of destruction of transfused as well as autologous platelets. PTP is more common in multiparous females due to previous sensitisation during pregnancy and is due to HPA antibodies boosted by the transfusion. Studies have also shown that pan specific HPA antibodies are present during the thrombocytopaenic phase and these transient antibodies are probably responsible for the autologous platelet destruction.

H&I laboratory investigations for PTP include confirmation of circulating HPA antibodies in the patient, typically by the flowcytometric Platelet Immunofluorescence Test (PIFT) and/or the Monoclonal Antibody Specific Immobilisation of Platelet Antigens (MAIPA) test.

PTP is treated with IVIg, with plasmapheresis as a second line treatment. Do not transfuse with more platelets as this would trigger more HPA antibodies.

FEBRILE NON-HAEMOLYTIC TRANSFUSION REACTIONS (FNHTR)

A febrile non-haemolytic transfusion reactions (FNHTR) is defined as a 1 - 2 degree Celsius increase in temperature during or soon after a transfusion. This may be accompanied by chills and rigors. A diagnosis of FNHTR can only be made after other causes of fever such as sepsis and haemolysis have been excluded.

FNHTR can be caused by HLA antibodies in the patient binding to their cognate antigens on leucocytes in the transfused product resulting in an immune response. Leucocyte derived cytokines released into the transfused product during storage as well as cytokines released by the recipient as a result of the transfusion have also been implicated.

H&I laboratory investigation of FNHTR includes HLA class I and II antibody testing of the patient typically by Luminex screening and SAB assays as indicated.

The incidence of HLA antibody induced FNHTR has decreased in the leucodepletion era.

HEPARIN INDUCED THROMBOCYTOPENIA (HIT)

Heparin is an anticoagulant (blood thinner) used mainly in the prevention and treatment of deep vein thrombosis, pulmonary embolism, arterial thromboembolism, heart attacks and unstable angina. A common side effect of heparin use is bleeding. A more serous complication is Heparin Induced Thrombocytopaenia (HIT). HIT is an immune complication of heparin therapy caused by antibodies to complexes of platelet factor 4 (PF4 - a positively charged platelet protein stored in platelet α-granules and released in high quantities at sites of platelet activation) and heparin - Anti-PF4/heparin antibodies. Pathogenic antibodies to PF4/heparin bind and activate cellular FcγRIIA on platelets to propagate a hypercoagulable state which can lead to life-threatening thrombosis.

The development of anti-PF4/heparin antibodies is common (8% to 50%), however, clinical complications of thrombocytopenia and thrombosis are far less frequent, affecting approximately 0.2%-3% of patients exposed to the drug. Once antibodies develop, some patients experience a profound thrombocytopenia and progress to life-threatening complications of thrombosis. The principal cellular targets for HIT antibodies are platelets, which express FcγRIIa receptors. Binding of HIT antibodies to platelet FcγRIIA elicits platelet activation via intracellular signalling involving the spleen tyrosine kinase and release of procoagulant microparticles. Platelet activation accompanied by intense thrombin generation.

Diagnosis of HIT requires demonstration of thrombocytopenia (platelet count < 150 x 10^9/L) and/or thrombosis at around the time of heparin therapy while excluding other causes of thrombocytopenia. In heparin-naïve individuals, Anti-PF4/heparin antibodies become detectable at a median of 4 days from start of heparin treatment. Thrombocytopenia and/or thrombosis develop 5 to 14 days after initial heparin therapy and on average, approximately 2 days (range 1-5 days) after antibody detection.

The H&I laboratory can contribute to the diagnosis of HIT through functional assays as well as immunoassays. Test for both HIT and PTP. If HIT is confirmed, then stop PTP investigation. Functional assays include platelet activation assays such as measuring release of radio-labelled serotonin from target platelets exposed to patient serum and a low concentration of heparin and platelet aggregation assays. These assays are highly specific for HIT but tend to be complex to perform and not highly sensitive.

Immunoassays on the other hand are relatively easy to perform. They detect the presence of anti-PF4/heparin antibodies using a variety of antibody capture platforms, including enzyme-linked immunosorbent assay and particle gel.

Treatment for HIT involves discontinuation of all sources of heparin and administration of one of the alternative agents such as Danaparoid or Bivalirudin.

Heparin antibodies should be Neg after 100 days.

GLANZMANN THROMBASTHENIA (GT)

Glanzmann Thrombasthenia (GT) is a rare platelet disorder characterised by excessive bleeding even though patients may have normal platelet counts. It is an inherited autosomal recessive disorder which results in qualitative and quantitative defects in the platelet membrane glycoprotein GPIIb/IIIa.

GPIIb/IIIa is a heterodimeric glycoprotein which acts the th3e receptor for fibrinogen. Activation of platelets causes a conformational change in GPIIb/IIIa which allow fibrinogen and other adhesion molecules, including von Willibrand factor to bind. This is an important step platelet aggregation and fibrin clot formation. Patients who have defective or deficient GPIIb/IIIa have deduced capacity for clot formation and which leads to the excessive bleeding.

Laboratory tests for diagnosis of GT is a platelets flowcytometric test for expression of GPIIb/IIIa.

Management of GT patients involves both preventive measures and treatment of specific bleeding episodes. Preventative measures include avoidance of antiplatelet agents such as aspirin and other anti-inflammatory drugs (NSAIDs) and hormonal contraceptives to control excessive menstrual bleeding.

Severe bleeding is treated with platelet transfusion and/or Recombinant factor VIIa (rFVIIa). Transfusion is also indicated as cover for surgical procedures. Iron or folate supplements may be indicated if bleeding results in anaemia. As GT patients are likely to be on life long transfusion support, use of ABO, HLA and Kell blood group matched platelets is indicated to avoid alloimmunisation which makes it difficult to find compatible platelets in the future. HLA matched platelets should be used for such patients even if not currently sensitised. Regular transfusion does carry risks including TRALI, blood borne pathogens and other transfusion reactions.

Post transfusion patients can be tested for HLA and GPIIb/IIIa antibodies. A travelling control bled on the same day is usually included in the test.

In cases of persistent and recurrent life-threatening bleeding which are refractory to current treatment, HSCT may be a clinical option. Gene therapy is currently experimental in this group of patients.

IDIOPATHIC THROMBOCYTOPAENIA PURPURA

Idiopathic Thrombocytopaenia Purpura (ITP) is a condition in which thrombocytopaenia (platelet count < 100) and purpura (bleeding underneath the skin) develop in patients with normal bone marrow and with no other causes of the thrombocytopaenia are present. The condition is associated with a characteristic purpuric rash and an increased tendency to bleed. ITP patients have detectable antibodies directed against several platelet antigens.

In children, ITP presents as an acute condition, often following an infection and is self-limiting with spontaneous resolution often within 2 months. In adults, ITP presents as a chronic condition which frequently relapses following treatment.

In pregnancy, treatment of ITP involves treating the mother to minimise bleeding by regular checking of platelet counts. Treatment is not required in the absence of bleeding unless platelet counts drop below 20. First line treatment is with steroid, primarily the corticosteroid prednisone. If effective, it leads to an increase in platelet count within 3-5 days. The duration of use should be kept to a minimum as steroid can be harmful to the baby. IvIG is a second line treatment if patient does not respond to steroids. Anti-D can be used in Rh-D Neg mothers. Evidence for the use of other steroid sparing alternative treatments such as Rituximab and thrombopoietin in pregnancy is limited. Some experts recommend early deliver, even caesarean, to reduce the risk of intracranial haemorrhage as a result of low platelet count.

KEY BLOOD COUNTS

Table 6 - Key Blood Counts

Count	Normal Range		Critical
Red Blood Cell – RBC (x 10^{12}/L)	M	4.5 – 6.5	
	F	3.8 – 5.8	
Haemoglobin (g/L)	M	140 – 180	<70
	F	120 – 160	<70
Hematocrit (%)	M	34 – 54	
	F	37 – 47	
Reticulocytes (%)	M	0.50 – 1.46	
	F	0.56 – 1.52	
Reticulocytes (x10^{9}/L)	M	26.1 – 96.7	
	F	28.8 – 94.1	

Platelet Count (x10^9/L)	M F	150 – 350 150 –350	<75 <75
White Blood Cell – WBC (x10^9/L)	M F	4.5 – 11 4.5 – 11	<1.5 & > 30.0 <1.5 & > 30.0
Lymphocytes (%)	M F	15.0 – 41.0 15.0 – 41.0	
Lymphocytes (x10^9/L)	M F	1.5 – 4.0 1.5 – 4.0	
T cells (%)			
CD34 (%)	M F	25 – 65 25 – 65	
CD4:CD8	M F	0.9 – 1.9 0.9 – 1.9	
B cells (%)			
NK Cells (%)			
Neutrophils (%)	M F	45.0 – 70.0 45.0 – 70.0	
Neutrophils (x10^9/L)	M F	2.0 – 7.5 2.0 – 7.5	
Monocytes (%)	M F	2.0 – 10.0 2.0 – 10.0	
Monocytes (x10^9/L)	M F	0.1 – 0.9 0.1 – 0.9	
Macrophages (%)			
Eosinophils (%)	M F	0.0 – 7.0 0.0 – 7.0	

Eosinophils (x10^9/L)	M F	0.0 – 0.5 0.0 – 0.5	
Basophils (%)	M F	0.0 – 1.5 0.0 – 1.5	
Basophils (x10^9/L)	M F	0.0 – 0.1 0.0 – 0.1	
Granulocytes			
CD34 counts			
Ferritin (µg/L)	M F	22 – 300 6.5 – 204	
Total serum protein (g/L)	M F	64 – 83 64 – 83	<30 <30
Transferrin (g/L)	M F	1.81 – 3.31 1.81 – 3.31	
Creatinine (µmol/L)	M F	64 – 104 49 – 74	>400 >400
Albumin (g/L)	M F	35 – 55 35 – 55	
Bilirubin (µmol/L)	M F	0.0 – 20.4 0.0 – 20.4	
C-Reactive Protein (mg/L)	M F	0.0 – 3.0 0.0 – 3.0	>=5.0 >=5.0
Urea (mmol/L)	M F	2.5 – 9.2 2.5 – 9.2	>=35 >=35
Uric Acid (µmol/L)	M F	210 – 450 150 – 360	

Serum sodium (mmol/L)	M F	136 – 145 136 – 145	<120 or >160 <120 or >160
Potassium (mmol/L)	M F	3.5 – 5.0 3.5 – 5.0	<2.8 or >6.2 <2.8 or >6.2
Bicarbonate (mmol/L)	M F	22 – 31 22 – 31	<10 or>40 <10 or>40
Calcium (mmol/L)	M F	2.2 – 2.6 2.2 – 2.6	<1.5 or>3.25 <1.5 or>3.25
Cholesterol (mmol/L)	M F	<5.2 <5.2	>6.3 >6.3
Tacrolimus – Whole blood (µg/L)	M F	5 – 20 5 – 20	

CHAPTER 5 HAEMOPOIETIC STEM CELL TRANSPLANTATION

Haematopoietic progenitor or stem cell transplantation (HSCT) refers to any process in which haemopoietic stem cells are given to a patient with the intention of repopulating the patients' haemopoietic system either in total or in part. HSCT has been used ever since it was discovered that leukaemia could be treated with radiation and the patient could be rescued from the subsequent aplasia with the infusion of stem cells from a compatible donor.

CLINICAL CONDITIONS TREATED WITH STEM CELL TRANSPLANTATION

LEUKAEMIA'S

ACUTE MYELOID LEUKEMIA (AML)

Acute Myeloid Leukemia (AML) is an acute onset leukaemia involving clonal expansion of myeloid cell line derived blast cells (precursors of polymorphonuclear leukocytes). The World Health Organisation (WHO) defines AML as leukaemia with bone marrow or peripheral blood blast percentage of more than 20%.

Patients often present with chromosomal abnormalities in marrow cells and maturational arrest of bone marrow myeloid cells in the earliest stages of development. AML manifests as pancytopenia, megaloblastic bone marrow and nucleated red cells in peripheral marrow.

AML commonly occurs following a prior hematologic disorder such as MDS, familial syndromes (germline mutations), environmental and drug exposures such as to alkylating agents used in treatment of a prior disorder.

If transplant indicated (intermediate or adverse risk AML), it is usually needed urgently with allo-HSCT the standard of care. High resolution HLA typing of patient and sibs, WMDA search and early initiation of the full search without waiting for related donors to all be typed may be required. Wherever possible, it is

recommended that the laboratory always have x2 potential donors identified and ready to be called.

ACUTE LYMPHOCYTIC LEUKEMIA (ALL)

Acute Lymphocytic Leukemia (ALL) is an acute onset leukaemia characterised by clonal expansion of lymphoid cell line derived blast cells in the bone marrow replacing the normal hematopoietic cells. Lymphoid precursor cells (i.e. B and T lymphoblasts) that are arrested in an early stage of development. ALL includes the precursor B lymphoblastic leukaemia and precursor T lymphoblastic leukaemia.

ALL is the most common childhood cancer. It also accounts for approximately 20 percent of adult acute leukaemia. Childhood B-cell precursor ALL has a multifactorial aetiology, with a two-step process of genetic mutation and exposure to infection playing a prominent role. Less is known about the aetiology of other ALLs.

HSCT is the standard of care for high risk patient in first complete remission and in other relapsed patients or patients who fail induction.

CHRONIC MYELOID LEUKEMIA (CML)

Chronic Myeloid Leukemia (CML) is the most common of the chronic onset myeloproliferative disorders and is typically a disease of the middle-aged or elderly. It is characterised by clonal expansion of the granulocytic cell line without the loss of their capacity to differentiate.

CML progresses through three phases - chronic, accelerated and blast. In the chronic phase mature cells proliferate. In the accelerated phase, additional cytogenetic abnormalities occur and in the blast phase, immature cells rapidly proliferate.

CML is characterized by a cytogenetic aberration consisting of a reciprocal translocation between the long arms of chromosomes 22 and 9 [t(9;22)] - the Philadelphia (Ph1) chromosome.

Use of stem cell transplantation for CML patients is reduced in the Imatinib era.

CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

Chronic Lymphocytic Leukemia (CLL) is the most common form of chronic lymphoid leukaemia. It is a monoclonal disorder characterized by morphologically mature but immunologically immature lymphocytes which slowly accumulate in the blood, bone marrow and lymphatic tissue.

CLL are clonal B cells arrested in the B-cell differentiation pathway, intermediate between pre-B cells and mature B cells. Morphologically, in the peripheral blood, these cells resemble mature lymphocytes.

CLL is usually an adult onset disease with the majority of patients diagnosed being males over 50 years of age. Many people with CLL lead normal and active lives for many years. Stem cell transplantation is therefore rarely used as a first-line treatment due to its associated risks.

Allo-HSCT is indicated for high risk patient and for patients who fail induction.

MYELODYSPLASTIC SYNDROME (MDS)

Myelodysplastic Syndrome (MDS) is a clonal haematopoietic disorder characterized by a hypercellular bone marrow with dysplasia (abnormality of development) and ineffective haematopoiesis in one or more cell lines. This may be accompanied by up to 20% myeloblasts.

MDS manifests as progressive cytopenia that occurs over months to years resulting from clonal expansion of mutant cells, predominating in the bone marrow, suppressing healthy stem cells.

MDS may occur de-novo or following exposure to alkylating agents and/or radiotherapy.

HSCT (Allo) is only indicated in high risk patients.

MYELOFIBROSIS

Myelofibrosis is a myeloproliferative disorder in which the bone marrow is replaced by fibrous tissue.

The fibrosis impairs the ability to generate new blood cells, leading to pancytopenia. The fibrosis also leads to blood forming in other organs such as the spleen and liver, leading to the classic symptoms of Myelofibrosis which include splenomegally and some degree of hepatomegaly.

Stem cell transplantation is generally not recommended for low risk patients but is the standard of care for high risk patient below the age of 45.

ADULT T-CELL LEUKEMIA/LYMPHOMA (ATLL)

Adult T-cell leukemia/lymphoma (ATLL) is a rare, aggressive T-cell lymphoma with a poor prognosis.

Patients present with generalized swelling of the lymph nodes hepatomegaly and splenomegaly accompanied by fatigue and anorexia. Patients may also have hypercalcemia, constipation and general weakness.

ATLL is linked to infection by the human T-cell lymphotropic virus 1 (HTLV-1). All potential live related donors must be tested for HTLV-1.

JUVENILE MYELOMONOCYTIC LEUKEMIA (JMML)

Juvenile myelomonocytic leukemia (JMML) is an aggressive hematopoietic disorder of early childhood caused by excessive proliferation of monocyte and granulocyte cell lineages.

JMML is characterized by overproduction of monocytic and granulocytic cells that infiltrate different organs, including the spleen, liver, lung and gastrointestinal tract. Affected children usually have pallor, fever and skin bleeding, which result from anaemia, leucocytosis and thrombocytopenia.

Approximately 90% of patients carry either somatic or germline mutations of PTPN-11, K-RAS, N-RAS, CBL or NF1 in their leukemic cells.

HSCT remains the only curative treatment for JMML. The urgency of transplant favours use of cord blood.

LYMPHOPROLIFERATIVE DISORDERS

HODGKINS LYMPHOMA (HL)

Hodgkin's disease is a potentially curable malignant lymphoma. It is characterized by the presence of Reed-Sternberg cells (large, multi-nucleated cells, with prominent eosin staining inclusions in the nucleus). Hodgkin's patients experience progressive enlargement of the lymph nodes, spleen and general lymphoid tissue. Hodgkin's disease is sometimes accompanied by symptoms such as fever, weight loss, fatigue and night sweats.

Hodgkin's disease occurrence is bimodal with incidence peaks in young adults (15-35) and older persons (over 55's).

Autologous transplantation is the standard of care relapsed/refractory Hodgkin's patients.

NON-HODGKINS LYMPHOMA'S

NHL comprises of all lymphomas except Hodgkin's lymphoma. NLH are a diverse group of diseases which have been divided into several subtypes based on whether the disease is indolent or aggressive and whether T or B cells are involved. Subtypes of NHL include Diffuse B Cell Lymphoma, Follicular Lymphoma, Mantle Cell Lymphoma, Burkitt Lymphoma, Peripheral T Cell Lymphoma and Adult T Cell Lymphoma.

BURKITT NON-HODGKIN LYMPHOMA

Burkitt NHL is a highly aggressive B-cell non-Hodgkin lymphoma characterized by the translocation and deregulation of the c-myc gene on chromosome 8. It occurs at a high frequency in patients with immunodeficiencies and in HIV positive patients.

The exact cause of Burkitt NHL is unknown. Theories include Epstein-Barr virus (EBV) and malaria infection as well as C-myc oncogene activation.

MANTLE CELL LYMPHOMA

Mantle cell lymphoma (MCL) is an aggressive B cell lymphoproliferative, non-Hodgkin lymphomas, derived from a subset of naive pregerminal center cells localized in primary lymphoid follicles or in the mantle region of secondary lymphoid follicles. It is characterized by the chromosomal translocation t(11;14)(q13;q32).

The cause of MCL is unknown but is thought to be associated with viral infection (Ebstein-Barr virus, HIV, human T-lymphotropic virus type 1, human herpesvirus 6), environmental factors (pesticides, hair dyes) and primary and secondary immunodeficiency.

FOLLICULAR LYMPHOMA

Follicular lymphoma is a type of non-Hodgkin lymphoma that most commonly presents as a painless, slowly progressive adenopathy (swelling of tear, sweat, hormonal and other glands).

The t(14;18) chromosomal translocation is a hallmark of follicular lymphoma.

Viruses, including the EBV, HTLV-1 and the herpesvirus associated with Kaposi sarcoma have been implicated in the aetiology.

First line treatment of Follicular lymphoma is chemotherapy including use of Rituximab. Reduced Intensity Conditioning (RIC) followed by Allo-HST has been shown to be effective in older patients and in patients who had previously undergone an Auto transplant as a bridge to Allo.

MULTIPLE MYELOMA (MM)

Multiple Myeloma (MM) is a bone marrow based plasma cell neoplasm (bone marrow plasma cells > 10%) characterized by high production of serum monoclonal protein (most commonly IgG) in the bone marrow and skeletal destruction causing bone lesions, fractures, bone pain, hypocalcaemia and anaemia.

MM is characterized by a proliferation of malignant plasma cells and a subsequent overabundance of monoclonal paraprotein (M protein) and can present as a single lesion (plasmacytoma) or multiple lesions.

The precise aetiology of MM is not established. Roles have been suggested for a variety of factors, including genetic causes, environmental or occupational causes, radiation, chronic inflammation and infection.

Current treatment involve treatment with the triple agents thalidomide, lenalidomide and bortezomib to CR potentially followed by Auto HSCT.

HAEMOGLOBINOPATHIES

THALASSAEMIA

The Thalassemia's are a heterogeneous group of hereditary autosomal recessive haemolytic anaemia's which have in common a decreased rate of synthesis of one or more haemoglobin polypeptide chains, resulting in anaemia.

Allo-HSCT with a matched related donor remains the only curative treatment.

SICKLE CELL

Sickle cell disease is a group of inherited disorders, the most serious of which is sickle cell anaemia, in which there is reduced oxygen carrying capacity of red blood cells due to a 'sickling' effect.

Sickle cell is a disease characterized by chronic haemolytic anaemia, episodic painful crises and pathological involvement of many organs.

Sickle Cell Disease is an autosomal recessive disease, its genetic basis being the homozygous expression of haemoglobin S.

First line treatment is with immunosuppressive therapy (IST) with antithymocyte globulin (ATG) and cyclosporine. Allogeneic stem cell transplantation is considered usually only if there is a matched sibling donor.

LANGERHANS CELL HISTIOCYTOSIS (LCH)

Langerhans Cell Histiocytosis (LCH) is a group of idiopathic disorders characterized by the excessive production of Langerhans cells, a type of white cell found mostly in skin and lymph nodes which are involved in preventing infections.

If the cells multiply and remain mostly in the skin, the result often is localized to the skin. In children this can manifest as rashes. When the cells migrate to other parts of the body, the symptoms are much more widespread and serious and the disorder can be life-threatening.

The aetiology of LCH is unknown. Langerhans cell proliferation may be induced by a viral infection, a defect in intercellular communication (T cell–macrophage interaction) and/or a cytokine-driven process mediated by tumour necrosis factor, IL-11 and leukemia inhibitory factor.

BONE MARROW FAILURE SYNDROMES

APLASTIC ANAEMIA (AA)

Aplastic Anaemia (AA) is a serious disorder of the bone marrow characterised by pancytopenia and a hypocellular bone marrow. AA can be idiopathic or secondary due to bone marrow damage by toxins, radiation, or immunologic factors.

Specific treatment of aplasia involves either immunosuppressive therapy (IST) with antithymocyte globulin (ATG) and cyclosporine or allogeneic stem cell transplantation with a matched related donor for Severe Aplastic Anaemia (SAA). Where HSCT is required, the preferred source of stem cells is bone marrow (BM) rather than peripheral blood stem ells (PBSC). Use of PBSC for transplants in SAA has been associated with significantly inferior survival with a concurrent two-fold increase in GvHD across all age groups but especially so in young patients. The lack of a need to eradicate an underlying malignancy means that non-myeloablative regimens can be used for SAA patients without the need for the increased cell dose obtained from PBSC, resulting in reduced GvHD with BM as the stem cell source.

FANCONI'S ANAEMIA (FA)

Fanconi's anaemia is the most common of the inherited bone marrow failure syndrome (IBMFS) with pancytopenia. Patients present with combination of aplastic

anaemia and physical anomalies including skin pigmentation and upper limb, gonadal and skeletal abnormalities.

Fanconi's anaemia is an autosomal recessive disease in more than 99% of patients (FANCB is X-linked recessive). Patient are homozygous for mutations in one of the 15 genes that cause Fanconi's anaemia.

HSCT is indicated in patients who are transfusion dependent or who have severe neutropenia and in patients with myelodysplasia or leukemia.

DIAMOND-BLACKFAN ANAEMIA (DBA)

Diamond-Blackfan anaemia is a rare inherited bone marrow failure syndrome (IBMFS) that manifests with pure red cell aplasia. The anaemia is usually macrocytic (large cells), with elevated foetal haemoglobin and increased red cell adenosine deaminase.

Diamond-Blackfan anaemia is (usually) an autosomal dominant disease caused by mutations in the genes that provide instructions for making several ribosomal proteins.

Indications for HSCT include nonresponse to steroids, transfusion dependency, progressive pancytopenia or MDS/AML.

IMMUNODEFICIENCIES

SEVERE COMBINED IMMUNODEFICIENCY (SCID)

SCID is a heritable immunodeficiency syndrome arising as a result of defects to several genes in the B and T cell arms of the adaptive immune response. Sufferers are extremely vulnerable to infectious diseases.

SCID arises as a result of defects to several genes in the B and T cell arms of the adaptive immune response.

Sibling HSCT has an overall survival rate of 90%. Transplantation may proceed without conditioning of GvHD prophylaxis given the severe immunocompromised state of the patient. In the absence of sibling haploidentical parents may also be an option for transplantation.

INBORN ERRORS IN METABOLISM (IEM)

Inborn Errors in Metabolisms (IEMs) are a large group of rare genetic diseases that generally result from a defect in an enzyme or transport protein which results in a block in a metabolic pathway. Most of the disorders are inherited as autosomal recessive genes though autosomal dominant and X-linked disorders are also present.

HURLER SYNDROME

Hurler syndrome (Mucopolysaccharidosis - MPS IH), is a genetic disorder which causes a deficiency of alpha-L iduronidase, an enzyme responsible for the degradation of glycosaminoglycans (GAGs) in lysosomes. Hurler syndrome is therefore known as a lysosomal storage disorder (LSD).

Hurler syndrome results in the build-up of GAGs (also known as mucopolysaccharides). Symptoms present at 3-6 months and include a large head with prominent frontal bones, elongated skull, flattened nasal bridge, widely spaced eye sockets and eyes that may protrude from the skull.

Hurler syndrome is an autosomal recessive resulting from two defective copies of the IDUA gene which encodes for the protein iduronidase. The gene has been mapped to the 4p16.3 site on chromosome 4.

Early HSCT is indicated to prevent multiple organ dysfunction and death.

SCHEIE SYNDROME

Scheie syndrome (Mucopolysaccharidosis - MPS IS), is a genetic disorder which causes a deficiency of iduronidase.

Scheie syndrome results in the build-up of glycosaminoglycans. It has milder symptoms compared to Hurler syndrome.

Scheie Syndrome is an autosomal recessive resulting from two defective copies of the IDUA gene which encodes for the protein iduronidase. The gene has been mapped to the 4p16.3 site on chromosome 4.

HURLER-SCHEIE SYNDROME

Hurler-Scheie syndrome (Mucopolysaccharidosis - MPS IH/S), is a genetic disorder which causes a deficiency of iduronidase.

Hurler-Scheie syndrome results in the build-up of glycosaminoglycans. It has symptoms intermediate between Scheie and Hurler syndromes.

Hurler-Scheie Syndrome is an autosomal recessive resulting from two defective copies of the IDUA gene which encodes for the protein iduronidase. The gene has been mapped to the 4p16.3 site on chromosome 4.

HUNTERS SYNDROME

Hunters syndrome (Mucopolysaccharidosis - MPS II), is an X-linked recessive genetic disorder which causes a deficiency of the lysosomal enzyme iduronate-2-sulfatase (I2S).

Hunters syndrome results in the build-up of the glycosaminoglycans heparan sulphate and dermatan sulphate in lysosomes of all body tissues.

Hunters syndrome is an X-linked recessive genetic disorder which preferentially affects males who inherit a defective X chromosome from the mother and do not have another X chromosome to compensate for the mutant gene.

MANNOSIDOSIS

Mannosidosis (classified as alpha and beta) is a lysosomal storage disorder, caused by a deficiency in alpha-mannosidase, a major exoglycosidase in the glycoprotein degradation pathway.

Mannosidosis is characterised by progressive lysosomal accumulation of mannose-rich oligosaccharides in all tissues, resulting in impaired cellular function and apoptosis.

Alpha Mannosidosis is caused by mutations in the MAN2B1 gene (short arm of chromosome 19) and Beta Mannosidosis is caused by mutations in the MANBA gene (long arm of chromosome 4).

LEUKODYSTROPHY

Leukodystrophies are a group of rare, progressive, metabolic, genetic diseases that affect the brain, spinal cord and often the peripheral nerves. Patient have an inability to degrade sulphated glycolipids and/or a deficiency in the lysosomal enzyme sulfatide sulfatase resulting in accumulation of sulfatide compounds in neural and in nonneural tissue, such as the kidneys and gallbladder.

Each type of leukodystrophy is caused by a specific gene abnormality that leads to abnormal development or destruction of the white matter (myelin sheath) of the brain.

AUTOIMMUNE DISEASES

Autoimmune Diseases comprise a large group of disorders characterized by pathologic immune reactions to autologous tissue. Autoimmune Diseases may be systemic (e.g. Systemic Lupus Erythematosus) or they may be organ specific, (e.g. Thyroiditis).

A small number of Auto-HSCTs have been performed for patients with Multiple Sclerosis (MS), Systemic Lupus Erythematosus (SLE) and childhood Crohn's with promising results.

SOLID TUMORS

GERM CELL

Germinal Tumours are neoplasms of the ovary and other uterine tissue or testis. The neoplasms may be benign or malignant.

NEUROBLASTOMA

Neuroblastoma is a metastatic malignant neoplasm often originating in the adrenal gland before spreading to other parts of the body. It is often asymptomatic before metastases. It has a characteristically early onset.

EWING'S SARCOMA

Ewing Sarcoma is a rare malignant neoplasm of bone and articular cartilage. The most frequently affected sites are the pelvis, femur, humerus, ribs and clavicle. Ewing Sarcoma is more common in young males, presenting between the ages of 10 to 20.

BREAST CANCER, OVERY CANCER, SMALL CELL LUNG CANCER AND RENAL CELL CARCENOMA

Breast Cancer is a metastatic malignant neoplasm originating in breast tissue. The vast majority of cases are carcinomas arising from the breast parenchyma or the nipple. Malignant breast neoplasms occur more frequently in females than in males.

ROLE OF THE STEM CELL MULTIDISCIPLINARY TEAMS (MDT'S)

Many key decisions related to stem cell transplantation and donor selection are made today by a multidisciplinary team (MDT). The stem cell MDT will typically include all haematologists who have current patients, clinical nurse specialists from the stem cell team, a H&I scientist, the data manager for the unit and sometimes the quality lead for the unit. The team would also typically have access to a dietician, though they may not attend all meetings. Increasingly, a patient advocate may also attend the MDT. The key function of the stem cell MDT is to review all current patients, starting with their clinical history, to determine if they are suitable or remain suitable, for stem cell transplantation. The donor availability is then reviewed, looking at related and unrelated donors including cord. The MDT will make a choice of donor to proceed with based on advice from the H&I scientist, taking into account all HLA and non-HLA factors. The MDT will also decide on the transplant protocol to apply depending on the disease status and stage and the donor availability.

ROLE OF HLA MATCHING IN STEM CELL TRANSPLANT OUTCOME

OVERVIEW OF ROLE OF HLA MATCHING

Of all the genetic and non-genetic factors that can influence stem cell transplant outcome, the most important remains HLA matching.

HLA matching is required for all forms of stem cell transplantation other than autologous transplantation. Matching is required at all the classical HLA class I and II loci, HLA-A, B and C, HLA-DRB1, DQB1 and HLA-DPB1 at high resolution allele level. Graft versus Host Disease (GvHD) is a major post-transplant complication in stem cell transplantation and can be initiated by as little as a single amino acid mismatch in the peptide binding groove of an HLA antigen. The degree of matching required will be influenced by the nature of the disease and the source of the stem

cells. Studies by Petersdorf et. al., and others have shown that the risk of GvHD increases with increasing numbers of mismatches in the Graft versus Host direction (i.e. HLA alleles present in the patient but absent in the donor). Similar studies have also shown that the risk of graft failure increases with increasing numbers of mismatches in the Host versus Graft direction (i.e. HLA alleles present in the donor but absent in the patient).

In autologous stem cell transplantation, the donor is the patient, making the HLA alleles the same. Any HLA typing undertaken is therefore not for matching for the transplant but rather for information should an allogeneic donor suddenly be needed or for supportive therapy such as the use of HLA matched platelets.

In the allogeneic setting, HLA matching is required at the high-resolution allele level, the 'best' matched donor being a syngeneic or genetically identical donor who will be allele matched at all the classical HLA loci. However, whilst the absence of HLA mismatches in the syngeneic setting reduce the risk of GvHD, it also reduces the desirable Graft versus Leukaemia (GvL) effect and is therefore associated with an increased incidence of relapse. In the absence of a syngeneic donor, a HLA matched sibling represents the most likely source of a HLA matched donor. A sibling donor has a ¼ chance of being HLA matched. Historically, some Transplant Centres limited their HLA typing to the low to medium resolution HLA typing i.e. first field DNA typing, at HLA-A, B and DR as sufficient to confirm a match between and patient and a sibling donor because of the strong linkage disequilibrium between HLA-B and C as well as between HLA-DR and DQ. This is the minimum required by European Federation for Immunogenetics (EFI) standards in related stem cell transplantation. However limiting typing to these loci may mean that the patient and donor may not be matched at HLA-C and HLA-DQ if one or more parents are homozygous for HLA-A, B, DR as the patient and donor could inherit different haplotypes which could be mismatches at the allele level when high resolution or second field typing is undertaken. There could also be crossover events between HLA-A and HLA-B and between HLA-DR/DQ and HLA-DP which would remain undetected if typing is not extended to all the classical HLA genes. For this reason, when matching siblings, it is also useful to determine the HLA types of the parents if possible. In addition, where parents share a haplotype, a parent could be matched to the patient and represent a potential source of stem cells. Historically, HLA-DPB1 matching was not always taken into account though has now largely changed.

For unrelated allogeneic transplantation with adult Peripheral Blood Stem Cells (PBSC) or Bone Marrow (BM), HLA matching was historically undertaken for HLA-A, B, C, DR and DQ at the high resolution second field level. A large study by the NMDP indicated that 8/8 matching, excluding HLA-DQB1, has the same outcome as 10/10 matching and is the minimum required for stem cell transplantation. Matching for HLA-DPB1 is now taken into account by many transplant centres though HLA-DPB1 mismatching is associated with increased GvL and therefore a lower risk of relapse making a degree of HLA-DPB1 mismatching desirable in some malignant diseases.

Transplantation with Cord Blood (CB) derived stem cells leads to a reduced incidence and severity of GvHD due to the relative immaturity of the immune system at birth. This can allow less stringent HLA matching for CB transplantation with one or two HLA mismatches tolerated. CB matching is carried out at high resolution at HLA-A, B, C and DR for a single cord. For double cord, matching is currently at the broad antigen level for class I and at the allele level for HLA-DR. HLA mismatches between the cord blood units is no longer considered to be relevant. Cell dose may be the more important factor.

The use of cord blood helps in the identification of donors for patients with rarer HLA types or uncommon haplotypes. Registry data does indicate that cord blood banks have a higher frequency of uncommon phenotypes than adult bone marrow registries.

The relative importance of matching at the individual classical HLA loci in stem cell transplant outcome is controversial with many apparently contradicting studies. This may be due to the wide range of disease groups, conditioning regimes and HLA typing techniques used over the years. Some studies have shown that in high-risk patients, transplantation with a donor with a single HLA allele mismatch leads to a better outcome than a prolonged search for a fully matched donor. An additive effect appears to exist such that the total number of mismatches may be more important than which specific loci are mismatched, though many transplant teams would prefer to avoid mismatches at class II (especially HLA-DRB1) over mismatches at class I.

Both HLA and non-HLA genes play a role in stem cell transplant outcome and therefore influence donor selection. HLA genes are the main genetic barrier to transplantation and therefore have the most influence on donor selection but non-HLA genes play a significant and increasingly understood role.

RELEVANCE OF HLA MATCHING AT THE ALLELE LEVEL

High resolution typing refers to a level of typing which is able to assign HLA alleles on the basis of differences in exons 2 and 3 for class I and exon 2 for class II. Alleles with identical sequences over these exons are all reported as part of a high-resolution HLA typing result though further testing over additional exons is often performed to further reduce ambiguities.

Allele level typing on the other hand refers to the unambiguous identification of the allele present.

Until very recently, most H&I laboratories performed high-resolution HLA typing by Sanger-based Sequencing (SBT). SBT has some limitations as it is not always able to resolve ambiguities. It often requires additional testing with group specific sequencing primers (GSSP) to help resolve these ambiguities but some do still remain. Recently, NGS techniques have been introduced. These tend to have much fewer ambiguities and superior phasing depending on the technique used, whole gene sequencing on the one hand versus shotgun sequencing which can have a small number of ambiguities.

The clinical advantages of high-resolution HLA matching over and above low to intermediate HLA matching in stem cell transplantation have been clearly demonstrated in reduced GvHD and improved overall survival. There is insufficient data at present to clearly define a clinical advantage of allele level HLA matching in stem cell transplantation over and above high-resolution HLA matching. The Anthony Nolan have some recently published data which shows superior one year mortality and morbidity and reduced GvHD for allele level matching compared to high resolution matching, especially when HLA-DPB1 was included in a 12/12 match. Potential reasons for this include:

- Allele level matching resolves remaining ambiguities relative to HR

- Allele level matching reduces variations in introns and untranslated regions which may be significant
- Allele level matching may be a surrogate for reduced SNP mismatches in other regions

RELEVANCE OF 'G' AND 'P' CODE IN HSCT

G code alleles are those HLA alleles that have identical nucleotide sequences across the exons encoding the peptide binding domains (exon 2 and 3 for HLA class I and exon 2 only for HLA class II alleles) while P code alleles include all those with, for HLA class I, identical protein sequences encoded by exons 2 and 3 and for HLA Class II, identical protein sequences as encoded by exon 2.

Examples of alleles that share the same G code include DQB1*03:01:01:01 with DQB1*03:19:01:01 and C*05:01:01:01 with C*05:37. Examples of alleles that share the same P code include DRB1*14:01:01 with DRB1*14:54:01:01.

For unrelated donor transplants where a patient appears to only have a 11/12 match with the only mismatch being a G or P coded allele, one strategy could be to first get the probability of the patient being able to get a full 12/12 allele match using the WMDA, NMDP or allele frequency tools. If the patient has a low probability of getting a better match, a literature seach would be conducted to see if there are any published data concerning permissible mismatches using the particular G or P code. If there are no specific references for the mismatch in question, there are general publications outlining reduced risk of transplants where the mismatches are outside of exons 2 and 3 for class I and exon 2 for class II. In all transplants with G or P code alleles other additional risk factors such CMV mismatching should be avoided.

ROLE OF HLA-DPB1 MATCHING

HSCT where HLA allele are matched 10/10 at HLA-A, -B, -C, -DRB1 and -DQB1 are mismatched at HLA-DPB1 in approximately 80% of cases and these mismatches have been shown to correlate significantly with non-relapse mortality. Finding donors matched 12/12 to include HLA-DPB1 can be difficult due to a recombination hotspot between HLA-DQ and -DP. However, the classification of HLA-DPB1 alleles into

permissive and non-permissive mismatches based on T-cell-epitope groups increases the odds of funding an unrelated donor for a patient. Classification of HLA-DPB1 into permissive and therefore tolerated and non-permissive mismatches has been shown to be significant in unrelated-donor HSCT.

Zino et al demonstrated in a paper published in Blood in 2004, through a series of EBV transformed B cell line cytotoxic assays, that subsets of HLA-DPB1 alleles shared T cell epitopes that determine their specific allo-response.

They divided HLA-DPB1 alleles into three groups, group 1 – Immunogenic, group 2 – intermediately immunogenic and group 3 poorly immunogenic. They hypothesised that expression of a group 1 or 2 allele would lead to clonal deletion of T cells specific for the epitope on that allele. Donors who shared those group 1 or 2 alleles were therefore likely to be permissive. Expression of a group 3 allele was predicted not to induce negative selection of alloreactive T cells specific for the shared epitope.

Broadly speaking, where patients and donors shared alleles from the same groups, the mismatch is permissive. Where patient alleles are in different groups, the mismatch is non-permissive.

Table 7 - Permissive and non-permissive HLA-DP mismatches

		Patient					
		1 & 1	1 & 2	1 & 3	2 & 2	2 & 3	3 & 3
Donor	1 & 1	P	P	P	HvG	HvG	HvG
	1 & 2	P	P	P	HvG	HvG	HvG
	1 & 3	P	P	P	HvG	HvG	HvG
	2 & 2	GvH	GvH	GvH	P	P	HvG
	2 & 3	GvH	GvH	GvH	P	P	HvG
	3 & 3	GvH	GvH	GvH	GvH	GvH	P

These data demonstrate that T cell epitope matching defines permissive and non-permissive HLA-DPB1 mismatching in HSCT. Selection of unrelated donors with permissive mismatches increases the donor options for stem cell patients.

Recently Petersdorf et al have proposed an additional model of HLA-DPB1 permissiveness based on the level of HLA-DPB1 expression due to a SNP in the 3' Untranslated Region (UTR). In this model, a donor with a HLA-DPB1 type that is highly expressed has a higher aGvHD incidence and donors with low expression have lower incidence of aGvHD.

A third model is based on HLA-DPB1 types being classified into two evolutionarily defined groups.

Non-permissive HLA-DPB1 mismatches are associated with significantly higher risks of treatment-related mortality compared to permissive mismatches. However non-permissive mismatches in the graft versus host (GvH) direction also significantly decreased the risk of relapse compared to permissive mismatches because of the GvL effect.

NULL HLA ALLELES

HLA null alleles are alleles for which no HLA products are expressed at the cell surface. Over 1,200 null alleles have been described across HLA class I and II (Mar 2021). Examples include A*01:04:01:01N and A*01:11N. The nomenclature for null alleles includes the 'N' suffix symbolising null. Other alleles which are alternatively expressed have the suffixes 'L' for low expression of HLA product, 'S' for products which are secreted rather than being expressed on the cell surface, 'C' for product found only in the cytoplasm, 'A' for product with aberrant expression where there is some doubt about the cell surface expression and 'Q' for products with questionable expression, where the mutation in the allele has previously been shown to affect normal expression.

Mutations in allele sequences which lead to null alleles include insertions, deletions and point mutation many of which lead to the introduction of stop codons, though some lead to the development of incorrectly spliced products. Many of these mutations are located outside of exons 2 and 3 for class I and outside of exon 2 for class II. DNA based HLA typing techniques that do not inspect the gemone outside of these regions therefore run a risk of misidentifying null alleles. Misidentifying a null allele for its fully expressed counterpart poses a significant risk in stem cell transplantation.

If a stem cell donor null allele is misidentified as a fully expressed product and therefore transplanted into a patient bearing the expressed antigen, the patient's antigen will be allogeneic for the donor's T cells and can lead to severe acute GvHD. In the reverse scenario, if a patient null allele is misidentified as a fully expressed product and therefore transplanted with a donor bearing the expressed antigen, the donors' antigen will be allogeneic for the patients' T cells and can lead to destruction of the donor stem cells and graft failure.

Donor selection for stem cell transplantation does not typically take DRB3, 4 and 5 into account. Patients and donors matched at HLA-DRB1 and DQB1 are also typically matched at DRB3, 4 and 5. Where a patient is DRB4*02:01N (al DR53 allele) for instance, provided patient and donor are matched at HLA-DRB1 and DQB1, the donor is also very likely to also be DRB4*02:01N. However, this needs to be checked, especially if DQB1 matching is not take into account, as transplanting a DR53 Pos. donor into this patient carries a risk of graft rejection. DRB3, 4 and 5 may also need to be undertaken fi the patient has antibodies directed against DRB3, 4 and 5.

Various studies have shown that low expressed alleles are expressed in sufficient numbers to result in T cell tolerance which means that in a stem cell transplant, misidentification of low expressed alleles in either the donor or the patient is likely to be tolerated.

Compared to the impact in stem cell transplantation, misidentification of null alleles in solid organ transplantation potentially has a reduced clinical impact. If a patient null allele is misidentified as a fully expressed product and therefore transplanted with a donor bearing the expressed antigen, this results in a mismatch which does not have

a clinical consequence provided the patient does not have HLA antibodies directed against the mismatch. The patient does however have a risk of developing donor specific antibodies for that mismatch. A donor null allele misidentified as a fully expressed product and therefore transplanted into a patient bearing the expressed antigen results in no humoral rejection and is well tolerated.

In the case of low expressed alleles, misidentifying a low expressed allele for its fully expressed variant presents very little risk to in solid organ transplantation. Even overlooking a low expressed allele such as HLA-A*24:01:01:02L in a donor carries only a small risk of humoral rejection by preformed anti-A24 antibodies due to the low expression.

A PUTATIVE NEW ALLELE

Discovery of a putative new allele in a stem cell patient means that the patient is very unlikely to be able to obtain a 12/12 unrelated donor. The first step upon discovering a putative new allele is to repeat and confirm the sequence using a buccal cavity sample to rule our disease driven mutations.

For a patient with a putative new allele that has a matched family donor, transplant can proceed with the related donor following confirmation that the sequences of the patient and potential donor are the same.

Where a matched related donor is not available, the decision on how to proceed to transplant will depend on the disease and the preferences/experience of the transplant centre. For diseases where mismatched transplants are indicated, some centres may prefer a related haplo donor over a 11/12 unrelated donor. If using a 11/12 unrelated donor, potentially permissible mismatches i.e. mismatches where the difference is outside exon 2 and 3 for class I or exon I for class II are preferred. Antibody screening of the patient is required to make sure the patient does not have HLA antibodies directed against the donor mismatches.

In all cases the sequence of the putative new allele must be submitted to the nomenclature committee for confirmation and assignment of an allele name.

ROLE OF NON-HLA GENETIC FACTORS IN STEM CELL TRANSPLANT OUTCOME

KIR MATCHING IN HSCT

KIR mismatching in HSCT plays a role in reducing relapse, mortality and GvHD, particularly in the mismatched setting such as in 11/12 unrelated transplants and in haploidentical related transplants. KIR mismatching may also potentially play a role in cord blood transplantation where HLA mismatched transplantation is more common though more studies are required to demonstrate this.

Some studies have shown that KIR ligand compatibility or incompatibility influence the outcome of HSCT. The ligands for KIR receptors are HLA class I molecules. These include HLA-C locus antigens with either Asn (Group 1 HLA-C antigens) or Lys (Group 2 HLA-C antigens) at position 80, the HLA-Bw4 epitope and some HLA-A antigens

The absence of a HLA-ligand in the patient and the presence of the corresponding inhibitory KIR in the donor means that inhibition of the donor NK cell reactivity cannot take place leading to the potential generation of NK alloreactivity against the patient target cells, resulting in alloreactivity in the graft-vs. host (GvH) direction. This is also the main mechanism contributing to the beneficial GvL effect by reducing relapse rates. In addition, this also contributes to a decrease in aGvHD risk by destroying recipient antigen presenting cells (APCs) and consequently to an improvement in OS. Conversely, the presence of a HLA ligand for KIR in the patient and its absence in the donor will result in alloreactivity in the rejection (HvG) direction.

The role of mismatches of the KIR antigens themselves, as opposed to mismatches of the KIR ligands, has not been extensively studied.

The KIR ligand calculator at https://www.ebi.ac.uk/ipd/kir/ligand.html can be used to calculate the KIR ligand GvH vs HvG mismatch.

Predicted Ligands for Patient				
Typing	B*07:02	B*44:02	C*07:02	C*05:01
Alleles	Allele listing	Allele listing	Allele listing	Allele listing
Ligand	Bw6	Bw4 - 80T	C1	C2
Exceptions				
Predicted Ligands for Donor				
Typing	B*08:01	B*35:01	C*07:01	C*04:01
Alleles	Allele listing	Allele listing	Allele listing	Allele listing
Ligand	Bw6	Bw6	C1	C2
Exceptions				
Mismatching in the GvH Direction				
HLA-B	KIR ligands are matched			
HLA-C	KIR ligands are matched			
Mismatching in the HvG Direction				
HLA-B	KIR ligands are (mis)matched in the HvG Direction (Bw4)			
HLA-C	KIR ligands are matched			

In summary, these ligands will be matched in the GvH direction and (mis)matched in the HvG direction.

Figure 5 - KIR Ligand Calculator

RELEVANACE OF MICA AND MICB

One study has shown that in a series of well-matched HLA-A, B, C, DRB1, DQB1 stem cell transplants, a higher rate of grades II – IV acute GvHD was observed in patients mismatched for MICA compared to those matched for MICA. The MICA mismatched patients had more gastrointestinal aGvHD than the matched patients, possibly reflecting the tissue distribution of MIC antigens.

MINOR HISTOCOMPATIBILITY ANTIGENS

HLA presents the major genetic barrier to stem cell transplantation. However, evidence that other genetic systems are involved includes GvHD and some degree of rejection even when transplanting with HLA identical siblings. A non-HLA system which is thought to contribute to this is the minor histocompatibility antigen (MiHA) system. Minor histocompatibility antigens comprise of peptides derived from proteins in which some degree of polymorphism exists such that there may be differences between the patient and donor repertoires. These peptides can be presented to the immune system by both HLA class I and II antigens. Minor histocompatibility antigens are target for cytotoxic T lymphocytes (CTL's) and mismatching them can lead to GvHD and to rejection depending on the direction of the mismatch.

The best characterised minor antigens are the Y chromosome derived HY peptide and the autosomal HA1 to HA5 peptides. Minor histocompatibility antigens such as HA1 and HA2 have restricted tissue distribution and are present normally only on haematopoietic cells. Others such as HY are more ubiquitously distributed, expressed for instance on gut epithelium. HA1 and HA2 are expressed on leukemic cells and some tumour cells, making them potential targets for cellular therapy. In mice, allogeneic stem cell transplantation donor CD8+ T cells specific for a MiHA found in the recipient has been shown to inhibit the division of leukemic cells. However, there is a risk in developing GVHD if the T cells are specific for MiHAs expressed ubiquitously on epithelial cells. Immune cell restricted MiHAs such as MiHA HB-1, are ideal targets for graft-versus- leukemia (GVL) since not all nucleated cells would be targeted by responding T cells.

Minor HLA antigens are restricted by certain HLA types. HA1 and HA2 for instance are presented by HLA-A2 and HY is presented by multiple class I and II HLA antigens.

CYTOKINES

The other non HLA genes thought to play a role in haematopoietic progenitor cell donor selection and transplant outcome are Cytokine genes. The belief that a variable ability to produce pro-inflammatory and other cytokines as a result of polymorphisms in the promoter and regions of these cytokine genes has lead to

many studies attempting to understand the role of cytokine polymorphisms in HSCT. Unfortunately, these studies have not lead to a consensus view of the role of cytokine genes in transplant outcome and therefore in donor selection. There is some tentative evidence that transplantation with donors who express genes that make them high producers of TNFα and IL-1 leads to increased GvHD. Levels of cytokine production may also have an influence on susceptibility to infection post-transplant.

NON-GENETIC FACTORS WHICH INFLUENCE HSCT OUTCOME

Outcomes for Haemopoietic Stem Cell Transplantation (HCST) are improving with improvements in conditioning regimes and GvHD prophylaxis and treatment. Potential outcomes for HSCT include Overall Survival (OS), Leukemia Free Survival (LFS), Rejection, Relapse, Non-Engraftment, GvHD, Infection and Transplant Related Mortality (TRM). These outcomes result from multiple inputs including genetic and non-genetic factors all of which influence donor selection.

The non-genetic factors which can influence HSCT outcomes and therefore donor selection include patient, donor and transplant related factors.

Donor components include donor age, gender and virology status (including donor CMV status). Of these, studies have demonstrated that the most important is donor age. Transplants using donors aged 18-35 fair best, followed by those using donors aged 35 – 45. Transplants using donors older than 45 have the worst outcome. HSCTs that use male donors do best from a GvHD point of view compared to those that use female donors. HSCT using female donors into male patients is associated with higher GvHD rates. However, HSCTs involving male donors into female patients are associated with higher rejection rates. Donor CMV matching to patient CMV is important for avoiding CMV transmission or patient CMV reactivation.

Patient factors which influence HSCT outcomes include disease stage, patient age, gender and virology status (including CMV status). Of these, the single most important patient factor is disease stage. For malignant diseases, patients transplanted early have the best outcomes, followed by patients with intermediate stage disease. Patients transplanted with advanced disease have the worst outcome. The influence of patient gender on HSCT outcomes are as described for donor

factors. i.e. HSCTs using female donors into male patients are associated with higher GvHD rates whilst those involving male donors into female patients are associated with higher rejection rates. In terms of age, younger patients have a significantly better outcome than older patients. As with donor factors, donor CMV matching to patient CMV is important for avoiding CMV transmission or patient CMV reactivation.

Transplant factors which influence HSCT outcomes include conditioning regimes, GvHD prophylaxis and treatment as well as infection control regimes. The use of less toxic conditioning regimes, particularly reduced intensity regime (RIC) have allowed older patients in particular to benefit from HSCT and have improved overall patient survival.

SOURCES OF HAEMATOPOIETIC STEM CELLS

There are potentially three main sources of haematopoietic stem cells for transplantation – bone marrow (BM), peripheral blood stem cells (PBSC) and umbilical cord blood stem cells.

BONE MARROW

Bone marrow (BM) is traditionally harvested from multiple well, spaced sites on the iliac crests under general anaesthetic. Multiple aspirates are taken to obtain a transplantable size. The median number of nucleated cells collected is normally 2 x 10^8/kg patient weight. Collection usually aims for a minimum CD34+ count of 2 x 10^6/kg patient weight.

BM is sourced from related donors or adult volunteers on registries. Registry searches and confirmation of donor type and medical clearance take time and so there is a lead up time (search time of 3 – 6 months) required when using BM compared to using cord blood for example. Bone marrow does however have the advantage of being able to return to the donor for future donor lymphocyte infusions (DLIs). BM is collected under general anaesthetic and so although a safe procedure, carries the same risks as any process that requires general anaesthetic. BM stem cells collected have a lower nucleated cell count than peripheral blood stem cells but much larger nucleated and CD34+ stem cell count than cord blood

stem cells. Engraftment with bone marrow haematopoietic stem cells is medium to fast, taking a median of around 21 days to neutrophil and platelet engraftment. BM transplantation carries a risk of causing GvHD and requires that patient and donor be well matched for HLA class I and II. BM transplantation carries a risk of transmitting infectious disease or acquired or congenital disorders if not uncovered as part of the donor work up.

PERIPHERAL BLOOD STEM CELLS (PBSC)

Peripheral blood stem cells (PBSCs) are collected after being mobilised from the marrow with granulocyte colony stimulating factor (G-CSF). G-CSF is administered over several days prior to collection. The stem cells are collected by apheresis. The median number of nucleated cells collected is normally 9×10^8/kg patient weight. Collection usually aims for a minimum CD34+ count of 7×10^6/kg patient weight. One advantage of PBSC over BM is that the donor can return for further apheresis if the number of cells collected is not sufficient.

Like BM, PBSC's are also sourced from related donors or adult volunteers on registries and have the same issues of long lead up time for donor work up as BM. The donor is available for future collections for donor lymphocyte infusions. The collection process for PBSC is however easier and safer as it does not require general anaesthetic. G-CSF has not been shown to have any harmful short-term effects though the long-term effects are unknown. Total nucleated cells collected by PBSC are generally much higher than BM collections. Engraftment with PBSC is faster than with BM, taking a median of around 15 days to neutrophil and platelet engraftment. PBSC carries a risk of causing GvHD and requires that patient and donor be well matched for HLA class I and II. PBSC, like BM, also carries a risk of transmitting infectious disease or acquired or congenital disorders if not uncovered as part of the donor work up.

CORD BLOOD

CORD BLOOD OVERVIEW

Umbilical cord blood (CB) stem cells are collected post-partum either in utero in the delivery room during the third stage of labour before the placenta is delivered or outside the delivery room, ex utero from the freshly delivered placenta. In general,

the in-utero collection method yields a larger volume and higher total nucleated cell count, though more recent studies have shown that with appropriate training it is possible to obtain high collection volumes and cell counts ex utero. Cord blood can be sourced from cord blood banks either in single or double units.

CB stem cells have the advantage of being immediately available. Collection is easy and harmless. A disadvantage is that the donor is not available for further collection should a donor lymphocyte infusion be required to rescue the patient from a failing graft. Total nucleated (median 3×10^7/kg patient weight) and CD34+ cell counts (median 1.5×10^5/kg patient weight) are much lower than those obtained from BM or PBSC collection. For this reason, CB was initially used predominantly for children. Use of double dose CB has increased the usage in adults. CB has a lower risk of transmitting infectious diseases compared to BM and PBSC but the risk of transmitting congenital disorders is unknown. Engraftment with CB haematopoietic stem cells is slower than with either BM or PBSC, taking up to 35 days to neutrophil engraftment and platelet engraftment can take even longer. Transplantation with CB derived stem cells does lead to a reduced incidence and severity of GvHD due to the relative immaturity of the immune system at birth. This allows less stringent HLA matching criteria for CB transplantation with one or two HLA gene mismatches at high resolution at HLA-A, B, C and DRB1 tolerated. Cell dose may be the more important factor for CB transplantation.

CORD BLOOD BANKING

The first successful cord blood transplant was performed in a patient with Fanconi Anaemia by Gluckman et al in 1988 using cord blood stem cells from a HLA matched sibling. The patient is still alive and well today. This success prompted the setting up of the first unrelated cord blood bank in New York by Rubinstein et al in 1991. The number of unrelated cord blood banks around the world has steadily expanded since then and today (checked Jan 2020) there are over 730,000 CB units in public cord blood banks worldwide. Cord blood banking involves the collection, processing, testing, banking, registration, selection and release of cord blood unit under strict quality-controlled conditions for ultimate transplantation.

Organisations setting up CB banks do so either in or associated with hospitals with large maternity units, often aiming for units with more than 5,000 births a year. A typical strategy in countries with a predominantly Caucasian population is to work

with hospitals based in regions with a large percentage of Black, Asian and Minority Ethnic (BAME) mothers. This increases the genetic diversity of the CB bank over that typically seen in the adult stem cell donor registries. Collection of CB from mothers requires full informed consent. In European Union member countries, the European Union Tissues and Cells Directive applies. This states under the heading of Consent that 'The procurement of human tissues or cells shall be authorised only after all mandatory consent or authorisation requirements in force in the Member State concerned have been met'. Umbilical cord blood stem cells are collected post-partum either *in utero* in the delivery room during the third stage of labour before the placenta is delivered or outside the delivery room *ex utero* from the freshly delivered placenta. Collection *ex utero* does have a higher risk of introducing microbial contamination. In general, the *in utero* collection method yields a larger volume and higher total nucleated cell count, though more recent studies have shown that with appropriate training it is possible to obtain high collection volumes and cell counts *ex utero*.

Processing mainly involves volume reduction to optimise freezer storage facilities. Volume reduction involves removal of red blood cells and plasma, leaving the stem cell in a buffy coat of around 21ml. Semi-automated closed systems such as Sepax and other systems are available for processing of cord blood units. Volume reduced units have the additional advantage over whole units of requiring much less DMSO for freezing and can, depending on the size of the recipient, be transfused without having to first wash the unit.

The current practice is to perform a number of tests prior to banking with further tests carried out if and when a unit is reserved for a potential patient. Pre storage tests include full cell counts, especially nucleated red cell counts, TNC and CD34+ counts, HLA typing, ABO Rh testing and bacteriology testing of the cord blood unit. Haemoglobinopathy tests may also be undertaken. Also pre storage, microbiology markers including HIV, HCV, HBV, HTLV and CMV testing is undertaken on the mother. At reservation, the additional tests carried out are in part driven by the requirements of the transplant centre making the reservation and the county that Centre is based in. Additional tests typically include maternal HLA type, confirmatory HLA type of the cord blood unit and microbiological tests on the cord blood unit.

Banking of a cord blood unit typically follows a full medical review, a review of all processing data and all test results on the mother and the unit. Cleared units are control rate frozen and stored in liquid nitrogen. Units suitable for transplantation are registered with national and international stem cell or dedicated cord blood registries. In the UK, the NHS cord blood bank, which is part of NHSBT and the Anthony Nolan, collect and bank cord blood. The NHS cord blood units are also registered with NETCORD and with the World Marrow Donor Association (WMDA). NETCORD runs an accreditation program for cord blood banks which many banks around the world are either accredited to or are seeking accreditation to.

CB banking has many advantages, one of the principle ones being the ready availability of CB units for stem cell transplantation. Sourcing of adult stem cells from registries can take anything from 3 – 6 months for the search, selection of potential donors, contact with those donors to confirm willingness to donate, performance of confirmatory typing and any additional tests and medical clearance of the donor. CB stem cells have the advantage of being immediately available. Collection is easy and harmless to the donor compared to adult bone marrow or stem cell donation which involve either a general anaesthetic and the risks that poses or the use of GCSF and the unknown long term risks of that process.

The targeting of hospitals with a high percentage of BAME mother means that CB banks typically have a higher representation of donations from BAME donors and a higher proportion 'rare' HLA types compared to adult stem cell registries. The British Bone Marrow Registry (BBMR) for instance has around 5% of donors registered as being from minority ethnic backgrounds whilst 20% of the cord blood units registered for searches by the NHS cord blood bank are from minority ethnic donors. In terms of HLA type, the NHS CB bank has a much higher HLA-A/B/DRB1 allele frequency of the rarer HLA types compared to the BBMR.

CORD BLOOD TRANSPLANTATION

Transplantation with CB derived stem cells does lead to a reduced incidence and severity of GvHD due to the relative immaturity of the immune system at birth. This allows less stringent HLA matching criteria for cord blood transplantation with one or two HLA gene mismatches tolerated. Cell dose may be the more important factor for cord blood transplantation.

CB units have a lower risk of transmitting infectious diseases compared to BM and PBSC due to the lack of exposure of the donor.

Transplantation with Cord Blood derived stem cells has a much lower incidence and severity of GvHD compared to BM and PBSC due to the relative immaturity of the immune system at birth. This can be explained by the lower cell numbers and the mostly naive repertoire of cord blood T cells. Lower GvHD is one of the main advantages of cord blood banking as the reduced incidence and severity of GvHD allows for a less stringent HLA match thereby increasing the pool of potential donations for a given patient. The Graft versus Leukemia (GvL) effect does appear to be preserved.

The main disadvantage of CB transplantation is that CB units have a much lower Total Nucleated Cell (TNC) count than adult stem cells from either BM or PBSC. Total nucleated cell counts have a typical median of 0.3×10^8/kg patient weight and CD34+ cell counts have a typical median of 0.2×10^6/kg patient weight. This is tenfold lower than is typically collected from adult stem cells. PBSC also has the advantage of being able to collect from the donor over several days until an adequate volume is collected. This option is not available to for CB. This disadvantage can be overcome to some extent by the use of double CB transplants. Double CB transplantation involves the transplantation of two cord units together in series to a patient. Data to date shows no increase in GvHD as a result of using two cord units rather than one. Interestingly, sustained haematopoiesis after double cord blood transplantation is usually by a single cord unit though the criteria that make one unit prevail over the other remains to be clarified.

The other disadvantage of the using a cord unit is that the donor is not available for DLI should one be required to rescue the patient from a failing graft. In addition, there is a lack of follow up of the donor, so it is not known if the donor later develops a congenital disorder. The haemoglobinopathy tests obtained at banking do help in this regard.

Engraftment with CB haematopoietic stem cells is also slower than with either BM or PBSC, taking up to 35 days to neutrophil engraftment and platelet engraftment

can take even longer. During this time the patient has a delayed immune reconstitution with associated risk of infectious morbidity and mortality. CB grafts also have a higher incidence of graft failure compared to BM and PBSC.

A number of studies comparing CB and adult stem cell transplant in children with AML showed similar 5-year disease free survival. In adults transplanted for malignant diseases, the mortality due to delayed engraftment appears to be counterbalanced by a reduction in acute GvHD so that the overall incidence of transplant related mortality, treatment failure and overall mortality are similar in patients receiving cord blood and adult stem cells. Despite this, CB transplantation in adults is still generally performed as a last resort if adult stem cells were not available. CB transplantation is used more frequently in children.

When sourcing donors for children, transplant units generally ask for a cord blood unit search at the same time they are undertaking an adult stem cell donor search. The indications for CB transplant are the same as those for adult stem cell transplant provided cell counts are adequate, though results with bone marrow failure syndromes are less satisfactory.

Guidelines for selecting cord blood units for transplantation recommend the following:

- Select a CB unit high-resolution HLA matched 8/8 at HLA-A, B, C and DRB1. TNC dose should be > 3×10^7/kg at freezing or 2.0-2.5 x 10^7/kg after thawing
- If selecting a CB unit high-resolution HLA matched 5/8, 6/8 or 7/8 at HLA-A, B, C and DRB1, TNC dose should be > 5×10^7/kg. HLA antibody testing must be undertaken to avoid donor specific antibodies (DSAs)
- Use of CB units < 3×10^7/kg patient weight at freezing and/or 4 or more HLA mismatches at HLA-A, B, C and DRB1 is not recommended
- CD34 counts should be treated with caution due to variations in technique. Aim for 1.5×10^5/kg at freezing or 1×10^5/kg after thawing
- If several units with the same degree of HLA match are available, the one with the highest cell dose should be chosen
- Cell viability and microbiology results must be taken into account
- In the UK a graft advisory panel is available to help with selection of cord units for 'complicated' patients

Future challenges for CB transplantation are to develop strategies to reduce the time to engraftment. Use of double cords is helping in that regard. Other strategies under investigation include the co- transplantation of stem cells from a haploidentical sibling and the ex vivo expansion of CD34+ cells. Aggressive early and pre-emptive therapy has been suggested as a means of overcoming the infection rates prior to engraftment.

STEM CELL DONOR SELECTION STRATEGIES

OVERVIEW

The algorithm to follow for donor selection will need to be agreed in consultation between the laboratory and the transplant team and should follow BSHI and EBMT guidelines for the different transplant indications.

Where an allogeneic donor is indicated, historically the patient were HLA typed at medium resolution for HLA class I and II though high resolution HLA class II typing was undertaken for acute leukaemia's and other urgent transplants to speed up unrelated donor searching if required. Any sibling donors and parents if available, were typed to low resolution for HLA class I and II initially and any that are matched then typed to high resolution class II. The emergence of Next Generation Sequencing (NGS) however now means that many centres to straight to high/allele resolution HLA typing of the patient and relate donors at the start of the donor search process.

For acute leukaemia's and other urgent transplants, an initial WMDA indicative search may be undertaken ahead of completing the sibling typing. If no matched sibling is found a formal WMDA indicative search is undertaken or the transplant team may decide to proceed straight to a formal unrelated donor search through the national registries depending on clinical urgency. A high-resolution HLA class II type of the patient is required before proceeding to unrelated donor search if not already performed. The WMDA indicative search results are now of such high quality that often, transplant centres select their preferred donors directly from the results of this search rather than waiting for the registry search results.

Where a registry search is undertaken, the registry will initially carry out a national search of all donors. If only 6 or fewer potential unrelated donors are found on the national registries an international search and a cord blood search are automatically requested depending on the agreement between the transplant centre and the national registry.

In most instances, three unrelated donors will be selected from the registry or WMDA searches and samples requested through the registry. The number of donors from whom samples are requested may be increased depending on the assessed likelihood of any units being matched. Choice of donor will be influenced by the registry with some registries having a higher number of deleted donors than others. Registries also differ in the speed of sample provision and in cost of sample provision. The ethnicity of the donor may also provide a clue as to which registries to prefer.

Given a choice of equally HLA matched unrelated donors on the search reports, factors that would be taken into account and the general order in which they will be taken into account are:

- Gender, with male donors preferred over female donors
- Age of donor, with young donors below the age of 40 preferred
- CMV status, with CMV matched preferred over mismatched and
- ABO compatibility with major blood group mismatches avoided if possible

Historically, the requested donors are HLA typed to medium resolution HLA class I and II to start with followed by high resolution class I and II typing of any matched donors. However, the emergence of NGS has meant that the laboratory will often proceed straight to high/allele resolution HLA typing. This may however not be required if the donor is already NGS typed by an accredited registry.

Generally, a fully matched sibling or related donors would be selected over a fully matched adult donor over a cord blood unit. For disease groups where a haplo identical transplant is a clinical option, a haplo sibling or parent donor will be preferred over a 11/12 matched unrelated donor and certainly over an 10/12.

Where a 1 or 2 mismatched transplant is being undertaken, the preferred order of mismatches varies by transplant centre. One option is to prefer mismatches at HLA-C before HLA-A before HLA-B and avoid mismatches at HLA class II if possible. Where a mismatched transplant such as a haplo, a 11/12 or a cord blood unit is being used patient HLA antibody testing will be carried out and any DSA avoided if possible. The HLA antibody testing is repeated at the time of donor work up. Where it is not possible to avoid DSA, a flowcytometric crossmatch can help to stratify the risk. Antibody reduction regimes have been carried out successfully by experienced centres in a few select cases.

Where a CB is being selected, TNC is a more important factor and would be taken into account alongside CD34 count and number of HLA mismatches. The basic standard for selecting cord blood units is:

- Select a CB unit high/allele resolution HLA matched 8/8 at HLA-A, B, C and DRB1. TNC dose should be > 3×10^7/kg at freezing or 2.0-2.5 x 10^7/kg after thawing
- If selecting a CB unit high/allele resolution HLA matched 5/8, 6/8 or 7/8 at HLA-A, B, C and DRB1, TNC dose should be > 5×10^7/kg. HLA antibody testing must be undertaken to avoid DSA
- Use of CB units < 3×10^7/kg patient weight at freezing and/or 4 or more HLA mismatches at HLA-A, B, C and DRB1 is not recommended
- CD34 counts should be treated with caution due to variations in technique. Aim for 1.5×10^5/kg at freezing or 1×10^5/kg after thawing
- If several units with the same degree of HLA match are available, the one with the highest cell dose should be chosen
- Take into account cell viability and microbiology results

Patients and donors must have their HLA types confirmed before proceeding to transplant. The initial registry type can be considered the first HLA type for unrelated donors with the laboratory HLA type being the confirmatory type (CT). A copy of the confirmatory HLA typing report must be sent to the registry. If there are any discrepancies between the registry type and the CT these must be highlighted.

Wherever possible but especially for the acute leukaemia's, a backup donor should be identified in case the preferred donor was to fall through.

PATIENTS WITH RARE/UNUSUAL HLA TYPES

Patients with 'rare' HLA types can be a challenge to find unrelated donors for. Situations that can give rise to patients with rare HLA type include

- Patients from BAME community
- Presence of a 'rare' or low frequency HLA alleles e.g. HLA-DRB1*04:08
- Presence of a low to intermediate HLA type which have several alleles present at a high frequency e.g. HLA-B15 or HLA-DRB1*04
- Presence of a rare HLA-B/Cw association
- Presence of a rare HLA-DR/DQ association
- Homozygosity for some HLA types such as HLA-C and HLA-DR

If a matched related donor is not available, the unrelated donor selection strategy for such patients should take into account the probability of any low to intermediate resolution HLA typed donors being matched at high/allele resolution. The strategy will therefore take into account the most likely regsit5ry from which to call donors or if calling from a predominantly Caucasian registry, the ethnicity of the donors on that registry. There may be a need to call more donors than the three which laboratories typically call in the first instance to increase the probability of finding a match.

URGENT TRANSPLANTS

The most important factors to take into account when sourcing stem cell donors for a patient are the patient diagnosis and risk factors and so therefore the urgency with which transplantation is needed. Factors to take into account at the time of unrelated donor selection from a seach report include:

- Availability of HLA typing information at HLA-A, -B, -Cw, -DRB1, -DQB1, -DPB1. This is even more important if the patient has a rare or unusual HLA type, unusual associations or if the patient has a HLA type which has several alleles present at a high frequency
- If HLA-DQB1 or HLA-Cw typing is not present, the likelihood of a match based on linkage disequilibrium must be taken into account
- The ethnicity of the donors may provide a clue as the most likely high/allele resolution HLA types

- Presence of molecular HLA type as opposed to serological type can provide some information as to when the donor was typed and therefore how reliable the HLA type may potentially be
- Select donors who have the most likelihood of being a 10/10 or even 12/12 high/allele resolution matched. For intermediate resolution HLA typed donors, this will require looking at the donor HLA string to make sure the patient HLA allele is included in the string
- A choice of a serologically typed donor with a likelihood of being a 10/10 or 12/12 matched may be preferable to a molecularly typed donor who does not carry the patient allele in their HLA string
- Take any null or low expression alleles into account, looking at GvH and HvG directions
- Given a choice of two mismatches, select the allele mismatch over the antigen mismatch
- Given a choice of two allele level mismatches consider if mismatch is permissible:
    - C*03:03 and C*03:04 – the differences are outside the peptide binding groove or α-helices
    - DRB1*14:01 and *14:54 - the differences are outside of exon 2
    - DQB1*03:01 and *03:19 - the differences are outside of exon 2
    -
- Given a choice of equally HLA matched donors to select from:
    - Male donors are preferred over female donors
    - If choice of female donors only, prefer those with no or fewer pregnancies
    - Younger donors below the age of 40 or 45 are preferred over older donors
    - CMV matched donors are preferred over mismatched donors, even if the donor is female
    - Major blood group mismatches are avoided wherever possible
    - If ABO and/or CMV data is not provided on the search report it may be possible to request these from some registries
    - For CMV positive patients, it may be possible to request current/recent results on CMV negative donors last tested more than a year ago
    - Select permissible HLA-DP matched donors if possible
- If using a mismatched donor test patient for HLA antibodies and avoid DSA
- If the patient has ATLL, test donors for HTLV1
- If the patient is HIV infected, test the patient and donors for CCR5

- The reputation of the registry is also a factor to consider. Some registries, such as th NMDP, have a higher number of deleted donors than others. Registries also differ in the speed of sample provision and in cost of sample provision. The DATRI and Saudi Arabian registry for instance are now the quickest to get stem cells from
- Send a copy of the final report to the registry
- Highlight any discrepancies if present and report to the donor registry
- Post-transplant, test for engraftment by chimerism
- If a mismatched donor has been used, test for HLA antibodies post-transplant

PATIENTS WITH METABOLIC DISEASES

HSCT is indicated for a number of inborn errors of metabolism, including Hurler syndrome, Scheie syndrome (Mucopolysaccharidosis - MPS IS), Hurler-Scheie syndrome (Mucopolysaccharidosis - MPS IH/S), Hunters syndrome (Mucopolysaccharidosis - MPS II), Mannosidosis (classified as alpha and beta) and Leukodystrophies.

Stem cell transplantation from siblings or fully matched unrelated donors are the standard of care. Mismatched donors are a clinical option though as there is no benefit from a GvL effect, it is not widely used. Factors to take into account at the time of unrelated donor selection from a seach report are similar to those described above for urgent donor selection.

CORD BLOOD SELECTION

AT TIME OF SELECTION

The most important factors are cell count and HLA match. At the time of reviewing the search report:

- Look at total nucleated cell count per kilogram patient weight
- Look at CD34 cell count per kilogram patient weight
- Look to see if high resolution HLA typing results already present at HLA-A, -B, -C and -DR
- Select the largest units in terms of TNC count with the lowest number of HLA mismatches

- HLA matching should be based on high resolution typing at HLA-A, -B, -C and -DR
    - Select an HLA matched 8/8 unit. TNC dose should be > 3 x 10^7/kg at freezing or 2.0-2.5 x 10^7/kg after thawing
    - If selecting HLA matched unit 5/8, 6/8 or 7/8 then TNC dose should be > 5 x 10^7/kg
    - CD34 counts should be treated with caution due to variations in technique. Aim for 1.5 x 10^5/kg at freezing or 1 x 10^5/kg after thawing
    - Cord units <= 4/8 are not recommended
- HLA-A and HLA-B mismatches are preferable to HLA-DRB1 mismatches
- Avoid mismatches that the patient has formed HLA antibodies against
- If there are multiple units avoid major blood group mismatches
- If present on the search report, review the volume as a surrogate of freezing method/date
- Check that the cord bank has FACT/NETCORD accreditation
- Request a cord blood report for promising units

Before reserving a unit, request the following if not already known from the unit report:

- From the unit report, determine collection date, processing/volume reduction and cryopreservation method
- Confirm that cord and patient do not have the same date of birth. There is a risk that paediatric patients may have donated cord which would obviously be matched to them
- Is the cord unit red cell replete or has the unit been volume reduced? Prefer volume reduced over red cell replete units
- If the bank is FACT/NETCORD was the unit collected before or after accreditation. An accredited bank could still have 'unaccredited' units
- Get gender of cord unit if possible and prefer male donors over female
- Get ethnicity of donor if possible
- Was the TNC determined pre or post volume reduction?
- Review colony forming unit (CFU) viability data
- Does the cord bank have post thaw viability data for this unit? Calculate the TNC when viability is taken into account
- Check microbiology results including of mother and cord blood unit, especially CMV
- Check mother's medical history if available

- Take non-inherited maternal antigens (NIMAs) into account. Mismatched CB transplants with NIMA matches have been shown to have better outcomes, in terms of the time to blood count recovery and overall patient survival
- Any post donation medical history of the cord blood donor?
- Check availability of DNA sample
- Depending on the cord bank and availability of DNA, request local high-resolution at HLA-A, -B, -Cw, -DRB1, -DQB1 and -DPB1 typing of any promising units
- If choice of multiple units what is known about the speed of provision of units of the cord bank?
- What is the cost of units for both reservation and for provision?
- For transplants undertaken treat a genetic condition, check for the presence of the mutation/SNP in the cord unit

AT TIME OF RESERVATION

Upon reserving a cord blood unit, request the following if not already:

- Confirmatory typing plus any extended or high/allele resolution typing required
- Some cord banks will also conduct HLA typing on the maternal sample at this stage
- The lab will provide the patient high/allele resolution HLA typing information is to the cord blood bank
- The cord blood bank will send an extended cord report to the lab. If upon review of the additional information the transplant centre wishes to proceed, a cord blood reservation form is completed
- Cord units are reserved for several weeks, typically 6 weeks but this can be extended
- The transplant unit will receive confirmation of the reservation from the cord bank together with instructions on how to organise delivery and the thaw instructions
- Upon reservation, the cord blood bank will carry out standard infectious marker tests, including HIV, HCV, HTLV and CMV, on any stored maternal samples to create the compete ‘donor file’
- The transplant unit can request additional infectious markers be tested for at this stage
- The cord blood bank will send an extended cord blood report, the ‘donor file’, to the transplant centre for review

If proceeding to transplant with the cord blood unit, the following steps are taken:

- The transplant centre sends a 'Request to Ship' form to the cord blood bank detailing shipping and transplant dates
- At this stage, some cord blood banks conduct chimerism testing on a line segment attached to the bang, to confirm the identity of the unit just before shipping
- The transplant centre receives the cord unit and sends a line segment to the laboratory for bio-identity confirmatory HLA typing on a line segment
- The transplant centre completes the transplant
- If bio-identity confirmatory HLA typing has not been performed on a line segment, the used bag is sent to the laboratory for urgent HLA typing using residual cells from the bag to confirm identity and HLA type of the transplanted unit

AUTO HSCT

Auto HSCT is the standard of care for HSCT for a number of conditions, such as Hodgkin's Lymphoma and Multiple Myeloma. Patients undergoing Auto HSCT do not have the problem of rejection or GvHD but it is nevertheless important that the H&I laboratory undertake HLA typing before treatment starts as such patients may need an urgent HLA matched blood products as part of their treatment should they have or develop HLA antibodies. In some conditions, such as multiple myeloma with specific molecular cytogenetic abnormalities, consolidative allogeneic HSCT following initial auto HSCT has been shown to extend progression-free survival (PFS) as well as overall survival (OS). Auto followed by Allo HSCT is another reason why it is important that the H&I laboratory undertake HLA typing for patients undergoing Auto HSCT.

HLA typing of potential sibling donors may also be undertaken for diseases where allo transplantation is a clinical option even if auto HSCT is the standard of care.

TRANSFUSION SUPPORT IN HSCT

RELEVANCE OF HLA MATCHED PLATELETS

Use HLA matched platelets for patients awaiting HSCT is relevant to avoid further sensitisation if already sensitised or to avoid sensitisation if not. This is particularly important where a mismatched transplant such as a 11/12, a haplo or a cord blood unit is being used. Where a mismatched unit is being used, platelets should be selected to avoid mismatched antigens, reducing the risk of the patient becoming sensitised to this mismatch.

RELEVANCE OF HPA ANTIBODIES

HPA antibodies have been known to cause delayed engraftment, especially of platelets. If a choice of donors is presented it may be possible to HPA type the donors. Donors who do not express the antigen to which the patient is sensitised should be selected if possible.

ROLE OF REDUCED INTENSITY CONDITIONING (RIC)

Myeloablative or high dose conditioning regimens are defined as regimes where alkylating agents +/- total body irradiation (TBI) are used at doses which do not allow autologous hematologic recovery. Examples of Myeloablative regimens include:

- Busulfan (high-dose 16 mg/kg), Cyclophosphamide (120 mg/kg) and high dose TBI (>800cGy)
- Busulfan (high-dose 16 mg/kg) and high dose TBI (>800cGy)
- Cyclophosphamide (120 mg/kg)) and high dose TBI (>800cGy)
- Busulfan (high-dose 16 mg/kg) and Cyclophosphamide (120 mg/kg) +/- ATG
- Busulfan (high-dose 16 mg/kg) and Melphalan (140mg/m^2)

These does can be toxic in older patients and have historically limited the availability of HSCT in older patients until the emergence of Reduced Intensity Regimes (RICs).

Reduced Intensity conditioning (RIC) regimes fall in between myeloablative and non-myeloablative regimens. RIC regimens differ from non-myeloablative regimens in that they cause cytopenia which may be prolonged and they do require stem cell support. RIC regimens differ from myeloablative regimens in the dose of alkylating agents or TBI. These are typically reduced by at least 30%. The introduction of reduced intensity conditioning has expanded the recipient pool for HSCT, which has importantly made transplant an option for the more commonly affected older age

groups. RIC is used mainly in the care of older patients and those with medical comorbidities and for patients with immune deficiencies and bone marrow failure syndromes.

Most RIC regimens combine fludarabine with an alkylating agent at reduced doses. Examples of include:

- Fludarabine, Cyclophosphamide and Melphalan
- Fludarabine, Melphalan and Alemtuzumab
- Fludarabine and Melphalan
- Fludarabine and Busulfan (low-dose 16 mg/kg)
- Fludarabine and TBI

Non-myeloablative regimens are defined as those which cause minimal cytopenia and do not require stem cell support. Examples of non-myeloablative regimens include reduced doses of:

- Fludarabine, Cyclophosphamide and Anti-Thymocyte Globulin (FLU + CY + ATG)
- Fludarabine, Cytarabine also known as Cytosine Arabinoside (Ara-C) and Idarubicin (FLU + CY + Ida)
- Cladribine and Cytarabine also known as Cytosine Arabinoside (Ara-C) (Cladribine + Ara-C)
- Total lymphoid irradiation + ATG
- Low dose TBI (<2 Gy) +/- purine analog

Transplant related mortality (TRM) after myeloablative regimens increases with increasing age and especially in patients over 50. Non-myeloablative conditioning regimens have reduced toxicity, thus making transplantation available in the older patient population. Non-myeloablative regimens also require a large number of donor T lymphocytes and donor CD34+ cells to facilitate donor engraftment, which also contributes to the GvL effect.

ROLE OF HLA ANTIBODIES

Presence of DSA, including to HLA-DP, in stem cell transplant patients has been associated with lower levels of engraftment using bone marrow and peripheral blood stem cells (only 30-40%) and using cord blood (60-70%).

The cut off for positive reaction on Luminex varies between centres but typically, an MFI above 1,500-2,000 would be considered positive. A patient who has DSA against a potential donor mismatch but where the MFI is below the cut off would not be considered a contraindication to transplant though the transplant team would be informed of the presence of potential DSA and antibody monitoring will be carried out in the weeks leading up to transplant. If the patient is having platelet transfusions it would be advisable to use HLA matched platelets to avoid further sensitisation though this does have a cost implication.

Presence of DSA above the positive cut off would generally be considered a contraindication to standard transplant protocols. Data on desensitisation protocols with such transplants is limited though a few successful transplants have been performed with a variety of antibody reduction protocols such as IvIG and Rituximab.

Post-transplant engraftment monitoring is vital in all transplants which take place in the presence of DSA irrespective of MFI levels. Days to neutrophil and platelet count engraftment must be tracked closely and chimerism testing must be undertaken at least monthly. Post-transplant HLA antibody levels should also be monitored.

HSCT IN SICKLE CELL DISEASE

Sickle cell disease (SCD) is a recessive hereditary red blood cell disorder (homozygosity for the haemoglobin S gene – HbS confers the disease) that is characterized by early mortality and severe morbidity, including debilitating painful crises and organ involvement, particularly chronic kidney injury that can progress to renal failure and dialysis. Sickle cell is prevalent in regions of the world where malaria is endemic as heterozygosity for the HbS gene confers some protection from malaria.

Mortality has improved significantly with improved care and particularly since the introduction of hydroxyurea therapy. Hydroxyurea is an antimetabolite that helps prevent the formation of sickle-shaped red cells. However, HSCT remains the only curative treatment currently available for SCD. Not only is HSCT curative, it abates progressive organ dysfunction.

HSCT for sickle cell is usually reserved for children with major complications, such as stroke and who have a fully HLA-matched sibling donor. Older patients with significant co-morbidities are usually excluded from HSCT. In a small clinical trial (the SCURT trial) of matched unrelated donor (MUD) transplantation, children had a 2-year survival of 79%. One-year incidence of GvHD was high. In general, a MUD donor is only used if there is no HLA-matched sibling donor and there is recurrent severe pain episodes despite hydroxyurea therapy and there is overt stroke or any neurologic deficit lasting >24 h. It is recommended that MUD transplants take place in the context of a clinical trial.

In the UK, use of other alternative donors such as 11/12 MUD and cord require approval from a national panel. In addition, there have been a small number of haplo transplants that have recently been undertaken successfully.

HSCT AFTER KIDNEY TRANSPLANT

Kidney transplant patients have a higher risk of malignancy compared to the general public. The most common post-transplant malignancies are lymphoproliferative disorders (non-Hodgkin lymphomas of B cell origin) and solid tumors. AML following solid organ transplantation is less frequent. Post-transplant AML is likely a direct consequence of drug toxicity.

The treatment of the AML to achieve remission can potentially lead to failure of the kidney graft. Where renal function is still acceptable in the renal transplant patient, HSCT may be an option for patients with AML following consolidation with immunosuppression. However poor renal function is a relative contraindication to HSCT.

A key consideration is immunosuppression strategy. Calcineurin inhibitors and other induction agents known to be nephrotoxic should be avoided. A reduced dose of immunosuppression is generally used in such patients to help reduce nephrotoxicity. G-CSF may be administered to reduce infection risk. Kidney function should be closely monitored throughout the treatment of the AML and through HSCT if undertaken. DSA monitoring and biopsy may be indicated if there are signs of poor renal function. Some patients may require temporary renal replacement therapy while undergoing treatment.

HSCT donor selection should be based on standard criteria. If a fully matched related donor was used for the kidney that donor may be a good option for the HSCT.

SECOND HSCT

SECOND TRANSPLANT DUE TO RELAPSE

Generally, outcomes of second transplant in leukemia patients after relapse of a 1st Allo-HSCT transplant are poor with low rates of disease free survival (~14%). aGvHD is frequently involved. Outcomes in non-malignant diseases such as AA a slightly better at around 40%.

Where a second transplant is being considered, the return of full patient haematopoiesis must be confirmed by chimerism studies. High/allele resolution patient HLA typing post relapse should be undertaken to confirm patient HLA typing has reverted to their original type and is not that of the donor type.

The donor identification strategy will be determined by the urgency of the transplant and should always be discussed with the stem cell team. If there are any 12/12 sibling prepared to donate this should be considered. If the stem cell unit have experience of haplo identical transplants, then donation from a sibling or parent could be a good option. A re-search of the adult and cord blood registries should be undertaken to determine if there are any new donors that may be 12/12. If there are no new donors, it may be possible to re-use the previous donor, depending on the reasons for the relapse. For instance, if the relapse was due to low cell harvest, it may be possible to do a bigger collected for the 2nd transplant.

A key consideration for second transplant is the induction and immunosuppression strategy which may be different from the 1st transplant.

SECOND TRANSPLANT DUE TO DONOR DERIVED LEUKEMIA

Chimerism studies should be undertaken to confirm the patient retains 100% donor chimerism and therefore that the leukemia is indeed donor derived. In this scenario going back to the original donor for a second harvest is not an option. There is a duty of care to inform the registry who may want to contact the donor as the donor may currently be asymptomatic and may need counselling a treatment themselves.

Donor options available to the patient for a second transplant depend on clinical urgency and need to be discussed with the stem cell team. If a 12/12 sibling or other related donor is available then this is a potential option. In conditions where haplo identical transplants are a clinical option then this can be considered. A search of the international stem cell adult and cord blood registries should be initiated early in case there are no related donors.

POST-TRANSPLANT MONITORING

POST TRANSPLANT MONITORING WITH CHIMERISM

The role of the H&I laboratory is to provide clinicians with accurate information of the engraftment status post-transplant by quantitatively determining the proportion of donor and recipient derived cells in the patient post-transplant. Most H&I laboratories use Short Tandem Repeat (STR) testing for this. STR's are short sequences of DNA, distributed throughout the genome which are repeated in tandem a variable number of times. The number of repeats of different STR markers varies between individuals, from 4 to 50 repeats for some STRs, giving a highly polymorphic system that can be used to uniquely identify donor derived DNA from patient derived DNA. With the exception of monozygotic twins, careful selection of a number of STR markers will enable most patients derived DNA to be distinguished from donor derived DNA.

Post stem cell transplant chimerism results are typically reported as % donor chimerism. A 100% donor chimera implies complete engraftment. A 0% donor chimerism implies no donor engraftment with all other percentages reported as mixed chimerism showing the proportion of donor engraftment. A longitudinal study of donor engraftment is of more value than a single static result and the H&I laboratory would typically test at agreed intervals and report a history of the chimeric status of the patient since transplant rather than a single test report. The relative changes in magnitude of the donor chimerism provide key information which helps clinicians to intervene and to monitor the patients' response to such intervention. Intervention options include changes in immunosuppression regimes and donor lymphocyte infusion.

Longitudinal STR chimerism analysis is particularly useful in reduced intensity conditioning (RIC) regimes where initial mixed chimerism post-transplant is relatively common. The frequency of testing is agreed between the H&I laboratory and the transplant centre. For myeloablative regimens, this is typically weekly in the first month post-transplant followed by monthly testing. For RIC regimes this is typically monthly.

While STR analysis can be performed on whole blood, many H&I laboratories will offer lineage specific STR analysis, separating T and B cells from myeloid cells. This approach increases the sensitivity of the technique and has proved useful as in some cases of mixed chimerism, the initial myeloid mixed chimerism may dominate and mask clinically significant changes in other cell subsets.

The use of STR for chimerism analysis has also proved useful in the case of double cord transplants where it is possible to see a mixed chimera consisting of patient and one or both cords early in the post-transplant period before one cord eventually expands to 100% present in the patient.

Stable mixed chimerism post-transplant does not necessarily indicate a need to treat particularly in diseases such as Aplastic Anaemia (AA) and other non-malignant conditions especially where RIC regimes have been used. STR results showing increasing donor proportion is good whilst STR results showing increasing recipient proportion may indicate relapse or graft rejection and may indicate a need to treat. A reduction in donor chimerism post-transplant is potentially a sign of a failing graft

and needs to be monitored closely. Weekly, fortnightly or at the very least monthly cell lineage based chimerism testing may be indicated if the donor chimerism level continues to fall. If the donor chimerism has not fallen too far it may be possible to save the graft by reducing GvHD prophylaxis if no GvHD present and/or by reducing immunosuppression if no infection present.

For non-malignant disease it may be possible that function remains acceptable even if donor chimerism has fallen. For malignant disease an MRD test may be indicated. A return to patient chimerism doesn't necessarily mean return of the malignancy.

A DLI is not possible with a cord blood unit so a second transplant may be indicated. For matched sibling and MUD donor transplants a DLI would be indicated if donor chimerism continues to fall with second transplant with the same or a new donor as the next step though this may have some funding implications.

GVHD POST-TRANSPLANT

OVERVIEW

GvHD is the most frequent post allogeneic stem cell transplant complication and is a major cause of morbidity and mortality. GvHD is a consequence of activation of donor T lymphocytes by recipient antigen presenting cells. It requires three conditions, transplantation of immunocompetent donor cells, histo-incompatibility between recipient and donor and immunocompromised recipient such that the recipient cannot mount an adequate immune response against the donor cells. A number of randomised controlled trials have compared the incidence of GvHD in bone marrow and peripheral blood stem cell transplants in the related and unrelated setting. In allogeneic sibling transplantation, the incidence of acute GvHD was the same in the majority of studies. There was however an increased incidence of chronic GvHD with PBSC compared to BM. In the unrelated setting, matched cohort comparison of BM and PBSC transplants report no difference in the incidence of acute and chronic GvHD. These were not however randomised controlled trials.

GvHD develops in a three-phase process. In Phase 1 the effects of the conditioning regimen leads to tissue damage and the generation of pro inflammatory cytokines including TNFα and IL-1. In Phase 2, donor T lymphocytes are activated by host

antigen presenting cells. The activated T cells produce cytokines including IL-2, leading to T cell expansion. Phase 3 is a cytokine storm in which a positive feedback loop of cytokine production leads to activation of effector cells such as CTLs and NK cells, which produce more pro inflammatory cytokines such as TNFα and IL-1.

GvHD has traditionally been classified into acute and chronic based mainly on the time to onset. GvHD arising before day 100 post-transplant was classified as acute and onset after day 100 classified as chronic. The current definition sets no time limits but is rather based on the presence of specific symptoms.

The organs primarily affected by GvHD are the skin, the gut and the liver. Skin GvHD manifests as a rash often affecting the palms and soles first, before spreading to the entire body surface. Gut involvement manifests as nausea and a watery diarrhoea, which in advanced disease can be bloody. Liver involvement can manifest as jaundice and is usually measured by the level of Bilirubin production.

Acute GvHD is traditionally classified in grades I to IV depending on the level of organ involvement. A new international classification of grades A to D is also in use. The traditional I – IV classification however continues to be the one most widely used. In brief, it classifies GvHD with only mild skin involvement as Grade I. Mild to moderate skin involvement with mild gut and/or liver involvement is classified as Grade II. Moderate to severe skin involvement with mild to moderate gut and/or liver involvement is classified as Grade III and Moderate to very severe skin involvement with moderate to severe gut and/or liver involvement is classified as Grade IV.

The two main mechanisms used to prevent GvHD are pre transplant graft T cell depletion of the stem cells and post-transplant immunosuppression of the patients. T cell depletion may be undertaken with for instance the use of alemtuzumab or Anti-Thymocyte Globulin (ATG). Graft T cell depletion is not very popular in the UK where patient immunosuppression appears to be the preferred method of GvHD prophylaxis. A common approach is to use a calcineurin inhibitor such as Cyclosporin-A (CsA) or Tacrolimus, in combination with short course Methotrexate (MTX). Another option is Sirolimus in combination with Tacrolimus and short course MTX. In reduced intensity transplants (RIC) Mycophenolate mofetil (MMF) has been used in combination with CsA.

Initial GvHD therapy post-transplant consists of a dose of Methyl-prednisolone. Patients refractory to this primary treatment have a poorer prognosis. Secondary treatment is with higher doses of Methylprednisolone or other immunosuppressive drugs not already used as induction therapy, including Tacrolimus, MMF or Sirolimus, ATG, monoclonal antibodies.

ROLE OF THE H&I LABORATORY IN GVHD CASES

The Laboratory should undertake chimerism studies to determine level of engraftment and help stem cell unit assess if immunosuppression can be safely altered to increase GvHD treatment

- If there is skin involvement in the GvHD, a biopsy sample from the affected and a non-affected area of the skin should be undertaken to determine if there are donor cell in the affected area
- A number of biomarkers have been shown to be associated with the development of GvHD and it may be possible to test for these early post-transplant before clinical symptoms of GvHD are manifest. These include:
    - Suppression of tumorigenicity 2 (ST2)
    - Elafin
    - Regenerating islet-derived 3α (REG3α) is
    - The cytokine CX3CL1, levels of which have been shown to be predictive of the development of GvHD in day 0-50 post-transplant

CHAPTER 6 KIDNEY AND PANCREAS TRANSPLANTATION

HISTORY OF CLINICAL SOLID ORGAN TRANSPLANTATION

1902

Karl Landsteiner classifies blood into three groups A, B and O. Group AB later added. This has been described as the beginning of modern Immunogenetics. Blood transfusion can legitimately be considered a type of transplantation

1905

First Successful Cornea Transplant was performed by Austrian surgeon Dr. Eduard Zirm

1908

French surgeon Dr. Alexis Carrel develops surgical techniques for sewing arteries and veins which are used in organ transplantation and other surgical procedures today

1916

Little and Tyzzer in analysing tumour transplants between mice demonstrated that several dominant genes influenced the outcome of allogenic tumour grafts. They were able to show that tumours transplanted from one strain of mice to mice of the same strain were accepted, whereas, tumours transplanted to a different strain were rejected. This was the first in a series of experiments by several researchers over many years that lead to the discovery of the Major Histocompatibility Complex (MHC) and its role in transplantation. Little, C. C., Tyzzer, E.E. (1916). Further experimental studies on the inheritance of susceptibility to a transplantable tumour, carcinoma (JWA) of the Japanese waltzing mouse. J. Med. Res. 33, 393-453

1937

Peter Gorer, working at the Jackson Laboratory discovered an antigen in mice which he named Antigen II. This was later discovered to be the same antigen that George Snell had named Fu and which he had demonstrated played a role in transplant rejection. In collaboration they discovered the genes encoding this antigen and named it H2 (H for histocompatibility and 2 for antigen II). This is the first early picture of what later came to be called the Major Histocompatibility Complex (MHC). The first published use of the term MHC was not until the 1970's

1940'S

British zoologist Peter Medawar used experimental skin transplants on animals to explain why burn victims from the bombing of civilians in England during World War II reject donated skin. This work enabled him to establish theories of transplantation immunity. Peter Medawar was awarded the Nobel Prize in Physiology or Medicine in 1960 for the 'discovery of acquired immunological tolerance'

1948

George Snell further characterised the MHC system. He carried out a series of mouse breeding experiments which showed that transplantability was determined by the presence of special antigens on the surface of the cell. He called these histocompatibility antigens. He also showed that these antigens were coded for by genes found within a limited area on chromosome 6. This area was called the major histocompatibility complex (MHC)

1953

James Watson and Francis Crick, drawing on their own work as well as the unpublished work of others including Maurice Wilkins and Rosalind Franklin, publish 'The Molecular structure of Nucleic Acids: A Structure for Deoxyribose Nucleic Acid' in the scientific journal Nature. vol 171 pp 737-738. This was the first published article which described the double helix structure of DNA. Watson, Crick and Wilkins were awarded the Nobel Prize in Physiology or Medicine in 1962 'for their discoveries concerning the molecular structure of nucleic acids and its significance for information transfer in living material'

1954

The first successful living-related donor kidney Transplant was performed. A kidney transplant between 23-year-old identical twins, one of who was dying from advanced glomerulonephritis was performed by Dr. Joseph Murray and Dr. David Hume, Brigham Hospital, Boston. Joseph Murray was awarded the Nobel price in 1990

1958

Jean Dausset describes the first Human leukocyte antigen that he named MAC using leukoagglutination techniques he had earlier described in 1952. He went on to propose a complex system which he designated HU-1 but which was later renamed HLA for 'Human Leukocyte Antigen'. Jean Dausset was awarded the Nobel Prize in Physiology or Medicine in 1980, together with George Snell and Baruj Benacerraf, for 'their discoveries concerning genetically determined structures on the cell surface that regulate immunological reactions'

1962

First successful deceased donor kidney transplant was performed by Dr. Joseph Murray and Dr. David Hume, Brigham Hospital, Boston. The patient received the new immunosuppressive drug azathioprine and lived for 21 months

1963

First successful lung transplant was performed by Dr. James Hardy at the University of Mississippi Medical Centre. That same year Jon van Rood discovered the Bw4 and Bw6 series of HLA antigens

1964

Julia and Walter Bodmer, with Rose Payne discovered the LA series of HLA antigens. The LA nomenclature was later to provide the last two letters of what became

known as the HLA system. Bernard Amos organised the first International Histocompatibility Workshop

1966

The first successful pancreas transplant from a deceased donor takes place. The recipient, who had uncontrolled diabetes and kidney failure, was a patient at the University of Minnesota Medical Centre

1967

First successful liver transplant was performed by Dr. Thomas Starzl, University of Colorado, Denver, CO

1967

First Successful Heart Transplant was performed by Dr. Christian Barnard, Groote Schuur Hospital, South Africa. The patient, 54-year old Louis Washkansky, received a heart from a 23-year-old woman who died in a car accident. The heart functioned until the patient died of pneumonia eighteen days later because of his suppressed immune system

1968

The WHO nomenclature committee for factors of the HLA system is set up by Bernard Amos. Also this year, Harvard University published the first definition of brain death

1976

The immunosuppressive capabilities of cyclosporine was discovered this year by J. F. Borel and colleagues

1981

First successful combined heart and lung Transplant was performed by Dr. Norman Shumway, Stanford University Medical Centre, Palo Alto, CA

1987/88

First successful intestine transplant performed in Germany by Dr E. Deltz. This was rapidly followed by successful intestine transplants in France and Canada.

1995

The first laparoscopic live donor nephrectomy was performed by Ratner et. al.

Ratner LE, Ciseck LJ, Moore RG, Cigarroa FG, Kaufman HS, Kavoussi LR. Laparoscopic live donor nephrectomy. Transplantation. 1995; 60: 1047–9.

1998

First short term successful hand transplant performed in France by a team assembled from different countries around the world and led by French Professor Jean-Michel Dubernard

1998

First reported case of the use of plasmapheresis for successfully transplantations across the blood group barrier.

Tanabe K, Takahashi K, Sonda K, et al. Long-term results of ABO-incompatible living kidney transplantation: a single-center experience. Transplantation 1998;65:224-8.

2003

First reported case of the use of Rituximab for ABO incompatible (ABOi) transplants.

Histocompatibility & Immunogenetics

Tyden G, Kumlien G, Fehrman I. Successful ABO-incompatible kidney transplantations without splenectomy using antigen-specific immunoadsorption and rituximab. Transplantation 2003;76:730-1.

2005

First successful partial face transplant carried out in France by Bernard Devauchelle, Benoit Lengelé and Jean-Michel Dubernard.

CAUSES OF KIDNEY FAILURE WHICH MAY LEAD TO KIDNEY TRANSPLANTATION

A number of kidney diseases can lead to end stage kidney failure requiring renal replacement therapy in the form of dialysis or kidney transplantation. The underlying immunology and treatment options of some of these kidney diseases can introduce confounding factors into laboratory tests.

ACUTE GLOMERULONEPHRITIS

Acute Glomerulonephritis (Acute GN) is a renal disease which results from an immunologic trigger of inflammation and proliferation of glomerular tissue, resulting in damage to the basement membrane, mesangium or capillary endothelium.

The pathophysiology involves glomerular lesions resulting from deposition or in situ formation of immune complexes. The kidneys may be enlarged by up to 50%.

The causal factors can be broadly divided into infectious and non-infectious groups (such as primary renal disease and conditions or agents).

ACUTE KIDNEY INJURY (AKI)

Acute Kidney Injury (AKI) is a sudden and rapid decline in renal function which clinically manifests as a reversible acute increase in blood urea nitrogen and serum creatinine levels over the course of hours to weeks.

The pathophysiology involves reduction in renal blood flow resulting in decreased glomerular filtration rate (GFR) - Normal GFR = 90 to 120 mL/min/1.73m2. Suitable transplant candidates are added to the waiting list if GFR <= 15

There are 3 main causes of AKI:

1. Prerenal failure caused by compromised renal perfusion
2. Intrinsic renal failure caused by diseases of the kidney itself, mainly the glomerulus and/or tubules
3. Obstruction of the urinary tract

ACUTE TUBULAR NECROSIS (ATN)

Acute Tubular Necrosis (ATN) is Acute Kidney Injury (AKI) in which the pathology lies within the kidney itself with tubule cell damage and cell death usually resulting from an acute ischemic (lack of oxygen) or toxic event.

The pathophysiology involves tubule cell damage and cell death.

ATN is caused for the most part by an acute event, either ischemic or toxic. Ischemic injury can be caused by heart failure, sepsis, anaphylaxis etc. Toxins that can cause ATN include cyclosporine and tacrolimus.

ALPORT'S DISEASE

Alport's Disease is a group of inherited, heterogeneous disorders involving the basement membranes of the kidney and frequently affecting the cochlea and eye as well. Alport's Disease is characterised by Haematuria (blood in the urine), Proteinuria (protein in the urine) and Hypertension.

The pathophysiology involves aberration of basement membrane.

Alport's Disease is caused by defects in the genes encoding alpha-3, alpha-4, or alpha-5 chains of type IV collagen of the basement membranes.

ANTIGLOMERULAR BASEMENT MEMBRANE DISEASE (ANTI-GBM)

Antiglomerular Basement Membrane Disease (anti-GBM) is an autoimmune disorder characterized by the presence of circulating pathogenic autoantibodies directed against proteins in the glomerular basement membranes which can lead to rapidly progressive glomerulonephritis.

The pathophysiology involves anti-GBM antibodies binding to an epitope of type IV collagen in glomerular and alveolar basement membranes. Complement is activated, proinflammatory cells and CD4+ and CD8+ cells are recruited to the site and subsequently, proinflammatory cytokines, chemokines and proteolytic enzymes are released. This results in endothelial damage with endothelial cell detachment from the underlying GBM and fibrin accumulation under the disrupted endothelial cells.

An environmental factor such as smoking or hydrocarbon exposure is presumed to be required to unmask the cryptic collagen antigen in the basement membrane.

In the laboratory, patients with anti-GBM tend to be auto crossmatch positive which can potentially lead to wrong interpretation of the crossmatch in the absence of an auto tests. However, treatment with Cyclophosphamide can cause a loss of B cells which makes B cell auto crossmatching impossible.

DIABETIC NEPHROPATHY

Diabetic nephropathy is a serious kidney related complication of type 1 and 2 diabetes characterized by persistent albuminuria, progressive decline in the glomerular filtration rate (GFR) and elevated arterial blood pressure.

The pathophysiology involves three major histological changes in the glomeruli:

1. Mesangial expansion directly induced by hyperglycaemia
2. Thickening of the glomerular basement membrane (GBM) occurs
3. Glomerular sclerosis caused by intraglomerular hypertension

The exact aetiology of diabetic nephropathy is unknown. Proposed mechanisms include hyperglycaemia causing hyperfiltration and renal injury, advanced glycation products and activation of cytokines.

FOCAL SEGMENTAL GLOMERULOSCLEROSIS (FSGS)

Focal segmental glomerulosclerosis (FSGS) is characterised by a segmental solidification of the glomerular tuft and segmental obliteration of glomerular capillaries (scarring or hardening of the glomeruli) which causes asymptomatic proteinuria or nephrotic syndrome with or without renal insufficiency.

The pathophysiology primarily involves injury inherent within or directed to podocytes. Involves podocyte fat process effacement, proliferation of mesangial, endothelial and epithelial cells in the early stages, followed by shrinkage or collapse of glomerular capillaries, all lead to scarring or glomerulosclerosis.

Proposed aetiology includes viral- or toxin-mediated damage or intrarenal hemodynamic changes such as glomerular hyperperfusion and high intraglomerular capillary pressure.

Treatment with Cyclophosphamide can cause a loss of B cells which makes B cell auto crossmatching impossible.

GOODPASTURE SYNDROME (GS)

Goodpasture Syndrome (GS) is a renal disease in which anti-GBM antibodies cause glomerulonephritis and also act against the alveolar basement membrane leading therefore in addition, to pulmonary haemorrhage.

The pathophysiology involves autoantibodies (anti-GBM antibodies) directed against the glomerular and alveolar basement membranes. These autoantibodies bind and activate the complement cascade, resulting in tissue injury.

The aetiology is likely due to environmental insult such as smoking, exposure to solvents or hydrocarbons, infections, in a person with genetic susceptibility.

Treatment with Rituximab will cause the B cell crossmatch to be positive or will cause sufficient loss of B cells to make B cell auto crossmatching impossible. Treatment with Cyclophosphamide can cause a loss of B cells which makes B cell auto crossmatching impossible.

HAEMOLYTIC UREMIC SYNDROME (HUS)

Haemolytic Uremic Syndrome (HUS) is a disease characterized by haemolytic anaemia (anaemia caused by destruction of red blood cells), acute kidney failure (uraemia) and a low platelet count (thrombocytopenia). It predominantly, but not exclusively, affects children.

HYPEROXALURIA

Hyperoxaluria is excessive urinary excretion of oxalate (kidney stones).

IGA NEPHROPATHY

IgA Nephropathy (also known as Berger's disease) is characterized by IgA deposition in the glomerular mesangium causing glomerulonephritis, a condition in which there is inflammation and proliferation of glomerular tissue, resulting in damage to the basement membrane, mesangium or capillary endothelium.

The pathophysiology comprises the formation of immune complexes which are deposited in renal mesangium and the production of autoantibodies. These induce glomerular injury through the release of pro-inflammatory cytokines and through chemokine secretion, leading to the migration of macrophages into the kidney.

In the laboratory, patients with IgA Nephropathy tend to be flow crossmatch positive due to auto antibodies. It is advisable to always set up an auto crossmatch test to exclude auto antibodies.

MEMBRANOUS GLOMERULONEPHRITIS

Membranous Glomerulonephritis (MGN) is a kidney disease triggered by an immune response, leading to immune complexes being deposited in the subepithelial space.

POLYCYSTIC KIDNEY DISEASE

Polycystic Kidney Disease is an inherited disorder multisystemic and progressive disorder characterized by cyst formation and enlargement in the kidney and other organs (e.g. liver, pancreas, spleen).

Polycystic Kidney Disease has a genetic basis with two main type. An autosomal dominant type caused by mutations in either the *PKD1* (polycystin 1) or *PKD2* (polycystin 2) gene and an autosomal recessive type caused by mutations in in the *PKHD1* (fibrocystin) gene.

RENAL ARTERY STENOSIS

Renal Artery Stenosis (RAS) also known as Renal Artery Emboli is a narrowing of one of the renal arteries, most often caused by atherosclerosis (build-up of plaque aka atheroma, inside the arteries) or fibromuscular dysplasia (abnormal growth within the wall of an artery).

SICKLE CELL NEPHROPATHY

Sickle cell nephropathy is a type of nephropathy (kidney disease or damage) associated with sickle cell disease which causes kidney complications as a result of sickling of red blood cells in the small blood vessels of the kidney.

ROLE OF THE RENAL MULTIDISCIPLINARY TEAMS (MDT'S)

Many key decisions about listing patients to transplant are made today by a multidisciplinary team (MDT). The clinical team will have standards that define the members required to make an MDT quorate. For a renal MDT this typically means at least one nephrologist, one surgeon, one H&I scientist, one donor coordinator nurse and one patient coordinator nurse. Other potential members include a psychiatrist and a patient advocate. The MDT would also typically have access to a cardiologist, though they may not necessarily attend all meetings.

Two key responsibilities of the renal MDT are to review all potential renal patients for suitability for addition to the deceased donor transplant waiting list and to review all live donor transplants. In considering patients for the deceased donor waiting list, the MDT will typically review the patient's current kidney status, including creatinine level and glomerular filtration rates (GFR), HLA type, HLA antibody status and matchability, cardiac condition, diabetes and any other relevant clinical history and potential immunosuppression strategy.

In considering patients and donors for live transplant, the MDT will review H&I laboratory results including any crossmatch results, as well as patient and donor clinical history. The MDT would consider whether or not a direct transplant is possible/desirable or whether the pair should be entered into the exchange scheme. The MDT may also review some patients post-transplant, though this may be taken up at a separate meeting such as a biopsy meeting.

ORGANISATION OF TRANSPLANTATION AND ORGAN SHARING SCHEMES IN THE UK

DEEMED CONSENT TO ORGAN DONATION IN THE UK

Deemed or presumed consent has been in place in Wales for some time and has in 2020 become law in England as well. Scotland too are passing a similar law though not Northern Ireland.

Deemed consent means that unless a donor chooses to opt out or they are in one of the excluded groups, they are considered a possible organ donor if they die in the right conditions for organ donation. Excluded groups include:

- People under 18 in England and under 16 in Scotland
- People who have been ordinarily resident in England for less than 12 months
- People who lack the capacity to understand the change

Deemed consent covers all organ transplants which took place under the old system, including heart, lung, kidney, liver, pancreas and bowel but nor Vascularised Composite Allografts (VCAs) i.e. limbs, face etc.

It is still possible to proactively opt into the organ donor register. All donors currently on the register will remain on unless they amend their registration. ODT have started a new opt out register where all UK residents can actively opt out of being a donor. All donors not on the opt in or opt out registers will be deemed to have given presumed consent. Their next of kin will still be asked and consented for the donation. Family support helps ensure important information about the donors medical, travel and social history is available.

In Wales, after an initial slow start, the consent to donate from families went up by about 15%, resulting in more donations. A similar increase in England and Scotland could have a significant impact on the waiting list if it is accompanied by an increase in transplantation. All parts of the transplant pathway will therefore have to be adequately funded from laboratory services to theatre time. An increase of around 500 transplants a year in England has been forecasted by ODT. This will have significant impact on H&I laboratory service provision both in terms of staff and non-pay resources that will need to be funded. There will be an increase in demand for out of hours testing which puts further strain on staff.

One additional potential advantage of the deemed consent approach was thought to be an increase in the number of donors from Black and Minority Ethnic groups (BAME) donors who are currently underrepresented on the donor register. Patients from these communities currently tend to wait the longest for organs. However, in the initial few months of existence of the opt out register, the largest proportion of UK residents who have registered to opt out are, unfortunately, from BAME communities.

A potential disadvantage to the deemed consent approach is that it may put off some donors who are happy to donate under a system that is manifestly voluntary but who might take offence at being presumed to have consented.

KIDNEYS

TYPES OF KIDNEY DONORS

Kidney donors can be live related, live unrelated or deceased. Live related donors are genetically related to the patient. Live unrelated donors can be any donor that knows the patient, such as spouses and friends or those who do not know the patient i.e., they are altruistic donors. An altruistic donor is someone who chooses to donate anonymously as a non-directed donor to any patient on the transplant waiting list. Deceased donor are further categorised into those who make Donation after Brain Death (DBD) and those who make Donation after Cardiac Death (DCD).

DBD is defined as organ donation from a donor who has suffered severe brain injury from trauma, cerebral vascular accident, anoxic event or other event such that there is no neurological reflex still present. DBD donors meet the legal definition of brain death and the time at which this is determined is the legal definition of the time of death. A DBD donor remains on ventilation until after organs are recovered for transplantation. The majority of organ including heart, lungs, liver, pancreas, kidneys and small bowel can be transplanted from a DBD donor.

DCD is defined as organ donation from a donor who has suffered devastating and irreversible brain injury and may be near death but does not meet formal brain death criteria as some neurological reflex is still present. However, the doctors have determined that determined that the patient has no chance of recovery and cannot

survive without mechanical ventilator. Once the family have made a decision to withdraw treatment, discussion about possible organ donation can begin. Once treatment is withdrawn, cardiac death is declared when there is no blood pressure, pulse, cardiac sounds or respiration. If the patient does not expire within 2 hours of treatment withdrawal, organ donation is no longer possible. DCD donors are typically only used for lungs, liver, pancreas and kidneys though a small number of heart transplants from DCD donors are now being done successfully in the UK.

DECEASED DONOR WAITING LIST

From a clinical point of view, all patients with end stage renal failure are potential transplant candidates provided the meet specific medical fitness criteria and informed consent about the mortality and morbidity associated with transplantation compared to dialysis has been obtained. There are however a number of absolute contraindications:

- Uncontrolled cancer
- Systemic infection
- Life expectancy < 2years

And a number of relative contraindications:

- Life expectancy < 5 years
- >50% Probability of graft loss in less than 1 year
- Predicted non-compliance with immunosuppression regime
- Predicted complications of immunosuppression

Laboratory tests and other data that would be required prior to listing include:

- ABO grouping and confirmation
- HLA typing and confirmation at all loci specified by ODT kidney advisory group to the minimum resolution specified
- HLA antibody screening on at least two samples taken not less than 24 hours apart
- List of unacceptable HLA mismatches together with reasons why
- Minimum HLA matching requirements
- Patient demographics, including DOB
- Potential age-related co-morbidities
- Patient height and weight

- Patient dialysis status
- Patient NHS status
- Patient cardiovascular risk
- Patient virology, including HIV, EBV, CMV Hepatitis B and C
- Other co-morbidities such as BMI and smoking history
- Organs required e.g. kidneys along, SPK, SIK etc.

KIDNEY ALLOCATION

In the UK, a new kidney allocation scheme was introduced in 2019. Extensive consultations took place over the last few years following which the scheme was updated to reflect the increased use of DCD donors and generally older, more challenging kidneys. The new scheme has been designed to better match patient and graft life expectancy, to give more priority to difficult to match patients and when matching is important, to consider all loci (HLA-A, B, Cw, DR, DQ). Key objectives of the new scheme include:

- To unify DBD and DCD schemes
- To match the 'quality' of the patient to the 'quality' of the donor
- To better tailor HLA match to age
- To attain geographical equity of access
- To reduce cold ischaemia times
- To reduce long waiting from dialysis on time on waiting list, whichever is earliest

The new involves a reduction in the number of matching Tiers from 5 to 2. Instead of Tiers A-E as in the old scheme, there will only be Tiers A and B.

- Tier A - Patients with matchability score=10 or 100% cRF or ≥7 years waiting time. Patients in this tier are prioritised solely based on waiting time
- Tier B - All other patients. Patients in this tier are prioritised by points based on donor-recipient risk index combinations, waiting time from earliest of dialysis or activation on the list, HLA match and age combined, location, matchability, total mismatch and blood group match

Of the point scoring items in Tier B, the new additions are the donor-recipient risk index combinations and the use of matchability. For the risk index, patients are given a risk score from R1 (low risk) to R4 (high risk) based on four factors:

- Patient age
- Whether or not they are on dialysis
- Waiting time from dialysis and
- Whether or not they are diabetic

Donors are also given a risk score from D1 (low risk) to D4 (high risk) based on seven factors:

- Donor age
- Donor height
- History of hypertension
- Female donor
- CMV Pos. donor
- Offering eGFR and
- Days in hospital

The new scheme awards more points to patients and donors in the same risk category. The rational for this is to better match patient and graft life expectancy. To ensure D4 older donors were utilised optimally, both kidneys from donors from these donors will be offered as dual kidneys to the centre with the highest priority patient listed.

The new scheme potentially has following advantages over the 2006 scheme:

- Patients listed on the waiting list after they started dialysis will see an increase in their waiting times and therefore more likely to get offers
- Prolonged waiting times will be reduced, particularly for patients who are difficult to match and/or highly sensitised
- The new scheme will potentially benefit patients from BAME communities who generally wait longer for a kidney. Transplant centres with a large proportion of BAME patients are likely to see an increase in activity
- Potentially, graft longevity will be better matched to patient longevity thus making better use of available kidneys

The new scheme could potentially have the following disadvantages:

- R4 and potentially R3 patients will have a significantly reduced probability of being offered the best kidneys in terms of donor age and co-morbidities. Even though the data shows that overall 5-year graft survival will be unchanged,

there is the potential for a perception that older patients are being disadvantaged
- Easy to match patients will wait on average longer than they currently do to accommodate the reduced waiting times for difficult to match patients

FAST TRACK KIDNEY ALLOCATION IN THE UK

A fast track kidney allocation scheme (FTKAS) for offering of previously declined kidneys was implemented in the UK in 2012 for DBD donors and 2013 for DCD donor. This scheme ensures organs that are at high risk of being discard are instead offered simultaneously to centres who wish to consider implanting higher risk kidneys. Centres have to opt into this scheme. Once the offer is made, each centre has 45 minutes to confirm whether or not they would like to accept it. Of all the centres who express a wish to accept, the kidney is allocated to the centre with the patient with the highest priority patient listed.

Kidneys from DBD donors are offered through the Fast Track Scheme under the following circumstances:

- If a SNOD or a member of the retrieving or transplanting team considers the kidney is to be unusable or
- If five kidney transplant centres decline a kidney-only offer or
- If the kidney was part of an SPK or other multi organ scheme and has accrued 12 hours of cold ischaemia time

Kidneys from DCD donors are offered through the Fast Track Scheme under the following circumstances:

- If a SNOD or a member of the retrieving or transplanting team considers the kidney is to be unusable or
- If three kidney transplant centres decline a kidney-only offer or
- If the kidney was part of an SPK or other multi organ scheme and has accrued 6 hours of cold ischaemia time
- If the kidney has been offered and accepted subsequently declined after treatment withdrawal but before retrieval

PAIRED/POOLED EXCHANGE SCHEME

The Human Tissue Act 2004 provides the legal framework for organ and tissue donation in the UK and made provision for paired/pooled kidney exchange and altruistic donation. These schemes have allowed live donor transplants where a patient-donor who are HLA or ABO incompatible and therefore unable to donate directly, can be entered into a national exchange scheme and potentially matched with another pair of patient and donor.

The scheme includes paired/pooled donation (PPD) and altruistic donor chains initiated by non-directed altruistic donors. IN the PPD scheme, donor and recipient pairs who are incompatible HLA and/or ABO blood group and unable to donate directly, are registered in a national scheme to achieve compatible transplants with other pairs. When two pairs are involved it is called 'paired' donation. When more than two pairs are involved it is called 'pooled' donation. Compatible donor-recipient pairs who may seek a better HLA or age match may also be registered in the scheme. This is often important in young patients and patients whose underlying kidney disease means that they would likely need another transplant in the future.

When a person chooses to donate anonymously as a non-directed altruistic donor to a recipient on the waiting list, that donor is known as an altruistic donor and their kind donation can kick start a chain. The donated kidney is allocated to a recipient in the paired/pooled scheme and in turn, the donor registered with that recipient donates to another recipient and so on. The chain ends when the last donor donates to a recipient on the national transplant list.

In the UK, living donor kidney matching runs are performed 4 times per year to identify paired/pooled exchanges and altruistic donor chains in optimal combinations. If there is a high priority recipient on the national transplant list, the kidney will be offered to that recipient prior to inclusion in the matching run.

The UK exchange scheme the ‘Living Donor Kidney Matching Run’ (LDKMR) is performed 4 times per year, in January, April, July and October. Entering a patient donor pair into the scheme requires the following:

- The patient must meet the criteria for listing on the organ donor register

- All relevant demographics
- Confirmed ABO
- Confirmed HLA type
- HLA antibodies on two separate samples
- List of unacceptable mismatches
- cRF
- Minimum permitted mismatches (000-222)
- Maximum age of donor

Once a matching run has been completed, the centres need to complete all medical checks and laboratory tests within 8 weeks. The laboratory tests are mainly the crossmatch though donor HLA typing CT may also be undertaken. The crossmatch consists of an initial and final crossmatch completed within 2 weeks of the transplant date. The initial crossmatch may be virtual.

ABO AND HLA INCOMPATIBLE KIDNEYTRANSPLANTATION

The overall median waiting time on the kidney waiting list in the UK is just under 2 years. Highly sensitised patients (calculated PRA > 85%) tend to wait on average much longer. Strategies that have been used in the UK to increase the number of transplants in this group have included the prioritising of such patients for well-matched kidneys based on the listing of all unacceptable mismatches, the use of marginal or extended criteria donors to increase the number of transplants in all patients on the waiting list and the use of paired exchange donations. Whilst these strategies have been useful, they do not always benefit all patients. In addition, even patients who are not highly sensitised but who have HLA antibodies against or are ABO incompatible with their live donor can fail to benefit from these schemes depending on how rare their HLA types are and what their blood groups are. Such antibody incompatible transplants can be undertaken with suitable desensitisation protocols.

Antibody incompatible transplantation refers to the transplantation of patient across the previously insurmountable ABO and HLA barriers (ABOi and HLAi). Normally, blood group incompatible transplantation is precluded by the presence of blood group isohaemaglutinin in the blood of the recipient, leading to hyperacute rejection and allograft loss. From the HLA viewpoint, Patel and Terasaki demonstrated in 1969 that hyperacute rejection can result from allograft injury caused by preformed donor specific anti-HLA antibodies (DSA), making a positive cytotoxic HLA crossmatch a

contraindication to transplant. It has however been shown as long ago as the 1980's that it is possible to transplant across an ABO incompatibility by antibody removal prior to transplant with many such transplants performed in Japan in particular but also in other countries. Similarly, removal of donor specific HLA antibodies to a point resulting in a negative crossmatch has been shown to avoid hyperacute rejection and permit HLAi transplantation. Removal of ABO and HLA antibodies to permit antibody incompatible transplantation is called desensitisation.

Careful patient selection for desensitisation is an important aspect of any antibody incompatible transplant program. Patients must be able to withstand whole volume plasma exchange, in some protocol's multiple times. In additional, patients must be assessed for the likelihood of a successful desensitisation and for protocols involving plasmapheresis, an estimation of the number of treatments required to achieve a negative crossmatch. For HLA incompatible transplantation, this requires knowledge of the antibody specificity and strength. Antibodies to HLA DR51, 52 and 53 for instance often prove difficult to remove. Some transplant centres will for instance only include patients awaiting first transplant whilst other may accept re-transplant patients but avoid previous HLA mismatches. HLA antibody screening and identification was historically by cell based techniques such as the Complement Dependent Cytotoxicity (CDC) assay. Currently however, most HLA antibody testing in the UK is by the solid phase Luminex bead based assays. Use of Luminex and other solid phase assays as part of the desensitisation protocols provides for precise characterisation of DSA and is one of the main reasons behind the success of antibody incompatible transplantation.

Early desensitisation protocols for HLA antibodies included the use of splenectomy. Typical protocols included plasmapheresis and IvIg with anti-CD20 (Rituximab) and splenectomy. Most patents on this protocol did achieve a negative crossmatch. However, patients on the same protocol without the splenectomy were later shown to achieve the same results. One protocol that became widely used for HLA desensitisation was high dose IvIg, typically given over the course of several months until a negative crossmatch is achieved. A common dose is 1g/kg patient weight. The exact mechanism of action of high dose IvIg is known. Proposed mechanisms include an anti-idiotypic effect of IvIg on HLA antibodies, saturation of the neonatal Fc receptor which would normally protect endogenous HLA IgG molecules and high dose IvIg is proposed to induce apoptosis in B cells. One disadvantage of high dose IvIg protocols is that they have been associated with thrombotic complications.

Another popular protocol is plasma exchange, with or without low doses of Ivlg. Plasma exchange protocols aim to physically remove HLA antibodies to a negative crossmatch before transplantation. Some plasma exchange protocols also incorporate antithymocyte globulin (ATG) and anti-CD20 antibodies such as Rituximab. The use of Rituximab is controversial as it removes B cells but not the antibody producing plasma cells. For this reason, some transplant centres did evaluate the use of Bortezomib, which has anti plasma cell activity. Depending on the strength of the DSA present, plasma exchange protocol can involve several sessions of whole volume exchanges. HLA antibody removal is effective, with the most commonly reported side effect being hypocalcaemia. Antibody removal by these protocols is however not specific to HLA IgG, removing other protective antibodies and clotting factor, potentially increasing the risk of infection or bleeding. This risk is reduced by the use of low dose Ivlg and by transfusion if required.

A newer approach to desensitisation is the use of filtration or immunoadsorption. Immunoadsorption involves an apheresis procedure in which the patients' blood is 'filtered' by passing it through a column containing beads coated with an IgG absorber such as Protein A which selectively filters out IgG molecules. This has few of the side effects of traditional plasma exchange. A single filtration session typically reduces HLA antibody MFI levels to half their pre filtration values. Several rounds of filtration are required to reduce antibody levels to achieve a negative crossmatch. HLA antibody levels rebound to some degree after each session as antibodies equilibrate between intravascular and extravascular spaces.

ABO desensitisation is by plasma exchange or by immunoadsorption. As in plasma exchange for HLA antibody removal, plasma exchange for ABO is also not specific to ABO IgG, removing other protective antibodies and clotting factor and requires administration of Ivlg in some protocols. Immunoadsorption for ABO desensitisation uses columns with a matrix of sepharose beads coated with blood group A or B carbohydrate antigens, typically in a double filtration configuration. As blood runs over the column, the blood type specific column removes isohaemaglutinin against the appropriate blood group. The column removes approximately 30% of anti-A or anti-B antibodies with each treatment. The columns are expensive but can be regenerated and reused for the same patient. Depending on the protocol in use, patients can proceed to transplant with an ABO antibody titre from 1:8 to 1:16.

H&I laboratories provide support for the HLA aspects of antibody incompatible transplant programs. Laboratory support for these programs can be grouped into support provided as part of the assessment and pre-treatment work up, support provided for treatment monitoring including the final pre-transplant crossmatch and support provided as part of the post-transplant monitoring.

H&I laboratory support provided as part of the pre-treatment work up includes HLA typing and antibody screening and identification. HLA typing is carried out at HLA-A, B, C, DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1 and DPB1. HLA antibody screening and identification is performed by solid phase assays such as Luminex single antigen beads (SAB) which give both the specificity and strength (MFI) for each antibody detected. H&I laboratories must be able to identify antibodies specific for HLA-A, B, C, DR, DQ and DP. It is particularly important to identify the MFI of DSA. Various studies have been conducted into the MFI value that correlates with a positive CDC when MFI value > 10,000 and positive flowcytometric crossmatch when MFI value are anywhere between 6000 and 10000 (One Lambda Luminex assay). This inter laboratory variation makes it important for each laboratory to establish the MFI values that correlate with positive crossmatch in their hands.

The support provided by the laboratory as part of the treatment monitoring depends on the protocol in use. High dose Ivlg protocols involves fewer, infrequent treatments whilst plasma exchange and filtration protocols may involve daily or alternate day treatment with HLA antibody testing before and after each treatment. This allows for an evaluation of the effectiveness of antibody reduction of each treatment, it allows an assessment of the antibody rebound between treatments and it helps to determine the number of additional or supplementary (such as increased immunosuppression) treatments that may be needed. The criteria for proceeding to transplant varies between transplant centres. Some will only proceed to transplant on the basis of a negative CDC and flowcytometric crossmatch, whilst others may proceed on the basis of a negative CDC, pos flowcytometric crossmatch provided the mean channel shift is below an acceptable level. The use of various immunosuppressive drugs such as Rituximab and alemtuzumab as part of the treatment protocol as well as the timing to the treatment and the availability of sample may make an actual crossmatch logistically difficult and some centres may proceed to transplant on the basis of a negative virtual crossmatch.

With high dose Ivlg protocols, post transplant antibody monitoring is typically weekly for the first month, then monthly. For plasma exchange and filtration protocols, antibody monitoring is more frequent. The Johns Hopkins protocol involves weekly monitoring for the first month, then at 2 and 3 months, then quarterly thereafter. However, depending on the patients post transplant course, initial monitoring can be daily for the first few days and weeks post transplant. Antibody levels can sometime rebound significantly post transplant in many cases often higher than the pre transplant levels. This does not necessarily imply imminent graft failure as many patients appear to accommodate the graft even in the presence of DSA. Treatment should therefore be on the basis of actual proven rejection rather than on antibody levels alone. Potential treatment options include post transplant plasma exchange or plasma filtration and enhance immunosuppression. Some protocols also treat with Ivlg and Rituximab though the use of Rituximab is not universal.

Patients are able to maintain grafts in the presence of DSA though Accommodation, the phenomenon in which a graft functions normally by acquiring resistance to immune-mediated injury despite the presence of anti-graft antibodies in the recipient. Proposed mechanisms for the Accommodation include the expression in the graft of several protective genes which block the activation of the transcription factor NF-KB, thereby suppressing induction of proinflammatory genes and inhibition of the membrane attack complex thereby disrupting the action of complement.

PANCREAS

PANCREASE ALLOCATION

Up until the introduction of the new kidney allocation scheme, the most significant change to the pancreas allocation scheme has been the addition of the simultaneous Islet and kidney transplant (SIK) category to the scheme to go alongside SPK. Some SPK patients sometimes cannot proceed due to co-morbidities and/or technical complications but would otherwise be suitable for islet alone and for kidney alone. The new category of SIK has been created for these patients.

There are a number of changes to the pancreas allocation scheme which flow directly from the changes to then kidney scheme. The new pancreas scheme is divided into three tiers A-C. Tier A will be incorporated into the Kidney Tier A and

will include SPK or SIK patients with 100% cRF, matchability score 10 or waiting time ≥3 years. Tier A will be ranked by waiting time from dialysis or listing, whichever is earliest.

Tier B will incorporate blood group identical and highly sensitised compatible patients ranked by Total Point Score TPS. TPS takes into account the following:

- Four or fewer HLA mismatches
- Points deducted per mm for Islet transplant
- Waiting time
- Second or subsequent Islet transplantation
- High cRF
- Dialysis
- Geographical proximity
- Donor BMI favour vascularised pancreas for low BMI donors
- Donor age match as a tie breaker

Tier C will incorporate other blood group compatible patients also ranked by TPS.

ROLE OF HLA MATCHING IN TRANSPLANT OUTCOME

HLA ANTIGEN LEVEL MATCHING

The primary role of the HLA molecules is to present pathogen derived peptides to T cells thereby eliciting a T cell mediated adaptive immune response. The T cell recognises both the HLA molecule and the peptide it presents, distinguishing self derived peptides from foreign peptides. It is this ability to restrict the T cell response, distinguishing self from foreign and permitting an immune response to be mounted against the foreign that makes the HLA antigens the main immunological barrier to transplantation, necessitating HLA matching.

The first successful living-related donor kidney transplant was performed in 1954 by a team in Boston, USA and involved a kidney transplant between 23-year-old identical twins. These identical twins were matched at all 6 classical HLA loci and the patient survived for many years. Many studies have since shown a strong correlation between the level of HLA matching at the Broad level for HLA-A, B and DR and

graft survival. In the UK in the 1990's, it was shown that the best outcome was achieved with kidneys that had no mismatches at HLA-A, HLA-B, and HLA-DR loci (000 mismatches). The next most favourable outcome was achieved with one mismatch at either A or B loci or one mismatch at both the A and B , but no mismatch at the DR locus (100, 010, or 110 mismatches).

A recent UNOS study has suggested a reduction of the influence of HLA matching in kidney transplantation in the Calcineurin Inhibitor era and with improvements in surgical techniques. This has however been refuted by a European CTS study (Opelz et al) which reviewed transplants over two decades from 1985 to 2004. The European study found a statistically significant association between graft survival and the number of mismatches across that period. They also found a significant association between the number of mismatches and the number of rejection episodes.

In the UK an updated HLA matching algorithm was implemented in 2006. The previous matching scheme was potentially iniquitous to patients from ethnic minorities, with potentially rarer HLA types, who are under represented on the donor panels. A system of 'defaulting' of rare HLA antigens to common equivalents was introduced. e.g. HLA-A80 in a patient was defaulted to HLA-A1 and HLA-DR103 was defaulted to HLA-DR1, making it much more likely that such patients will be transplanted.

The current UK scheme comprises 5 tiers as far as HLA matching is concerned. Tiers A comprises 000 matched highly sensitised paediatric patients and 000 matched HLA-DR homozygous paediatric patients. Tier B comprises all other 000 matched paediatric patients. Tier C comprises 000 matched highly sensitised adult patients and 000 matched HLA-DR homozygous adult patients. Tier D comprises of all other 000 matched adult patients and all well matched paediatric patients (100, 010, and 110 HLA-A, -B, and -DR mismatches). All other patients are in Tier E. A point system is then used to prioritise within each tier based on factors including waiting times.

The emphasis that the scheme places on prioritising 000 matched paediatrics highlights the impact of mismatching of HLA antigens on the development of alloantibodies and therefore the ability to re-transplant in later years should the graft

fail. The data the current UK algorithm is based on four identified levels (graded levels 1 – 4) of HLA matching which were found in a review of transplant outcomes based on the UK 1998 algorithm, to correlate well with increasing risk of transplant failure. Level 1 comprises 000 HLA-A, B and DR mismatches, Level 2 comprises 0 HLA-DR and 0 or 1 HLA-B mismatch, Level 3 comprises 0 HLA-DR and 2 HLA-B mismatches or 1 HLA-DR and 1 HLA-B mismatch and Level 4 comprises 1 HLA-DR and 2 HLA-B mismatches or 2 HLA-DR mismatches. The UK data showed that HLA-A mismatches had no effect on transplant outcome.

The impact of matching for HLA-C, DQ and DP in kidney transplantation has been reviewed in a small number of studies. One study has suggested a potential influence of HLA-C mismatching on the number of acute rejection episodes in the presence of 1 additional HLA-B mismatch. The role of HLA-DQ mismatching in the presence of a HLA-DR match has received relatively little study in recent years. One study in the 1980's and another in the 1990's found no significant correlation between HLA-DQ mismatching and graft outcome in the presence of a HLA-DR match. A small number of studies have shown a role for HLA-DP matching in re-transplant patients but no significant role in first transplants even in the presence in preformed donor specific antibodies.

HLA matching for kidney transplantation is generally at the Broad antigen level and not at the allele level. One study has shown a correlation between allele level mismatched in HLA-DRB1 and the number of rejection episodes though the study found no correlation with long term survival. Certainly the ability to identify allele specific antibodies using the current generation of solid phase HLA antibody detection techniques presents the ability to list allele specific antibodies as unacceptable mismatches. In addition, a number of studies have shown that the use of structural epitope matching techniques such as HLAMatchmaker is predictive of positive crossmatches. Duquesnoy has shown that in 0 HLA-DR matched, HLA-A and/or HLA-B mismatched transplants, the number of epitope mismatches correlates significantly with 5 year graft survival.

The impact of HLA matching in kidney transplantation continues to evolve in the desensitisation era with the presence of preformed donor specific alloantibodies no longer the absolute contraindication to transplantation that it once was. The UK BSHI/BTS guidelines require that laboratories are capable of identifying HLA antibodies to HLA-A, B, C, DR, DQ and DP so that donors who should be negative

can be identified for crossmatching. However desensitisation protocols may permit transplantation even in the presence of donor specific antibodies for any of these HLA loci.

Not all solid organ transplantations require the level of HLA matching that is the norm in kidney transplantation though most require HLA antibody definition. Pancreatic transplantation is one exception which does requires the same level of HLA matching as kidney transplantation. With cardiothoracic transplantation, HLA matching is not necessarily undertaken but antibody definition is required and if present then a prospective crossmatch or retrospective crossmatch within 48 hours is required.

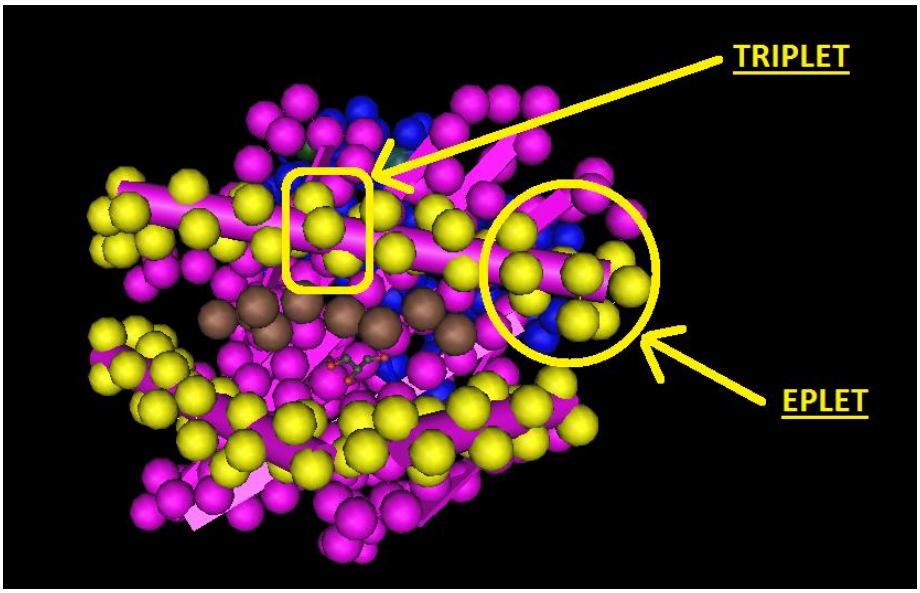
HLA matching prior to liver transplantation is not required and prospective crossmatching is not indicated. Some centres do carry out HLA antibody identification to aid in setting immunosuppression levels. HLA matching is not necessarily undertaken for small bowel and intestinal transplantation.

EPITOPE MATCHING

It has long been known that antibodies do not bind to the whole of the HLA antigen but instead bind to specific epitopes on the antigen surface. Each HLA antigen potentially has many sites or epitopes that can bind antibody. These epitopes may be private to a given HLA antigen or they may be shared by more than one HLA antigen, i.e. they may be public. Several studies have demonstrated a potential application to solid organ, especially renal transplantation.

Several epitope systems have been described, including the Terasaki system which is based on use of Luminex SAB to absorb and elute samples and identify the specific epitope the antibody is reacting with, the Duquesnoy HLAMatchmaker in silico system and the Kosmoliaptsiset system which is based on interlocus and intralocus comparison of patients and donors to identify amino acid differences but also crucially, the physiochemical properties of the amino acid mismatches and the role these may play in clinical outcome.

There are two versions of HLAMatchmaker, the Triplet and Eplet versions. The Triplet version treats HLA class I antigens as linear sequences of sequences of 3 amino acids in antibody accessible regions of the HLA molecule. Amino acids in positions such as the β pleated sheet are considered not to be in antibody accessible regions and are specifically excluded. The Eplet version is based on an analysis of the three dimensional structure of the HLA molecule rather than on linear sequences of amino acids. Instead of epitopes being defined by three amino acids in a linear sequence, eplets are defined as all the amino acids within a 3 to 3.5Å radius of each polymorphic residue position.



The applicability of epitope matching to kidney transplantation, particularly using HLA Matchmaker, has been extensively studied. In the antibody testing and crossmatching setting, studies have shown that a higher number of epitope mismatches between donor and patient has a statistically significant association with presence of Luminex positive HLA antibodies and with a positive crossmatch. In one large study, Duquesnoy et al, showed that in 0DR mismatched kidney transplants, 5 years outcomes for transplants with kidneys that had up to 4 Triplet mismatches, irrespective of the number of antigen mismatches, had the same organ survival rates as those patients transplanted with no HLA-A or B antigen mismatches.

Another study showed that matching at the epitope level rather than the antigen level, potentially increased the number of donors that could be considered compatible for a sensitised patient.

The number of epitope mismatches between patient and donor has also been shown to be statistically associated with the frequency of de novo donor specific antibody formation post-transplant.

ROLE OF NON-HLA GENETIC FACTORS IN TRANSPLANT OUTCOME

MINOR HISTOCOMPATIBILITY ANTIGENS

There have only been a small handful of studies into the influence of minor histocompatibility antigens in solid organ transplantation, with most of the available data being based on the HY antigen rather than the autosomal minor histocompatibility antigens. In fully matched kidney transplants, minor histocompatibility antigens are thought to contribute to immune rejection processes which, along with many other factors, lead eventually to long term graft loss. HY antigens are ubiquitously expressed on renal tubular epithelial cells and thus functions as a target for destruction. In one study, HY specific cytolytic T cells were detected in a female patient transplanted with a kidney from her HLA matched brother. In another study, the presence of de novo anti HY antibodies in female patients transplanted with kidneys from male donors correlated with the incidence of acute rejection.

In the largest study of the role of minor histocompatibility antigens in kidney transplantation, Gratwohl et al reviewed over 150,000 deceased donor transplants and found in a multivariate analysis that when compared to all other combinations of sex, transplant of kidneys from male donors into female patients was associated with an increased risk of graft failure at both 1 and 10 years.

MICA & MICB

Post transplant de novo anti MIC antibodies have been shown to be associated with graft loss in kidney transplantation, with acute rejection episodes in heart

transplantation and with chronic rejection in lung transplantation. MIC mismatching has also been shown to associate with aGvHD in stem cell transplantation.

In one large retrospective study involving over 1900 transplants, the presence of pre transplant MICA antibodies was found to associate significantly with an increase in graft failure. This was also evident in well matched transplants in patients with no pre-transplant anti HLA antibodies. In another smaller study, anti MICA antibodies were eluted from kidneys which had been lost due to acute as well as chronic rejection. These antibodies were eluted with and without anti HLA antibodies. Where donor material was available, the study could identify the possible immunising donor MICA epitope. In one study of the relevance of MICA in heart transplantation the presence of MICA antibodies was significantly higher in those patients with severe acute rejection than those without rejection. The study found that in most cases the appearance of the MICA antibodies preceded the development of acute rejection. Histological evidence showed an upregulation in MICA. The role of MICA and MICB in lung transplantation has shown that the development of antibodies to either MICA alone or to MICA and HLA together, correlated significantly with the development of bronchiolitis obliterans syndrome in chronic rejection.

KIR

In solid organ transplantation, NK cells are known to infiltrate allograft, suggesting that activation of NK cells may be critical in the immediate post transplant period. However, one recent study found that there was no associated between KIR ligand mismatch and the incidence of acute rejection though they did find that certain KIR receptor - HLA class I ligand combinations were more frequent in patients with stable grafts when compared to patients with acute rejection. This reflects the outcome of a much larger CTS study involving over 2,700 deceased donor kidney transplants which found no effect of ligand matching on graft survival.

ROLE OF HLA ANTIBODIES IN GRAFT SURVIVAL

ROLE OF HLA ANTIBODIES IN THE STAGES OF GRAFT REJECTION

HLA alloantibodies have been implicated in all three stages of allograft rejection – Hyperacute (including Accelerated), Acute and Chronic rejection. Hyperacute rejection occurs within minutes of transplant, acute rejection occurs within days to

weeks, while chronic rejection occurs months to years post transplant. Antibody mediated rejection has a different pathology to T cell mediated rejection. Antibody mediated rejection preferentially attacks the peritubular and glomerular capillaries in contrast to T Cells which typically infiltrate the tubules and arterial endothelium.

Hyperacute rejection occurs almost immediately after the kidney is transplanted and is usually due to the presence of pre-formed donor specific anti-HLA antibodies. The mechanism of hyperacute rejection involves deposition of antibodies against HLA antigens expressed on the endothelium of the glomeruli and the graft microvasculature. This leads to activation of the classical complement cascade causing endothelial necrosis, platelet deposition and local coagulation. Hyperacute rejection is usually accompanied by C4d deposition, though this may be negative early on. Hyperacute rejection is not typically reversible and requires the immediate removal of the graft. Accelerated rejection usually occurs within days and is due to a memory or anamnestic response to pre-formed antibodies directed against mismatched donor antigen which are absent at the time of transplantation. Improved antibody screening and identification techniques as well as improvements in crossmatch techniques have significantly reduced the incidence of hyperacute and accelerated rejection.

Acute rejection occurs days to weeks (or months in the case of late acute rejection) post transplant and may contain a cellular as well as a humoral component. Acute humoral rejection is caused by pre-existing donor specific anti-HLA antibodies which are negative at the time of the pre-transplant crossmatch and/or de-novo donor specific antibodies. Diagnosis of Acute antibody mediated rejection involves identification of rapid graft dysfunction, accompanied by the presence of circulating anti-donor HLA antibodies and biopsy evidence of C4d deposition in the peritubular capillaries. C4d may also be deposited in the glomeruli though this is variable. Acute antibody mediated rejection is reversible with treatment such as plasmapheresis and intravenous immunoglobulin plus increased immunosuppression (with for example tacrolimus and mycophenolate mofetil). The Anti-CD20 antibody Rituximab is also used in some cases.

The role of alloantibodies in chronic rejection is increasingly being recognised. Recent data from the 14th international histocompatibility workshop demonstrated that four year deceased donor kidney allograft survival was 20% less in patients with donor specific antibodies compared to donors with no HLA antibodies. Chronic

rejection is characterised by slow progressive loss of renal function with endothelial antibody deposition leading to endothelial injury and glomerular basement membrane duplication characteristic of transplant glomerulopathy.

H&I laboratory tests carried out in support of the prevention or management of antibody mediated rejection in renal transplantation include HLA antibody screening and identification pre-transplant, crossmatching at the time of at the time of transplant as well as post transplant antibody monitoring either as part of a routine and regular program or at least at the time of suspected rejection episodes.

Current UK standards (BSHI/BTS 2010) require that HLA antibody screening and identification techniques used must be sufficient to identify antibodies to HLA-A, B, C, DR, DQ and DP. In addition, the testing must be able to distinguish IgG allo and IgM allo and auto antibodies. HLA antibody screening and identification has historically been by cell based techniques such as the complement dependent cytotoxicity (CDC) assay or a cell based flowcytometric assays. Currently however, most HLA antibody testing in the UK is by the solid phase Luminex bead based assays. These assays are much more sensitive than the traditional CDC techniques and require careful consideration of the clinical significance of Luminex positive, CDC negative tests. Pre-transplant, this level of testing helps provide a list of unacceptable mismatches for renal patients, eliminating positive crossmatches and the impact this has in extending cold ischaemia times.

Once a patient is listed for transplantation, HLA antibody testing must be undertaken every three months and 2 – 4 weeks after potentially sensitising events such as blood transfusion. This helps build up a full antibody profile for the patient, avoiding the risk of an unexpected positive crossmatch. HLA antibody investigation is to the same level of detail as described for pre listing. i.e. Antibodies to HLA-A, B, C, DR, DQ and DP must be identified and the testing must be able to distinguish IgG allo and IgM allo and auto antibodies. The unacceptable mismatches listed with UK transplant must be updated.

In the live donor transplant setting initial crossmatches may be undertaken well in advance of the actual transplant as part of the donor selection process, with a final crossmatch at the time of transplantation. The purpose of any crossmatch test is to determine whether the patient has any preformed HLA antibodies which will react

with antigens expressed by the donor and therefore helps to inform the immunological risk. In the UK, crossmatching is generally undertaken by CDC and/or flowcytometric assays. Cell separation techniques are used to ensure that reactivity to T and B cells can be distinguished. Tests used are also able to distinguish between IgG and IgM antibodies. In some centres, a virtual crossmatch is undertaken as the initial crossmatch in certain carefully selected cases.

At the time of transplant, antibody screening of the 'current' patient sample as well as pre-transplant crossmatching by CDC and flowcytometric techniques have significantly reduced the incidence of hyperacute rejection.

Post transplant, donor specific HLA antibodies contribute to acute humoral rejection characterised by rapid graft dysfunction, accompanied by the presence of circulating anti-donor HLA antibodies and biopsy evidence of C4d deposition in the peritubular capillaries. C4d may also be deposited in the glomeruli though this is variable. In addition, the role of HLA antibodies in chronic rejection is also increasingly recognised. Laboratory HLA antibody screening can be used to monitor patients with suspected rejection for circulating anti-donor HLA antibodies.

In HLA incompatible transplant, the role of H&I laboratory tests is crucial in monitoring the desensitisation to achieve a pre-transplant negative crossmatch as well as to closely monitor the patient post transplant for a rebound in antibody levels to help clinicians respond with therapies to reduce humoral rejection.

MECHANISMS FOR HLA ANTIBODY REMOVAL

PLASMA EXCHANGE

Plasma exchange protocols aim to physically remove HLA antibodies to a negative crossmatch before transplantation. Some plasma exchange protocols also incorporate antithymocyte globulin (ATG) and anti-CD20 antibodies such as Rituximab. Depending on the strength of the DSA present, plasma exchange protocol can involve several sessions of whole volume exchanges. Patients need careful assessment as to whether or not they are healthy enough to withstand several rounds of plasma exchange. The H&I laboratory can help determine the

number of rounds that may potentially be needed by undertaking HLA antibody titre tests.

HLA antibody removal is effective with the most commonly reported side effect being hypocalcaemia. Antibody removal by these protocols is however not specific to HLA IgG, removing other protective antibodies and clotting factor, potentially increasing the risk of infection or bleeding. This risk is reduced by the use of low dose IVIg and by transfusion if required.

FILTRATION

Filtration or Immunoadsorption involves an apheresis procedure in which the patients' blood is 'filtered' by passing it through a column containing beads coated with an IgG absorber such as Protein A which selectively filters out IgG molecules. This has few of the side effects of traditional whole volume plasma exchange. A single filtration session typically reduces HLA antibody MFI levels to half their pre-filtration values. Several rounds of filtration are required to reduce antibody levels to achieve a negative crossmatch. HLA antibody levels rebound to some degree after each session as antibodies equilibrate between intravascular and extravascular spaces. The H&I laboratory can help determine the number of rounds that may potentially be needed by undertaking HLA antibody titre tests.

IVIG

IVIg is typically given over the course of several months until a negative crossmatch is achieved. A common dose is 1g/kg patient weight. The exact mechanism of action of high dose IVIg is unknown. Proposed mechanisms include an anti-idiotypic effect of IVIg on HLA antibodies, saturation of the neonatal Fc receptor which would normally protect endogenous HLA IgG molecules and induction of apoptosis in B cells.

One disadvantage of high dose IVIg protocols is that they have been associated with thrombotic complications.

RITUXIMAB

Rituximab is an anti-CD20 recombinant chimeric murine-human monoclonal antibody. It binds to CD-20 which is expressed on precursors and mature B cells but not on plasma cells. Binding triggers a series of cytotoxic immune response resulting in the elimination of B cells and the reduction in antibody formation. As B cells play an important antigen presentation role to helper T cells, their reduction through Rituximab also reduces other immune responses.

BORTEZOMIB

Bortezomib is proteasome inhibitor used mainly in the treatment of relapsed multiple myeloma. Proteasomes are thought to support the immortal phenotype of multiple myeloma by rapidly degrading pro-apoptotic factors. Bortezomib binds to the catalytic site of proteasome with high affinity and specificity, inhibiting its action and permitting the activation of programmed cell death. Bortezomib induces apoptosis in plasma cells, which are immune to Rituximab and is therefore very useful in desensitisation protocols in solid organ transplantation.

APPLICATION OF H&I LABORATORY TECHNIQUES

PRE-TRANSPLANT

COMPLEX UNACCEPTABLE ANTIBODY LISTING

DQA antibodies cannot be listed as unacceptable with ODT but do cause a positive crossmatch and can lead to rejection. They therefore need to be taken into account when listing unacceptable mismatches and when accepting a kidney for a patient.

If an antibody can be confidently assigned to DQA i.e. all the DQA beads in the Luminex assay are positive and one or more of the non-masked DQB beads are Negative, then the DQB is not listed as unacceptable but would be reviewed at the time of a donor offer. For some patients, it may be possible to list a particular DQB as unacceptable in order to avoid donor offers with a particular DQA type e.g. It is possible to list DQB*05 as unacceptable to avoid offers from DQA*01 donors. It partly depends on the MFI and the risk of a positive crossmatch. A risk assessment is

carried out at the time of a donor offer to assess whether or not the crossmatch is likely to be positive. For this, the donor DQA type would need to be known.

MANAGING HIGHLY SENSITISED PATIENTS

Highly sensitised patients should be maintained on the waiting list as even a small chance of a deceased donor offer is better than no chance. Options to increase the chances of the patient getting a deceased kidney donor offer include:

- Repeating the HLA antibody in case there have been any changes
- Consider de-listing any unacceptable mismatches that have not been detected in the last 2 years and that are not repeat transplant mismatches
- Review the Luminex MFI cut-off for listing unacceptable mismatches for this patient
- If CDC screening has not been undertaken, do CDC to identify Luminex Pos., CDC Neg. specificities which could potentially be removed from the list of unacceptable mismatches
- HLA antibody screening titres may be undertaken to determine which specificities titre out
- Third party crossmatches may be undertaken to help predict crossmatch outcome

In addition to keeping the patient on the deceased donor waiting list, if the patient has a live donor, consider a live donor workup. If any potential live donors are HLA and or ABO incompatible but otherwise willing to donate, enter the patient and their donors into the paired/pooled exchange for at least two rounds.

If no offer is made through the paired/pool exchange then depending on the expertise in the local unit, the patient can be considered for antibody removal as part of an ABOi or HLAi transplant. Options for antibody removal include:

- High dose IVIg. This will require regular, perhaps monthly HLA antibody removal
- Plasma-exchange/Apheresis filtration. This will require HLA antibody screening titre to predict the number of rounds of antibody removal that may be required, together with pre and post antibody removal cycle tests

AT TIME OF TRANSPLANT

FACTORS THAT CAN AFFECT THE CROSSMATCH RESULTS

ANTIBODIES TO DENATURED ANTIGENS ON BEADS

Although it is possible to have single Luminex beads reacting with very high MFI, these can also be denatured antibodies or non-specific binding to the beads, especially if the FCXM is negative.

In a live donor situation there is often time to investigate denatured antibodies either in your own lab or by sending the sample away to a lab with validated denatured antibody testing protocols. Acid treatment to strip the Luminex beads of bound antigen should result in a negative Luminex test if the antibodies are real HLA antibodies. If however the antibodies are binding non-specifically to the beads then acid treatment will not significantly reduce the MFI of the supposed DSA bead.

Where non-specific binding is confirmed and a repeat of the FCXM remains negative then it is safe to proceed to transplant.

PAN REACTIVE LUMINEX BEADS

Occasionally, the Luminex antibody test can be pan reactive. Possible steps that can be taken in these situations include:

- Repeat the Luminex screen to see if there are been an error
- If Luminex ID beads available, test with those as well
- I would also test with the alternate Luminex kit to see if there is a kit specific issue
- If denatured bead protocol available would test to see if these antibodies are real
- If C3d/C1q available to lab would test with those or
- I would do CDC screening if Luminex Class I to see if these antibodies are cytotoxic
- I would do high res typing of the patient to see if they have null or low expression at this locus to explain the antibody formation pattern
- For completeness would do auto-crossmatch to see if negative

- Would titre the antibody screening to see if any specificities titre out
- Would do third party crossmatch to determine any specificities are crossmatch compatible
- Ultimately would only transplant as part of a high risk immunosuppression protocol with post-transplant antibody monitoring

PROSPECTIVE PRE-TRANSPLANT TEST DECISION MAKING

ANTIBODY SCREENING RESULTS: NO DETECTABLE ANTIBODIES

Transplant can proceed without further pre-transplant testing provided this result has been seen on at least two samples bled at different times, the last sample tested was bled within the last three months and it is confirmed that patient has not had any sensitising events since the last sample was bled. Samples must be collected pre-transplant for retrospective crossmatching though these may potentially be stored and tested only if the patient is having issues

ANTIBODY SCREENING RESULTS: CDC IGM LOW TITRE POS., IGG NEG, LUMINEX NEG.

Transplant can proceed without further pre-transplant testing provided the last sample tested was bled within the last three months and it is confirmed that patient has not had any sensitising events since the last sample was bled. Samples must be collected pre-transplant for retrospective crossmatching though these may potentially be stored and tested only if the patient is having issues

ANTIBODY SCREENING RESULTS: CDC NEG, LUMINEX NON DSA WEAK POS. WITH PEAK MFI BELOW 2000

Transplant can proceed without further pre-transplant testing provided the last sample tested was bled within the last three months and it is confirmed that patient has not had any sensitising events since the last sample was bled. Samples must be collected pre-transplant for retrospective crossmatching though these may potentially be stored and tested only if the patient is having issues

ANTIBODY SCREENING RESULTS: CDC NEG, LUMINEX NON DSA POS. WITH PEAK MFI ABOVE 2000 AND LESS THAN 5000 BUT LUMINEX SPECIFICITY CRF <75%

Minimum of pre-transplant SAB testing required assuming there have been no sensitising events since the last sample was bled. If DSA's remain negative and MFI levels of non DSA have not changed significantly transplant can proceed without a wet pre-transplant crossmatch. Samples must be collected pre-transplant for retrospective wet crossmatching though these may potentially be stored and tested only if the patient is having issues

ANTIBODY SCREENING RESULTS: CDC HIGH TITRE IGM POS., IGG NEG, LUMINEX NEG.

Minimum of pre-transplant SAB testing required. If patient remains Luminex SAB Neg. then transplant can proceed without a wet pre-transplant crossmatch. Samples must be collected pre-transplant for retrospective crossmatching though these may potentially be stored and tested only if the patient is having issues

ANTIBODY SCREENING RESULTS: CDC NEG/NT, LUMINEX DSA WEAK POS. (BELOW 2000)

Minimum of pre-transplant SAB testing required. If patient remains Luminex SAB Weak Pos. then transplant can proceed without a wet pre-transplant crossmatch. Samples must be collected pre-transplant for retrospective crossmatching

ANTIBODY SCREENING RESULTS: CDC NEG/NT, LUMINEX DSA POS. (ABOVE 2000)

Minimum of pre-transplant Flowcytometric crossmatch required. A CDC crossmatch may also be undertaken if Luminex SAB peak MFI > 5000 provided Renal Unit are happy to proceed to transplant with a CDC Neg. Flow Pos. crossmatch

ANTIBODY SCREENING RESULTS: RE-TRANSPLANT PATIENT WHO IS CDC AND LUMINEX NEG.

If full antibody history is available and patient has remained Neg throughout then minimum of pre-transplant SAB testing required. If patient remains Luminex SAB Neg. then transplant can proceed without a wet pre-transplant crossmatch. If there are repeat mismatches these must be highlighted to the renal unit. Samples must be collected pre-transplant for retrospective crossmatching though these may potentially be stored and tested only if the patient is having issues

ANTIBODY SCREENING RESULTS: RE-TRANSPLANT PATIENT WHO IS CDC NEG AND LUMINEX POS.

Minimum of pre-transplant Flowcytometric crossmatch required. A CDC crossmatch may also be undertaken if Luminex SAB peak MFI > 5000. If there are repeat mismatches these must be highlighted to the renal unit. If the repeat mismatches are DSA this raises the risk level of the transplant. Interpretation of the crossmatch results must take into account any current immunosuppression and whether or not the previous graft is still in-situ

ANTIBODY SCREENING RESULTS: CDC NEG, LUMINEX NON DSA POS. WITH PEAK MFI ABOVE 5000 AND LUMINEX SPECIFICITY CRF >75%

Minimum of pre-transplant Flowcytometric crossmatch required. A CDC crossmatch may also be undertaken if Luminex SAB peak MFI > 5000.

ANTIBODY SCREENING RESULTS: CDC POS.

All patients who are CDC screen Pos. require a CDC crossmatch. A Flowcytometric crossmatch may also be carried out at the same time to help inform the immunosuppression strategy. If the CDC is negative but the Flow is positive, the transplant may still be able to proceed depending on the MCS/RMF provided the patient is able to tolerate enhanced immunosuppression. In such circumstance, if possible, a pre-transplant plasma exchange may be undertaken followed by close post-transplant antibody monitoring. If DSA antibody levels are rising post-transplant plasma exchange can be undertaken

CROSSMATCH SERUM SELECTION

One possible policy is to select all samples from the last 12 months, the peak cRF% sample if older than the last 12 months and any samples collected post potential sensitising events if prior to the last 12 months.

CROSSMATCH RESULTS AND IMMUNOLOGICAL RISK

Patel and Terasaki showed in 1969 that a positive cytotoxic crossmatch between a patient and donor due to pre-formed donor specific HLA antibodies (DSA) was associated with hyperacute rejection in kidney transplantation. This lead to a positive cytotoxic crossmatch becoming an absolute contraindication to transplantation in the absence of antibody removal. More recently however, improvements in immunosuppression and antibody definition have lead to a shift from a simplistic Pos/Neg. veto on transplantation to one of an assessment of the immunological risk posed.

HLA antibodies are known to contribute to all categories of rejection, including the Hyperacute, Accelerated, Acute and Chronic phases. Hyperacute rejection occurs within minutes and is due to pre-formed DSA present at the time of transplantation. Accelerated rejection takes place within days and is usually due to a memory or anamnestic response to pre-formed DSA which are absent at the time of transplantation. Acute rejection occurs within days or weeks and is usually due to de-novo DSA. Terasaki and others have also demonstrated a role for DSA in chronic rejection, though the timing of antibody formation and chronic rejection do not always coincide.

Consideration of the immunological risk associated with kidney transplantation needs to take into account the timing, duration, priming source, titre and specificity of any DSA. Antibodies formed as a result of transfusion are often IgM and IgG and may not be long lasting. Antibodies formed after transplantation or pregnancy are often IgG and involve immunological memory.

Gebel et al have proposed three levels of immunological risk associated with kidney transplantation, High, Intermediate and Low. In the UK, these categories have been incorporated into the BHSI/BTS guidelines on clinically relevant alloantibodies. Though the guidelines deal mainly with anti-AHLA antibodies, other antibodies such as anti-ABO and anti-endothelial cell antibodies are also relevant.

High immunological risk is indicated when the patient has circulating antibodies specific for mismatched donors HLA antigens. An example is a T + B cell Complement Dependent Cytotoxicity (CDC) current sample positive crossmatch in the presence of HLA class I DSA specific for the mismatched donor antigen. This carries a high risk of Hyperacute rejection in the absence of desensitisation. Another

example of a High risk transplant is a B cell CDC current sample positive crossmatch in the presence of HLA class II DSA specific for the mismatched donor antigen. Transplants can also be high risk if only the historical sample is positive. For example a T + B cell CDC historical sample positive, current sample negative crossmatch in the presence of HLA class I DSA specific for the mismatched donor antigen or a B cell only CDC historical sample positive, current sample negative crossmatch in the presence of HLA class II DSA specific for the mismatched donor antigen are both High risk. Both have the potential to trigger an anamnestic or memory T and/or B cell response resulting in Accelerated rejection. In most cases, High risk transplants would be avoided unless as part of a validated desensitisation program.

Intermediate immunological risk is indicated by a flowcytometry positive, CDC negative crossmatch result with both current and historical samples in the presence of HLA class I DSA. Examples include T + B cell flow Pos, CDC Neg. current and historical samples in the presence of HLA class I DSA specific for the mismatched donor antigen.

Another example of an Intermediate risk transplant is a B cell only positive CDC current and historical sample in the presence of weak IgG HLA class I DSA specific for the mismatched donor antigen.

With Intermediate risk transplants, a clinical assessment is needed on the relative risk of proceeding to transplant with augmented immunosuppression and close post transplant monitoring versus remaining on the waiting list in the hope of obtaining a better match or taking part in a paired exchange if a live donor is available. Patients do die on the waiting list. This decision will be influenced by the experience of the transplant team and the H&I laboratory when it comes to monitoring and managing such patients, as well as the length of time the patient has already been on the waiting list, the CRF and the matchability of the patient.

Low immunological risk is indicated when the crossmatch result is negative and the patient is unsensitised. Low immunological risk is also indicated when the crossmatch result is negative, even if the patient is sensitised, provided the sensitisation is clearly shown to IgM only, is clearly shown to be Auto only or is otherwise considered to be non HLA.

Low immunological risk is also indicated when the crossmatch result is negative and any HLA antibodies present in the current and historical samples of the patient are not donor specific. In addition, Low immunological risk is also indicated when the crossmatch result is negative and HLA class I or II IgG DSA are detected in either the current or historical samples of the patient, provided these are detectable by Luminex SABs only.

Finally Low immunological risk is indicated when the crossmatch result is positive but the patient is either unsensitised or any HLA antibodies present in the current and historical samples are not donor specific.

Assessment of the immonological risks associated with a transplant requires close co-operation between the H&I laboratory and the transplant team. The lab should be informed of all potential sensitising event, including previous transplants, skin grafts, transfusion, pregnancies, miscarriages if known and recent infections or vaccinations. The laboratory crossmatch report to the transplant team should include appropriate advice on the clinical relevance of the results.

COMPLEX CROSSMATCH INTERPRETATION

Crossmatch results, especially FCXM results, do not always correlate linearly with MFI, especially when reviewed across different patients. However in general, presence of high MFI DSA should be predictive of a positive crossmatch and absence of DSA should be predictive of a Negative crossmatch.

If a crossmatch is Positive in the absence of DSA, potential reasons for the positive reaction would need to be investigated. Potential laboratory issues such as sample mix up and technical problems would need to be ruled out. If in doubt, the crossmatch would need to be repeated.

Clinical reasons such as patient underlying condition and treatment should also be investigated. Patients with autoimmune conditions often have a B Cell Positive crossmatch due to autoantibodies. If an auto crossmatch has not been set up this can

be done to rule out auto antibodies. If the patient is on rituximab this can cause a B cell positive crossmatch. ATG can cause a positive T cell crossmatch.

Upon full investigation, if the crossmatch is still positive in the absence of DSA and auto antibodies and patient treatment have been ruled out then the positive reaction can be attributed to non-HLA and is not a contraindication to transplant. Post-transplant monitoring would be required.

If a crossmatch is unexpectedly Negative in the presence of high titre DSA, then like the situation where an unexpected positive reaction is seen in the absence of DSA, the potential reasons for the Negative reaction would need to be investigated. The patient antibody profile and donor type would need to be carefully reviewed to determine if the antibody is truly DSA. For instance are all the Luminex beads for that specificity actually positive? Could this be an antibody to an allele not expressed in the donor? Or has an antibody been assigned to DQB that is actually a DQA antibody and is therefore not actually DSA? Similarly, is an antibody actually to DPA but has been assigned to DPB?

Potential laboratory issues such as sample mix up and technical problems would need to be ruled out. If in doubt, the crossmatch would need to be repeated.

Upon full investigation, if the crossmatch is still negative, especially by CDC then it is safe to proceed with the transplant. Close post-transplant monitoring will be required.

RELEVANCE OF HIGH MFI LEVELS FOR DP ANTIBODIES

The decision to proceed to transplant or not would always be made in consultation with the renal unit, taking into account factors such as first or second transplant, is the DP DSA a repeat mismatch, the level of matching of the kidney at all other loci, the likelihood of the patient getting another offer that was otherwise as well matched, the CDC and Flowcytometric crossmatch results, the MFI level of the DP DSA and whether or not the patient was able to tolerate enhanced immunosuppression.

If this patient is going for first transplant or the DP DSA is not a repeat mismatch, the transplant can proceed if the CDC crossmatch is negative. If the CDC is negative but the Flow is positive, the transplant may still be able to proceed depending on the MCS/RMF provided the patient is able to tolerate enhanced immunosuppression. If possible, a pre-transplant plasma exchange may be undertaken followed by close post-transplant antibody monitoring. If DSA antibody levels are rising post-transplant plasma exchange can be undertaken.

CROSSMATCH RESULT INTERPRETATION

CDC CURRENT AND HISTORIC T CELL POS B CELL POS, FXCM CURRENT AND HISTORIC T CELL POS B CELL POS, LUMINEX CLASS I DSA IN CURRENT AND HISTORIC SAMPLES

Very High Risk – Transplant veto

CDC CURRENT AND HISTORIC T CELL NEG B CELL POS, FXCM CURRENT AND HISTORIC T CELL POS B CELL POS, LUMINEX CLASS II DSA IN CURRENT AND HISTORIC SAMPLES

High Risk

CDC CURRENT AND HISTORIC T CELL NEG B CELL NEG, FXCM T CELL POS B CELL POS, LUMINEX CLASS I DSA IN CURRENT AND HISTORIC SAMPLES

Intermediate Risk

CDC CURRENT AND HISTORIC T CELL NEG B CELL NEG, FXCM T CELL NEG B CELL POS, LUMINEX CLASS II DSA IN CURRENT AND HISTORIC SAMPLES

intermediate Risk

CDC T CELL NEG B CELL POS, FXCM NT, LUMINEX WEAK CLASS I DSA IN CURRENT AND HISTORIC SAMPLES

High Risk

CDC NEG, FCXM T CELL NEG B CELL NEG, LUMINEX CLASS I AND/OR II DSA POS IN CURRENT AND HISTORIC SAMPLES, CDC SCREEN NEG

Standard Risk – None complement fixing antibody

FCXM T CELL NEG B CELL POS, CDC NT, LUMINEX CLASS I AND II DSA NEG

Standard Risk if results confirmed. Repeat to confirm. Test auto if not tested already

FACTORS WHICH MAY CONFOUND THE CROSSMATCH RESULTS

Goodpasture, Lupus and patients with some other autoimmune conditions can have autoantibodies which can confound HLA crossmatch tests. In addition, some of these patients are treated with plasmapheresis, cyclophosphamide or Azathioprine, Rituximab. There are a number of other clinical conditions and treatment which may also confound the crossmatch results.

Interpretation of crossmatch results in such patients can be complex. It may be necessary to repeat some unexpected positive crossmatch results to confirm and to review the HLA antibodies and potentially test with an alternative Luminex kit. Each H&I laboratory needs to evaluate the DSA levels which correspond with a positive crossmatch in their hands but typically, depending on the kit used, the flow would not be expected to be positive due to HLA DSA of below 2000 MFI.

A flow Pos. crossmatch in the absence of HLA DSA is considered to be standard risk according to BSHI/BTS guidelines and is not in of itself a contraindication to transplant. However, the positive reaction would ideally need to be investigated before proceeding with a transplant.

To investigate the positive flow result further, the flow crossmatch may need to be repeated and an auto crossmatch set up to determine if the reactivity is due to autoantibodies.

If autoantibodies and treatment are ruled out as potential causes of the positive flow the HLA DSA should be investigated further by for instance testing with an alternative Luminex antibody kit if not tested already and the patient should also be screened for non-HLA antibodies. Non-HLA antibodies, such as anti-angiotensin, anti-epithelial and anti HNA antibodies can cause a positive flow crossmatch and do contribute to rejection episodes post-transplant.

A CDC crossmatch may also be useful in determining the level of immunological risk. The ultimate decision to proceed or not will be made in consultation with the renal unit and as part of a high-risk strategy, taking into account clinical urgency.

ATG

Anti-Thymocyte Globulin (ATG) is an anti-human T cell antibody immunosuppressive used for transplant induction in highly sensitised patients and in the prevention and treatment of acute rejection in kidney and cardiac transplantation. Use of ATG in a patient pre-transplant will cause a Positive T Cell Crossmatch.

RITUXIMAB

Rituximab is an anti-CD20 recombinant chimeric murine-human monoclonal antibody. It binds to CD-20 which is expressed on precursors and mature B cells but not on plasma cells. Binding triggers a series of cytotoxic immune response resulting in the elimination of B cells and the reduction in antibody formation. Use of Rituximab means that crossmatches need to be interpreted with care. The anti-CD20 means that the B cell CDC and flowcytometric crossmatch will likely be positive. It can also potentially reduce the number of B cells available in the crossmatch.

IVIG

The exact mechanism of action of IVIg is unknown but the proposed mechanisms include an anti-idiotypic effect of IVIg on HLA antibodies, saturation of the neonatal Fc receptor which would normally protect endogenous HLA IgG molecules and induction of apoptosis in B cells. IVIg is derived from pooled human plasma and can therefore cause a positive crossmatch if administered before crossmatch sample is taken.

CYCLOPHOSPHAMIDE

Cyclophosphamide, an alkylating agent, is one of the most efficacious immunosuppressive drugs available. Cyclophosphamide is used for treatment of autoimmune disorders such as SLE and multiple sclerosis. Treatment with Cyclophosphamide can cause a loss of B cells which makes B cell auto crossmatching impossible.

TRANSPLANTING ACROSS A POSITIVE FLOWCYTOMETRIC CROSSMATCH

A B Cell Pos. Flow crossmatch in the presence of HLA class II DSA in the current or historic sera would be considered an intermediate risk. Further testing such as auto-crossmatch may be required if not done already to eliminate auto antibodies and if not done yet, a Luminex SAB test would be carried out in the B cell weak Pos. sample to determine if the flow reactivity was due to HLA.

Enhanced immunosuppression and post-transplant antibody monitoring would be recommended to identify and potential changes in DSA. Frequency of testing could be 7, 14 and 28 days post-transplant, followed by 3, 6, 9 and 12 monthly testing if stable and also testing following any rejection episodes.

MARGINAL DECEASED DONOR OFFER FOR PATIENT WITH LIVE DONOR

Patients being worked up for a live donor transplant are suspended from the ODT waiting list 1 month before the transplant to avoid this scenario. Ultimately the decision is made by the patient after full discussion with the clinician. It partly depends on an assessment of the offered deceased kidney in terms of DBD/DCD,

donor age, level of match etc. Use of the deceased donor avoids an operation on the live donor and that live donor can still be kept in reserve for a future date.

PROBLEMS ON CALL

The main role of the consultant clinical scientist on call is to provide support to the state registered scientists who man the on-call rota, providing advice with interpretation of any complex results. They may also be required to discuss options and provide advice to the transplant team depending on the results.

Staff on the on call rota would normally be expected to be able to correctly select the sera to crossmatch based on written policies and to be able to interpret and report straightforward results but they know they have the assurance of a consultant clinical scientist on the other end of the phone should they need them.

POST-TRANSPLANT

PASSENGER LYMPHOCYTES AND KIDNEY TRANSPLANTATION

Passenger lymphocytes are donor derived lymphocytes that are transferred into the patient during solid organ transplant. Passenger lymphocytes can cause conditions such as severe haemolytic anaemia in minor ABO mismatched transplantation in a condition known as Passenger lymphocytes syndrome(PLS) and Solid Organ associated GvHD.

In PLS, transplant of an O+ kidney into an AB+ recipient for example represents a minor ABO mismatch. Donor derived Passenger lymphocytes by be present and may produce anti-ABO antibodies directed at the patient's AB+ red blood cells and can lead to sever haemolytic anaemia.

The condition is often self limiting after a few week but can be supported with blood transfusion if required. In severe cases, use of Ritiximab may be indicated.

In SO-GvHD, passenger lymphocytes from the transplanted organ, mismatched with the patient in the GvH direction but matched in the HvG direction may engraft and lead to GvHD which is often difficult to control as there is bone marrow involvement.

BIOLOGICAL PROCESSES INVOLVED IN COLD ISCHAEMIA AND REPERFUSION INJURY

An extended period of cold ischaemia time (over 21hrs) is associated with delayed graft function (DGF). This is as a result of varying degrees of cold ischaemia and reperfusion injury despite improvements in hypothermic preservation solutions that have been developed to maintain tissue viability and reduce the accumulation of toxic substances.

A prolonged cold ischaemia time followed by reperfusion leads to the production of reactive oxygen species which induce adhesion molecule expression and increased production of cytokines, resulting in infiltration of neutrophils, followed by mononuclear cell infiltration and the upregulation of HLA antigens, all contributing to an inflammatory response which causes acute tubular necrosis.

Steps that can be taken to reduce cold ischaemia time include detailed and regular three monthly solid phase HLA antibody testing such as by Luminex to provide a detailed antibody history that would allow some carefully selected patients to proceed to transplant on the basis of a virtual pre-transplant crossmatch, with a retrospective crossmatch. For patients where a wet pre-transplant crossmatch is indicated, it is possible to request peripheral blood from the donor ahead of organ retrieval to allow a crossmatch can be performed and the cold ischaemia time kept to a minimum.

POST SOLID ORGAN TRANSPLANT MONITORING

Solid organ transplantation has become an established therapy for the treatment of organ failure. Early allograft survival has improved dramatically over the last 3 decades with improvements in surgical and laboratory techniques as well as improvements in immunosuppressive regimes such as the use of the calcineurin inhibitors (CNI) Cyclosporin A and later Tacrolimus and the introduction of

Sirolimus. Late graft loss however remains fairly static with 10 year graft survival at around 50% for kidneys and even lower for other organs.

Various techniques exist for monitoring graft function post transplant for signs non function and rejection, some of which do not typically involve the H&I laboratory. In the case of kidney transplantation, these include monitoring of serum creatinine levels for signs of a rise. This may be accompanied by monitoring of other signs of kidney dysfunction such as haematuria and proteinuria levels. The results of these may indicate a need for a biopsy. Biopsy signs of rejection include amongst other things, interstitial lymphocytic infiltration, interstitial fibrosis, tubulitis, tubular atrophy, vascular occlusion, thickening of glomerular capillaries, expansion and duplication of the glomerular basement membrane and C4d deposition. Other assays undertaken to assess renal function post-transplant include monitoring levels of cytotoxic T lymphocyte transcripts such as granzyme B and perforin.

The role of the H&I laboratory in post kidney transplant monitoring mainly involves testing for HLA antibodies post transplant but may also include testing for other antibodies such as anti-MICA or anti-endothelial cell antibodies. HLA antibodies have been implicated in all stages of allograft rejection from the hyperacute stage, to the acute and chronic phases. Hyperacute rejection occurs within minutes of transplant and is usually due to the presence of pre-formed donor specific antibodies. Acute rejection occurs within days to weeks, while chronic antibody mediated rejection occurs months to years post transplant. Recent data from the 14th international histocompatibility workshop demonstrated that four year deceased donor kidney allograft survival was 20% less in patients with donor specific antibodies compared to donors with no antibodies. Care is however needed as the time between detection of circulating donor specific antibodies and allograft failure does vary considerable in different studies, with some grafts functioning normally many years post the detection of DSA. Patients with non donor specific HLA antibodies also had a significant reduction in graft survival compared to non sensitised patients. In other studies, patients with HLA and MICA antibodies alone were shown to have significantly reduced allograft survival compared antibody negative patients. Patients with MICA antibodies alone also had reduced allograft survival though the effect was less than in patients with HLA antibodies alone.

The BSHI/BTS 2010 guidelines recommend that post-transplant antibody monitoring should be performed at agreed regular intervals, at the time of biopsy and in cases of

suspected rejection. Antibody testing should also be undertaken at times of declining graft function when there is no other clinical cause. The guidelines do not however recommend a testing frequency.

The testing frequency is agreed between the H&I laboratory and the transplant centre. One example is to test at 1 week post transplant, then again at 1, 3, 6 and 12 months post transplant, then annually. Some labs collect samples at three monthly intervals as in the pre-transplant setting but store these rather than routinely test them unless the patient experiences declining renal function. This strategy allows a retrospective review of the patients post transplant antibody history should rejection be suspected without the costs of routine and regular screening of all post transplant samples. For patients who undergo HLA antibody incompatible transplantation, the BTS/BSHI guidelines recommend a post transplant testing frequency that matches the risk of adverse immunological events, especially for those patients who have antibody removal prior to transplant. The testing frequency for antibody incompatible transplantation with antibody removal in some protocols is daily for the first week, followed by weekly for the first month and monthly thereafter.

Post-transplant antibody testing in the UK is mainly carried out by Luminex solid phase assays. Any of the three types of Luminex assays may be used depending on the clinical scenario. Routine screening in suspected cases of rejection may use the mixed bead screen method or the phenotype panel system, with specificity confirmed by single antigen bead assays as required. Desensitisation programs will likely use single antigen bead methods. The Luminex solid phase assays have the advantage of also giving results for anti MIC antibodies.

Circulating donor specific antibodies together with C4d+ staining in biopsy in the presence of declining graft function indicate antibody mediated rejection. Early diagnosis is required if the graft is to be salvaged. Treatment options include removal of antibody by plasmapheresis, use of Ivlg, use of anti-proliferative agents and Rituximab.

The H&I laboratory may also be involved in testing for antibodies to organ specific markers in other solid organ transplantation. Antibodies directed against the endothelial cell antigen vimentin for instance have been shown to be associated with the development of transplant associated vasculopathy in cardiac transplantation.

POST-TRANSPLANT COMPLICATIONS

MANAGEMENT OF A POST-TRANSPLANT PATIENT WITH A RISING CREATININE AND C4D POSITIVE BIOPSY

A rising creatinine and C4d positive biopsy is suggestive of antibody mediated rejection if DSA are present. A new sample would be requested for follow up antibody investigation if the last sample was more than 3 months ago. All the HLA antibody tests since the transplant and including the transplant sample itself will be reviewed and the DSA levels tabulated and graphed to show changes in the DSA level over time.

The clinicians will be advised to consider intervention if DSA levels are over 5000 MFI. Interventions to consider include starting on rituximab and IVIg and monitoring antibody levels. If antibody levels do not come down or graft dysfunction is detected, then plasmapheresis or immunoadsorption and possibly Bortezimab would be considered.

The H&I laboratory would undertake Luminex SAB titre tests to predict the number of plasmapheresis or immunoadsorption rounds that may be required and if these proceed, pre and post plasmapheresis/immunoadsorption Luminex SAB testing to determine if DSA MFI levels are reducing.

BK VIRUS POST-TRANSPLANT

Renal failure from BK virus (BKV) infection is a relatively common challenge in kidney transplant recipients and is associated with the potent immunosuppression used to reduce rejection. If left untreated, BKV infections can lead to kidney allograft dysfunction or loss.

BKV replication typically begins early after transplantation and/or after treatment for rejection when immunosuppression levels are at their greatest. The primary treatment for BKV nephropathy is a reduction in immunosuppression. This however runs the risk of increasing rejection. It is therefore vital that screening is undertaken

early to detect and prevent BKV nephropathy. Reduction in immunosuppression results in a significant increase in BKV-specific IgG antibodies, the emergence of BKV-specific cellular immunity, clearance of the BKV and increase in graft function as measured by a reduction in creatinine levels.

From the laboratory perspective, a kidney biopsy remains the gold standard for diagnosing BKV nephropathy. Luminex DSA levels should also be monitored closely during any period of reduction in immunosuppression.

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER

A post-transplant lymphoproliferative disorder (PTLD) is a potentially fatal complication of kidney and other solid organ transplantation. PTLD after solid organ transplantation almost always derives from recipient lymphoid cells. The majority of cases are associated with EBV infection or reactivation as a result of the patient being in an immunocompromised state.

Laboratory diagnosis of PTLD is mainly based on histology rather than H&I tests, though screening for an increase in EBV DNA levels may give an early indication. First line treatment mainly involves a reduction in immunosuppression. More recently use of Rituximab has shown some promise.

As the first line treatment is a reduction in immunosuppression, Luminex SAB DSA levels should be monitored closely during any period of reduction in immunosuppression.

PANCREAS AFTER KIDNEY

Patients listed for pancreas after kidney (PAK). A note will be made of all mismatches with the kidney and these will be listed as unacceptable if the patient has DSA directed against them of if there are gaps in the antibody history. A limited study in heart patients has shown that in the absence of detectable HLA-antibodies, repeat mismatches are not associated with worse graft or patient survival. Therefore if a full antibody history is available mismatches to which the patient has not

developed HLA antibodies will not be listed as unacceptable. However if the offered organ has a repeat mismatch this will be highlighted to the transplant team.

One key consideration for PAK is the type and level of immunosuppression required. This needs to be discussed between the two transplant teams.

KIDNEY AFTER STEM CELL TRANSPLANTATION

- Acute kidney injury (AKI) is a common post HSCT complication with an incidence ranging from 20% to 73%
- Contributory risk factors to the development of AKI post HSCT include use of development of sepsis, use of cyclosporine and other nephrotoxic drugs, myeloablative induction and development of graft-versus-host disease (GVHD)
- The main management approach for the avoidance of AKI in stem cell transplant patients is the avoidance of risk factors which contribute to AKI
- Involvement of a nephrologist in the post-transplant management of HSCT patient may be desirable. HSCT patients should be periodically monitored for Creatinine levels
- For completeness, it may be desirable to screen for BK virus
- Where AKI is suspected, depending on the rate of loss of renal function, pre-emptive listing prior to dialysis may be desirable for such patient
- A kidney transplant from the stem cell donor may be undertaken with minimal or no maintenance immunosuppression if 100% donor chimerism is confirmed
- Initial immunosuppression is still required to avoid memory T and B cells which have potentially survived
- If it is not possible to obtain a kidney from the stem cell donor then discussions are required between the stem cell and kidney transplant teams to agree an induction and immunosuppression strategy for any kidney transplant

PREGNANCY AFTER KIDNEY TRANSPLANTATION

Many patients, male and female, have their fertility affected by kidney disease and pregnancy after transplant is not uncommon. For females who are thinking of having children post-transplant, an important consideration is the choice of immunosuppression. Many of the drugs used for induction and immunosuppression pose a risk to the unborn child. For this reason, females of child bearing age who are about to undergo transplantation have a pregnancy test to confirm they are not pregnant before starting treatment.

An ideal profile of the potential kidney transplant mother includes "normal" or "good" kidney function (usually defined as glomerular filtration rate, GFR ≥ 60 ml/min), scant or no proteinuria (usually defined as below 500 mg/dl), normal or well controlled blood pressure (one drug only and no sign of end-organ damage), no recent acute rejection, good compliance and low-dose immunosuppression, without the use of potentially teratogen drugs (mycophenolic acid and m-Tor inhibitors) and an interval of at least 1–2 years after transplantation.

In pregnancy post-transplant there are risks to the unborn child, risks to the kidney graft and risk to the health of the mother.

There are risks to the child associated with the length of the gestation, maternal health, risk of disease transmission and importantly, risks from the immunosuppression in use. In addition, there are risks associated with the original kidney disease for which the mother underwent transplantation. The original kidney disease is an independent risk factor for preeclampsia, prematurity, low birth weight and neonatal death. The impact of these on the pregnancy is influenced by the degree of renal dysfunction, any pre-existing hypertension and the extent of proteinuria.

The mean gestational age for pregnancy after transplant tends to be shorter (34 weeks) than for other pregnancies and so plans should be made to manage early delivery. The risk posed by calcineurin inhibitors such as cyclosporin and tacrolimus is unknown though there are some concerns. Mycophenolate mofetil (MMF) and Sirolimus (rapamycin) are considered harmful and current recommendations are to avoid their use for 6week before pregnancy.

Risks to the graft include risk to long term graft function and risk of rejection. A rising serum creatinine level, associated with proteinuria, significantly increases the risk for irreversible graft loss as a result of the pregnancy. Achieving the correct blood immunosuppression maintenance levels in pregnancy can be a challenge as a result of changes in blood volume, thus increasing the risk of rejection. Rejection can be treated with corticosteroids.

Risks to the mother include hypertension which must be treated to maintain normal blood pressures to reduce the risk of preeclampsia. Preeclampsia can cause severe maternal and foetal complications such as renal failure, HELLP syndrome (haemolysis, elevated liver enzymes, and thrombocytopenia), seizures, liver failure, stroke and potentially death for the mother. Other comorbidities which can be present in maternal transplant patients include gestational diabetes, anaemia and infections such as urinary tract infections.

It is noted from several small studies that women who become pregnant after donating a kidney have little cause for concern.

From the point of view of the H&I laboratory, the key contribution that is made is to help monitor HLA donor specific antibody levels as immunosuppression is changed and tailored for pregnancy and also at the time of biopsy to help interpret the biopsy results.

COMPLEX KIDNEY TRANSPLANT SCENARIOS

KIDNEY TRANSPLANTATION IN SICKLE CELL PATIENTS

Renal complications are a well-known cause of morbidity and mortality in Sickle Cell Disease (SCD). SCD is a genetic disorder resulting from the presence of a mutated form of haemoglobin, haemoglobin S (HbS), which causes red blood cells to develop a rigid, sickle-like shape under reduced oxygen conditions, leading to haemolysis and vaso-occlusion. SCD results in a range of renal complications, including various tubular and glomerular functional changes and potentially, gross anatomic alterations of the kidneys. Kidney injury as a result of sickle cell is known as Sickle Cell Nephropathy (SCN).

The underlying mechanisms that lead to SCN relate mainly to hypoxia (lack of oxygen) and ischaemia (lack of blood supply). Hypoxia and Ischaemia as a result of RBC sickling can lead to impairment of solute reabsorption and urinary concentrating ability. They can also lead to impairment of renal acidification and potassium secretion resulting in hyperkalaemia and in incomplete tubular acidosis.

There are very few studies specifically looking at progression to End Stage Renal Disease (ESRD) secondary to SCN. Prognosis is poor in ESRD even on dialysis. Kidney transplantation offers the best long-term outcome in such patients. SCD patients tend to be transfusion dependent and can potentially develop antibodies to HLA. As patient DJ has a long history of transfusion, his antibody history would need to be carefully reviewed with respect to his sibling donor's HLA type.

An initial and final pre-transplant crossmatch test would be required. The initial crossmatch can be virtual if the sibling donor is fully HLA matched or if there are no DSAs. There is a ¼ chance his sibling could be fully matched. If there are a choice of compatible sibling donors, it may be useful to screen the donors for the APO-L1 gene. APOL1 encodes an apolipoprotein. A variant of the gene found commonly in persons of West African descent, confers resistance to sleeping sickness. Homozygosity for this variant has however been shown to increase kidney disease risk. Where there is a choice, kidney donation must be from a sibling who does not have the risk mutation of APO-L1.

Patients transplanted for SCN often receive peri-operative transfusions if anaemic or exchange transfusions if not. Post-transplant patients are also at higher risk of sickle cell crisis and therefore receive post-transplant exchange transfusions. Early and ongoing post-transplant DSA level monitoring is therefore required.

There is a significant risk of recurrence of SCN.

Patients with HIV can be considered for kidney transplant if they meet all the normal criteria for kidney transplantation and in addition, they must be on a stable highly active anti-retroviral treatment (HAART) regimen and have viral suppression for at least 6 months with CD34 count greater than 200. Compliance with the immunosuppression regime is vital for successful transplantation. Stability on HAART is a good indicator. Phycological review may also be useful. The increased risk of transplant must have been explained to HIV patient which they must accept the risk.

All HIV positive patients must be discussed at MDT prior to listing. HIV patients are not suitable for some immunosuppression strategies such as ATG, therefore the MDT discussions should include the unacceptable mismatches strategy. Typically, all specificities with MFY greater than the positive cut off for the laboratory (~2000 MFI) must be listed as unacceptable.

At the time of a donor offer, the risks of transplant versus maintenance on dialysis are again carefully reviewed. Marginal kidneys may not be the best option for patients who are stable on dialysis.

A pre-transplant wet crossmatch is required for all HIV patients irrespective of DSA status. The crossmatch must include samples from as much of the patient history as possible to give a complete risk assessment.

Post-transplant, the HIV patients should be reviewed by the HIV team who should advise specifically on dosing of HAART. Regular post-transplant antibody monitoring is recommended, especially at times of suspected rejection.

KIDNEY TRANSPLANTATION IN DIABETIC PATIENTS

Diabetes is one of the most important causes of chronic kidney disease (CKD) and in patients with advanced diabetic kidney disease, kidney +/- pancreas transplant is the treatment of choice. The prognosis of diabetic patients with end stage kidney disease (ESRD) and without the possibility of live donor kidney transplant or early deceased donor transplant is poor. Therefore, for these patients all effort should be

made to secure a live transplant, either directly or through the live donor sharing scheme (paired exchange).

There are no randomized controlled trails comparing forms of transplantation in type 1 diabetic patients with end stage kidney failure. Live donor kidney appears to have the best outcomes even over deceased donor SPK. Live donor is also preferable where the patient requires PAK.

Type 2 diabetic patients with end stage kidney failure should be offered a live donor transplant if they have a donor as outcomes are better than deceased donor transplant which is in turn better than dialysis.

KIDNEY TRANSPLANTATION IN PAEDIATRIC PATIENTS

There are a number of additional factors which should be taken into account in paediatric kidney transplantation which may not always be considered in adult transplantation. The most obvious is that the patient will likely need a second transplant in their lifetime and so steps should be taken to reduce the risk of alloimmunisation from the first transplant. For instance if a paediatric patient was not being transplanted with kidney from one of their parents in the first transplant, the parental HLA mismatches could be listed as non-antibody defined unacceptable mismatches so that the patient could potentially be protected from forming HLA antibodies against those mismatches, making the parent acceptable future direct donors.

KIDNEY ALLOGRAFT REJECTION

BANFF CATEGORIES

In 1991 a group of renal pathologists, nephrologists and transplant surgeons met in Banff, Canada to agree a standardised international classification system of renal allograft biopsies. A Banff conference is held every year and the original Banff classification scheme has evolved significantly since first introduced. The most recent review has 6 categories:

- Normal or Nonspecific Changes

- Antibody-Mediated Changes
- Borderline Changes
- T-cell Mediated Rejection
- Interstitial Fibrosis and Tubular Atrophy
- Other Changes Not Considered to be Due to Acute or Chronic Rejection

An adequate biopsy sample for assigning of Banff categories must have a minimum of 7 glomeruli with one artery. Ideally the sample should have 10 or more glomeruli with at least two arteries.

Category 1 shows no change or nonspecific changes.

Category 2, Antibody mediated changes, are classified into three groups, 2a active antibody mediated changes, 2b chronic active antibody-mediated rejection and 2c C4d staining without evidence of rejection. The first two groups 2a and 2b both require 3 things:

- Evidence of tissue injury such as microvascular inflammation
- C4d staining in peritubular capillaries
- Presence of donor specific antibodies (DSA)

Category 2c has C4d without evidence of circulating DSA.

Category 3, borderline changes are biopsies which are suspicious for acute rejection and involves either minor interstitial inflammation with foci of tubulitis or moderate to severe interstitial inflammation (T cell inflammation), both with no arteritis (inflammation of the walls of arteries).

Category 4, T-cell mediated rejection, are classified into two groups, 4a Acute T-cell-mediated rejection and 4b Chronic active T-cell-mediated rejection. Interstitial inflammation >25%, mild to moderate intimal arteritis and tubulitis and presence of arteritis. Chronic active T-cell-mediated rejection additionally requires chronic allograft arteriopathy.

Category 5, Interstitial Fibrosis and Tubular Atrophy, as the name implies, requires the presence of mild, moderate or severe interstitial fibrosis and tubular atrophy.

Category 6, Other Changes Not Considered to be Due to Acute or Chronic Rejection, involves changes seen on biopsy that are not due to acute or chronic rejection. Examples include:

- BK virus nephropathy
- Posttransplant lymphoproliferative disorders
- Calcineurin inhibitor nephrotoxicity
- Acute tubular injury
- Recurrent disease
- De novo glomerulopathy
- Drug induced interstitial nephritis
- Pyelonephritis (inflammation of the kidney tissue and renal pelvis)

This patient has been classified as 2a - Active antibody mediated changes. This category implies the patient has evidence of evidence of tissue injury, is C4d Pos., and the laboratory has demonstrated the presence of DSA. If creatinine levels are also rising, the renal unit may choose to enhance the immunosuppression and/or undertake plasma exchange. The H&I laboratory can support this course of treatment by undertaking more DSA studies. In some circumstances it may also be informative to undertake a post-transplant crossmatch to compare with the crossmatch done at the time of transplant.

CHRONIC ALLOGRAFT NEPHROPATHY (CAN)

The definition of Chronic Allograft Nephropathy (CAN) has changed over the years. Initially it was used almost interchangeably with chronic rejection. However 'chronic rejection' implies an alloimmune mechanism whereas the histological features of CAN have been shown to be present in post 1 year protocol biopsies even in the absence of alloimmune processes. CAN has come to be defined as an end point of tubular atrophy and interstitial fibrosis in the graft resulting from a number of immune and non immune processes. Typical histological features also include fibrous intimal thickening and the presence of glomerular lesions.

The mechanisms which contribute to the development of chronic allograft nephropathy can be divided into three broad categories, Input causes such as pre-existing chronic donor condition, Immune causes such as acute and chronic rejection and Load causes such as donor – recipient size disparity.

Input mechanisms which contribute to the development of CAN may be donor specific, recipient specific or related to the transplant process itself. Increased donor age has been shown to be associated with increased rates of CAN. Kidneys from older donors may have a reduced ability to withstand stress and may already be carrying some structural damage. They also typically have delayed renal function compared to kidneys from younger donors. This is compounded if the donor has a history of hypertension which, in itself, increases pressure in the glomerulus, causing injury to glomerular cells and leads to glomerulosclerosis – a scarring of the glomerulus. Glomerulosclerosis disturbs the filtering process of the kidney and allows proteinuria - leaking of protein from the blood into urine. In addition, any pre-existing chronic kidney condition in the donor may be carried over to the patient and may contribute to the development of CAN. The type of donor, Donation after Cardiac Death (DCD) versus Donation after Brain Death (DBD), makes differing contributions to CAN.

Patient input factors shown in studies to be associated with the development of CAN include increased patient age, male gender, high body mass index (BMI) and a diabetic status. Like hypertension, diabetes can cause glomerulosclerosis and therefore loss of graft function leading ultimately to CAN. Diabetes pre transplant does carry the additional distinction of being a good predictor of post transplant cardiac events.

The transplant process itself contributes, as an input factor, to the development of CAN. Many of the stages of the transplant process could potentially injure the kidney, triggering pro-inflammatory responses which may further harm the kidney. Injury can occur during donor maintenance prior to retrieval, during the retrieval process and during cold and warm ischaemia. The role of ischaemia in CAN is highlighted by the reduced incidence of CAN in transplantation from live donors, which typically have a much shorter ischemic period, compared to those from deceased donors. Prolonged warm and cold ischaemia leads to the upregulation of HLA antigens and adhesion molecules and to the increased production of cytokines, all contributing to inflammatory responses which cause acute tubular necrosis, one

of the symptoms of CAN. Another common mechanism of injury leading to the development of CAN is reperfusion injury. During reperfusion the resupply of blood to an organ that has been deprived of oxygen leads to the production of reactive oxygen species (ROS) which contribute to kidney injury. Taken together, ischaemia and reperfusion injury (IRI) are the biggest non immune cause of delayed graft function. Delayed graft function correlates very strongly with CAN. DGF is contributed to by all the input factors including existing chronic donor kidney condition and IRI as well as a number of immune mechanisms such as acute rejection.

The immune mechanisms which contribute to CAN include acute and chronic alloantibody mediated rejection (AMR) as well as acute and chronic cellular rejection and leads to a distinct set of histological feature, chiefly, Transplant Glomerulopathy (TG), characterised by a doubling of the glomerular basement membrane (GBM). Late acute rejection that is recurrent and resistant to treatment is a key predictor of the development of CAN. The number of acute rejection episodes correlates well with the number of HLA mismatches, even in the CNI era. HLA matching reduces the incidence of both early and late acute rejection. The relationship between pre-existing donor specific anti HLA antibodies (DSA) and the development of hyperacute or accelerate rejection is well documented. The level of panel reactive antibody (PRA) of non donor specific anti HLA antibodies in circulation pre transplantation and the development of donor specific anti HLA antibodies post transplantation are the strongest independent risk factors of acute rejection. Studies show that the presence of class II or of class I and II antibodies together may be more predictive of this antibody mediated rejection (AMR) than the presence of class I antibodies alone. A key surrogate of AMR is the presence of C4d staining in the peritubular capillaries, though not all patients with anti-HLA antibodies will necessarily exhibit C4d deposition (The Banff classification of renal allograft pathology has been changed recently to capture the role of C4d staining.). Acute cellular rejection involves diffuse infiltration of the interstitium with CD4+ and CD8+ T cells and macrophages with an alloreative immune response initiated at the graft endothelium. This leads to cytotoxic T cell activation, NK and monocytes infiltration and antibody formation. Immune responses are mainly directed against HLA though responses involving antibodies against other antigens such as the MHC like class I A and B (MICA and MICB) and antiendothelial cell antibodies have been reported. Alloreactivity involves both the direct and indirect pathways of antigen presentation with the indirect pathway in particular believed to play a dominant role in the development of CAN whilst the direct pathway is believed to be predominantly involved in early acute cellular rejection. As it requires donor antigen presenting cells which will not be long lived post transplant.

The incidence of true immunological chronic cellular rejection is reduced in the CNI era in patients who are compliant with their Immunosuppression regimes. When present however chronic cellular rejection is associated with a significantly increased risk of CAN. Recent data from the 14th international histocompatibility workshop (Terasaki et al) demonstrated that four year deceased donor kidney allograft survival was 20% less in patients with donor specific antibodies compared to donors with no HLA antibodies. The evolution of chronic antibody mediated rejection in believed to begin with the development of DSA, followed by C4d in the peritubular capillaries (PTC), then the development of TG and other graft pathology and finally graft dysfunction.

The use of desensitisation protocols to transplant patients across the ABO and HLA barriers as part of ABO incompatible and/or HLA antibody incompatible transplant programs will present interesting new long term data on the role of immune responses in the development of CAN. These patients are potentially at higher risk of AMR if not in the acute phase then certainly in the chronic phase. In addition, de-novo IgG HLA alloantibodies developed post transplant in addition to functioning as a pathogenic factor in the development of CAN may also be a surrogate marker for ongoing indirect T cell immunity.

The non immune post transplant load factors believed to play a role in the development of CAN include drug toxicity, including CNI nephrotoxicity, donor – recipient size disparity, hypertension, hyperlipidemia, proteinuria, infection, life style and patient disease recurrence.

The CNI's CsA and Tacrolimus cause damage by constricting the afferent arterioles leading to localised ischaemia and eventually to glomerular collapse. On the other hand, these CNI's are beneficial in that they protect against the effects of immune injury. The contribution of these CNI to the development of CAN is therefore a function of the balance between their toxicity and their immunosuppressive capabilities. To reduce toxicity, CNI's can be replaced with alternatives such as Mycophenolate mofetil (MMF).

Differences in donor – recipient size have been shown to have a small effect on development of CAN with very large recipients showing significantly reduced graft survival in multivariate studies. Female recipients of male kidneys have been shown to have slightly better graft survival than sex matched transplants. This effect is believed to be due to 'nephron dose' with low numbers of available nephrons leading to hyperfiltration in the remaining nephrons to meet the excess load, eventually leading to nephron exhaustion. Hypertension, which is very common in kidney patients post transplant, also leads to hyperfiltration causing glomerulosclerosis and is significantly associated with graft failure. The presence of pre transplant hypertension in the patient and/or donor, of delayed graft function at the time of transplant and the use of calcineurin inhibitors (CNI) all contribute to the development of post transplant hypertension. Similarly, hyperlipidemia is very common in post transplant patients and is significantly associated with development of CAN. It is contributed to by the presence of pre transplant hyperlipidemia, by rapid post transplant weight gain and by the presence of diabetes. Development of post transplant diabetes is a significant risk factor for graft failure.

Proteinuria (leaking of protein from the blood into urine) may contribute to CAN through the toxicity of filtered protein to the tubules.

Infection plays a significant role in the development of CAN firstly because it may lead to the altering or otherwise lowering of Immunosuppression therefore increasing the immune response but also because infection can also directly cause CAN. Renal transplant patients who develop opportunistic infections after 6 months usually have higher serum creatinine levels. The polyoma virus, BK, which is highly prevalent in the population but normally asymptomatic in immunocompetent persons, can become reactivated in immunocompromised patients and lead to allograft nephropathy through viral replication in the tubules, leading to apoptosis or necrosis of tubular epithelial cells. Kidneys from CMV positive donors have been shown to be associated with a small but significant reduction in allograft survival though it is not clear this is directly due to the development of CAN. The role of CMV reactivation may well be limited to the effect this has on managing immunosuppression levels and therefore the knock on effect that potentially has on immune reactivity.

Lifestyle choices, particularly non compliance with immunosuppressive regimes and smoking, can contribute significantly to the development of CAN. Studies have

shown that smokers are more likely to require allograft biopsy one year post transplant than non smokers. One study found that in histological analysis, the amount of smoking correlated with the severity of vascular fibrosis and intimal thickening observed. The effect of donor smoking on the development of CAN is unclear.

Finally, CAN may be caused by patient disease recurrence. Renal transplantation does not after all cure the underlying disease. Patients whose primary disease was Glomerulonephritis are at risk. The contribution of disease recurrence to CAN looks set to increase, particularly as improved immunosuppression increases the half life of transplanted kidneys.

The current definition of chronic allograft nephropathy (CAN) is that it is the common end point of a series of immune and non immune insults to the kidney resulting ultimately in graft failure. It is contributed to by donor, patient and transplant process input factors, by immune factors involving acute and chronic humoral and cellular processes and by load factors post-transplant including drug toxicity and infection.

CHAPTER 7 CARDIOTHORACIC TRANSPLANTATION

LISTING PATIENTS FOR HEART TRANSPLANTATION

Patient HLA type and antibodies are not used as part of the listing but form part of the immunological risk assessment of any offer. Therefore prior to listing, laboratory tests and other data that would be required include:

- ABO grouping and confirmation
- HLA typing and confirmation
- HLA antibody screening by CDC and Luninex on at least two samples taken not less than 24 hours apart
- Patient demographics, including DOB
- Potential age related co-morbidities
- Patient height and weight
- Patient renal function
- Patient NHS status
- Patient virology, including HIV, EBV, CMV Hepatitis B and C
- Other co-morbidities such as BMI, smoking history, hypertension, vascular disease
- Current or historic malignancy status
- Listing category e.g. Super Urgent, Urgent, Non Urgent
- Once listed, 3 monthly HLA antibody screening by CDC and Luminex

THE HEART ALLOCATION SCHEME IN THE UK

The most recent change, due to go live in Oct 2016, is the introduction of the Super Urgent Heart Allocation (SUHAS) Scheme. This is for a category of patients who already meet all of the criteria for listing on the Urgent Heart Allocation (UHAS) Scheme but who in addition, are critically ill on short-term VAD's with which they cannot be released to go home and who are not suitable for long term VAD's. The aim of introducing this new category is to ensure that patients with the greatest need will have the highest priority for a donor heart.

The effect on the H&I does not change much as the work involved would be similar to that already being done for the UHAS. The pre-listing tests do not change nor do

the regular 3 monthly antibody tests. One thing to make sure of if only one HLA antibody has been tested is to make sure a second sample, bled at least 24 hours apart is taken and tests. Once listed, in when an offer is made the carries out the immunological risk assessment to advice on the level of risk based on BST/BSHI guidelines and any locally agreed variations.

Criteria for the UHAS include:

- Patients on mechanical circulatory support devices (MCSD)
- Patients with Intra-Aortic Balloon Devices (IABD)
- Patients on Extra-Corporeal Membrane Oxygenation (ECMO)
- Patients on some muscle contraction medication (inotropes)

THE LUNG ALLOCATION SCHEME IN THE UK

Recent changes to the lung allocation scheme in the UK involve changes to organ sharing between the UK and Ireland. The other change which is really more of a clarification, is changes to organ offering for heart patients who also require lungs.

The organ sharing scheme with Europe now explicitly states the EU and Ireland. This does not affect the UK H&I labs much as only organs not placed in the UK are offered for sharing across the EU.

The clarification for heart transplant patients who also need lungs is that if an urgent heart patient also needs lungs, the transplant centre will inform the duty office who will then advice all transplant centres with lung patients above the heart patient in the lung transplant waiting list. The decision on whether or not the heart transplant patient will also get the lungs will then be made by all the involved transplant centres in consultation.

There is minimum direct effect on the H&I laboratory of this change/clarification except perhaps being asked to be involved in the immunological risk assessment of a patient involved in these decisions.

ROLE OF HLA MATCHING IN CARDIOTHORACIC TRANSPLANTATION

Heart allocation is based on blood group and size match taking into account clinical urgency and HLA antibody risk stratification and not based on HLA type matching. Therefore, in this scenario, list the patient with the initial HLA type and use that and the antibody screen as part of the immunological risk assessment and do the CT at the time of offer or immediately post-transplant.

RELEVANCE OF HLA ANTIBODIES IN CARDIOTHORACIC TRANSPLANTATION

RISK ASSESSMENT

A prospective wet crossmatch would not be required. A risk stratification based on the BST guidelines would be carried out as follows:

- No DSA = Level I Standard Risk
- DSA < 2000 MFI = Level II Low Risk
- DSA > 2000, < 5000 = Level III Intermediate Risk. Low risk of hyperacute rejection but significant risk of early rejection and antibody mediated graft damage. Immediate pre-transplant antibody reduction may be considered when feasible
- DSA > 5000, = Level IV High Risk

The CDC test results would also be used as part of the risk stratification. Luminex Pos. CDC Neg. with cumulative DSA less than 8000 would not be considered a contraindication in our local unit.

A retrospective CDC and Flow crossmatch will be carried out and a Luminex SAB test on the day of transplant sample.

CUMULATIVE DSA MFI MORE THAN 5000

Depending on the clinical urgency and logistical practicalityNkg2, a prospective wet crossmatch may be undertaken. A prospective Luminex SAB test may be required

depending on the date bled of the last test (greater than 3 months since last sample) and any sensitising events since that sample.

A risk stratification based on the BST guidelines would be carried out at the time of any offer as follows:

- No DSA = Level I Standard Risk. Prospective wet crossmatch not required
- DSA < 2000 MFI = Level II Low Risk. Prospective wet crossmatch not required
- DSA > 2000, < 5000 = Level III Intermediate Risk. Low risk of hyperacute rejection so Prospective wet crossmatch not required. However significant risk of early rejection and antibody mediated graft damage, therefore pre-transplant antibody reduction may be considered
- DSA > 5000, = Level IV High Risk. Transplant veto unless CDC Neg. CDC test results are used as part of the risk stratification. Luminex Pos. CDC Neg. with cumulative DSA less than 8000 would not be considered a contraindication in our local unit and would not require a prospective wet crossmatch
- A retrospective CDC and Flow crossmatch will be carried out and a Luminex SAB test on the day of transplant sample.

KIDNEY AFTER HEART TRANSPLANT

Chronic kidney disease is common in heart and lung transplant patients and for these patients, pre-emptive listing prior to dialysis is desirable.

Patients listed for kidney after heart/lung will have full HLA antibody testing prior to listing. A note will be made of all mismatches with the heart and these will be listed as unacceptable if the patient has DSA directed against them of if there are gaps in the antibody history. Studies have shown that in the absence of detectable HLA-antibodies, repeat mismatches are not associated with worse graft or patient survival. Therefore if a full antibody history is available then mismatches to which the patient has not developed HLA antibodies will not be listed as unacceptable. However if the offered kidney has a repeat mismatch this will be highlighted to the renal unit.

One key consideration for kidney after heart/lung is the type and level of immunosuppression required. This needs to be discussed between the heart and renal teams to agree a strategy.

CARDIOTHORACIC PATIENT RETROSPECTIVE CROSSMATCH INTERPRETATION

WEAK B CELL POS

The transplant team would immediately be informed of the results. Further testing such as auto-crossmatch may be required if not done already to eliminate auto antibodies. A Luminex SAB test would be carried out in the B cell weak Pos. sample to determine if the flow reactivity was due to HLA. Post-transplant antibody monitoring would be recommended to identify and potential changes in DSA.

LUMINEX DOES NOT CORRELATE WITH CROSSMATCH

Historic Luminex DSA Pos., CDC Neg, FXCM T & B Cell Pos. on its own would be considered intermediate risk and should only proceed if absolutely necessary, depending on urgency. Would want to know about the priming source for these antibodies, particularly want to know if transfusion related.

The current sample is however DSA SAB Neg but still FXCM Pos. Would want to do FXCM Auto to exclude auto antibodies. Would also want to ask cardiothoracic team about treatments such as Rituximab which could help explain Pos. B Cell FXCM in the absence of DSA.

The CDC is IgG Neg so no risk of hyperacute rejection. If confirmed that priming source of historic Pos. is transfusion related and current FXCM Pos. is due to treatment, can proceed to transplant with HLAi protocol and close post transplant follow up.

CARDIOTHORACIC PATIENT POST-TRANSPLANT FOLLOW UP

TESTING INTERVALS

A retrospective CDC and Flow crossmatch will be carried out and a Luminex SAB test on the day of transplant sample. In addition, post-transplant HLA antibody testing by Luminex will be carried out at day 14 and 28 at a minimum and 3, 6, 9 and 12 monthly thereafter and also following any signs of acute rejection.

SUSPECTED AMR POST HEART TRANSPLANT

AMR is indicated in the presence of circulating DSA, graft dysfunction and C4d positive biopsy. However in this case, the Luminex screen and SAB are Neg. This needs to be confirmed/repeated, ensuring testing has been done with adsorb and with EDTA. If confirmed the C4d is likely due to non-HLA antibodies. Anti-endothelial, anti-vimentin and anti ABO and other non-HLA antibodies have been shown to be implicated in acute rejection in heart transplant patients.

If non-HLA antibodies are present this can be treated by changes in the immunosuppression regime.

HEART TRANSPLANT FOLLOW UP

A new sample would be requested for follow up antibody investigation. All the HLA antibody tests since the transplant and including the transplant sample itself will be reviewed and the DSA levels tabulated and graphed to show changes in the DSA level over time.

The clinicians will be advised to consider intervention if DSA levels are over 5000 MFI. Interventions to consider include starting on rituximab and IVIg and monitoring antibody levels. If antibody levels do not come down or graft dysfunction is detected, then plasmapheresis or immunoadsorption and possibly Bortezimab would be considered.

The H&I laboratory would undertake Luminex SAB titre tests to predict the number of plasmapheresis or immunoadsorption rounds that may be required and if these

proceed, pre and post plasmapheresis/immunoadsorption Luminex SAB testing to determine if DSA MFI levels are reducing.

LUNG TRANSPLANT FOLLOW UP

A new sample would be requested for follow up antibody investigation. All the HLA antibody tests since the transplant and including the transplant sample itself will be reviewed and the DSA levels tabulated and graphed to show changes in the DSA level over time.

The clinicians will be advised to consider intervention if DSA levels are over 8000 MFI. Interventions to consider include starting on rituximab and IVIg and monitoring antibody levels. If antibody levels do not come down or graft dysfunction is detected, then plasmapheresis or immunoadsorption and possibly Bortezimab would be considered.

The H&I laboratory would undertake Luminex SAB titre tests to predict the number of plasmapheresis or immunoadsorption rounds that may be required and if these proceed, pre and post plasmapheresis/immunoadsorption Luminex SAB testing to determine if DSA MFI levels are reducing.

CHAPTER 8 LIVER AND OTHER ABDOMINAL ORGAN TRANSPLANTATION

INTESTINAL ORGAN ALLOCATION SCHEME IN THE UK

Intestinal organ allocation is primarily on the basis of ABO, age and size matching. Other factors taken into account are clinical urgency, waiting time, requirement for other organs such as kidney or liver and HLA sensitization. The recent change that happened this year was for paediatric patients to be preferred for paediatric donors. This was achieved by increasing the point allocated to a paediatric patient for a paediatric donor from 1000 to 5000.

From the H&I laboratory perspective this does not change what the lab does. Risk stratification is based on 4 levels from no detectable HLA antibodies (level I standard risk), to MFI < 2000 (level II low risk), to MFI between 2000 and 8000 (level III intermediate risk) and MFI > 8000 (level IV high risk). A positive crossmatch in the absence of a liver transplant from the same donor constitutes a high risk.

SO-GVHD POST LIVER TRANSPLANT

Solid organ associated graft versus host disease (SOA-GvHD) is a rare and potentially lethal complication of transplantation. It presents initially with fever and skin rash but can very rapidly advance to affect multiple systems, including the bone marrow and other non-transplanted solid organs. It is a particular risk in liver (and small bowel) transplantation. One hypothesis is that the risk factor for SOA-GVHD is related to the amount of lymphoid tissue (passenger lymphocytes) in the donor organ. Other risk factors include greater HLA match between donor and recipient and patient age greater than 65 years.

SOA-GVHD has an acute phase and a chronic phase. The acute phase is defined as occurring within the first 100 days after transplant. The chronic phase is defined as occurring more than 100 days after transplant. The same symptoms typically seen in stem cell GvHD are often present, including diarrhoea, intestinal bleeding and cramping abdominal pain. The involvement of the bone marrow leads to severe pan cytopenia.

Symptoms of SOA-GvHD are usually quite subtle and are often confused with early infection in the immunocompromised patient. Diagnosis of SOA-GVHD is implied by biopsy-proven histologic features and presence of donor chimerism. H&I laboratory investigation of a suspected case of SOA-GvHD involves chimerism testing by STR to detect donor derived cells in the patients. This could be done from a blood sample. Where the SOA-GvHD involves a skin rash, affected and unaffected areas of skin can be tested to detect donor derived cells in mixed chimerism with patient cells.

Treatment of SOA-GVHD is controversial, with the limited evidence given the rarity and high mortality rates. Treatment is usually initialized with high-dose corticosteroids and methylprednisolone. Second line agents such as tacrolimus and basiliximab are also used. In addition, antibiotic, antifungal and antiviral prophylaxis should be started for infection prevention.

KIDNEY AFTER LIVER TRANSPLANT

Chronic kidney disease is common in non-renal transplant patients and for these patients, pre-emptive listing prior to dialysis is desirable.

Liver allografts are resistant to AMR from preformed HLA alloantibodies when compared to other allografts. DSA is most often cleared a few months post liver transplant marking the liver as an ‘immune privilege’ organ. Where patients have been transplanted with simultaneous liver and kidney in the presence of low levels of DSA (MFI<5,000), the liver has had a ‘protective’ effect on the kidney though high levels of DSA have been shown to result in graft rejection of both the liver and kidney.

Patients listed for kidney after liver will have full HLA antibody testing prior to listing. A note will be made of all mismatches with the liver but these will not necessarily be listed as unacceptable. However if the offered kidney has a repeat mismatch this will be highlighted to the renal unit.

One key consideration for kidney after liver is the type and level of immunosuppression required. This needs to be discussed between the liver and renal teams to agree a strategy.

CHAPTER 9 OTHER TRANSPLANTED ORGANS

CORNEA TRANSPLANTATION

Corneal transplant is indicated where it will help maintain or restore vision or restore the integrity of the eye globe, where it will relieve pain or help with the removal of an infectious or noxious agent.

H&I lab involvement in corneal transplantation is limited. HLA matching is not undertaken for allocation. There have been a handful of studies that have reported some benefit for HLA epitope matching but these need follow up.

VASCULARISED COMPOSITE ALLOTRANSPLANTATION SERVICES

The term Vascularised Composite Allotransplantation (VCA) refers to the transplantation of a graft with multiple types of tissue including bone, muscle, nerve, skin and blood vessels and is gaining acceptance as a solution for complex reconstructive problems. Examples include upper limbs, face, abdominal wall and uterus transplantation.

In contrast to traditional organ transplantation, VCA other than uterus transplantation is further complicated by the fact that the transplanted organ is external and therefore visible. This requires that the organ not just be immunologically compatible but is also aesthetically compatible in size, age, gender and skin colour. The donation of externally visible organs such as face and hands is a difficult subject to discuss with the families at the time of counselling.

VCA transplantation require ABO matching. HLA matching also taken into account but is not the primary focus given the scarcity of organs. VCA patients, especially burns victims, are often multi transfused and may have undergone several skin grafts and are therefore often HLA alloimmunised with high levels of antibodies which do need to be taken into account. The incidence of acute rejection in hand and face transplantation is reported to be around 80%, which is much greater than the rate is

traditional solid organ transplantation (SOT). Data on antibody mediated rejection and chronic rejection is more limited.

In the absence of published guidelines for VCAs, many of the standards applied to SOTs are being applied. A laboratory which has been asked to develop services in support of VCAs should seek advice, input and potentially protocol sharing from a laboratory already offering such services. The laboratory needs to ensure they sign up to the relevant EFI and UKAS accreditation standards and the incorporate the relevant training into their competency systems. The demand is likely to remain low and so may not represent a significant staff resource impact but if 24/7 access to virtual and/or wet crossmatching is required then this would have to be factored into the design of the service.

The laboratory needs to be capable of providing HLA typing to intermediate resolution across all 11 HLA loci and needs to be able to undertake HLA antibody screening and identification with solid phase technology such as Luminex. While waiting for a transplant, patients should be screened every 3 months as is the case for SOT. All patients should be discussed at Multi-Disciplinary Team (MDT) meetings.

Ability to perform a virtual crossmatch is required in addition to wet crossmatching, primarily flowcytometric crossmatching. Ability to undertake CDC crossmatching is also a potential option. A small number of hand transplants have taken place with pre transplant desensitisation in the presence of flowcytometric positive crossmatch results, followed by post-transplant plasma exchange is indicated. Tolerable cold ischaemia times are yet to be fully established but need to be kept to a minimum. Crossmatching using PBL is vital if pre-transplant crossmatching is required.

ATG is a common induction treatment in VCAs as the transplanted organ contains many donor APCs which lead to direct and indirect antigen presentation. Maintenance immunosuppression tends to derive from protocols developed for SOT and generally include tacrolimus, MMF and prednisolone.

Post-transplant monitoring follows the same antibody monitoring used for SOT. HLA antibodies should be tested for at least once in the first year or when graft

rejection is suspected. In transplants where there is DSA present at the time of transplant, a more frequent post-transplant sampling regime must be agreed with the transplant unit. A potential regime as used un cardiothoracic transplantation is to test at 2 weeks, 1, 3, 6 and 12 months and annually thereafter.

Some transplant centres are experimenting with stem cell transplantation as a means of inducing stable mixed chimerism and therefore significantly reducing immunosuppression requirements. Where such protocols are used, the laboratory may need to be able to provide chimerism testing services.

Kidney dysfunction represents a major post-transplant complication in VCA patients due to the high levels of immunosuppression used in VCAs. Strategies such as reducing calcineurin inhibitor levels or using alternative, non-nephrotoxic immunosuppressive agents help to mitigate this risk.

B. SERVICE MANAGEMENT

CHAPTER 10 MANAGEMENT IN THE HEALTHCARE SETTING

THE NHS CONSTITUTION

The NHS constitution sets out the principles and values of the NHS and describes the rights of users and the responsibilities of providers. The NHS constitution lists the core values of the NHS. These include the patient being at the core of the service, respect and dignity for all who work in the NHS or who come into contact with the NHS, a commitment to provide the highest standards of care, to show compassion as that care is being provided, to help improve lives and to make the best use of resources.

The constriction sets out 7 key principles that underpin these core values:

1. That the NHS will provide a comprehensive service available to all without discrimination
2. That the NHS will remain free at the point of use based on clinical need
3. That providers will aspire to the highest standards of professionalism and excellence
4. That care will be centred around the needs of patients
5. That the NHS will work in partnerships with other organisations, social services and communities to provide healthcare
6. That the NHS will provide value for money
7. That the NHS will be accountable to the public for th services it provides

"The NHS, in partnership with other organisations, social services and communities, providing a comprehensive, free, value for money, patient centred service with the highest professional standards and accountability."

LEADERSHIP

LEADERSHIP MODELS

Most leadership models incorporate some aspects of the Sandhurst Military academy model developed in the 1970's which posits that effective leaders focus on three needs, the needs of the task, the needs of the team and the needs of individuals

within the team. The needs of the task include clear objectives and systems and processes for delivering those objectives. The team requires that clear communication lines be established, roles and responsibilities clarified and the interactions of the team and teamwork be made as effective as possible. Finally, as each individual is different, effective leaders pay attention to how each individual in the team is feeling and behaving and what they need to be effective members of the team.

There are a number of traits which every good leader must have. Other than actual task competence, these traits include the ability to manage stress and conflict in the team, good communication skills and the ability to provide feedback to the team on their performance.

Conflict is an inevitable part of work and at times as different parts of the team or individuals within the team can develop different goals and needs, leading to clashes. The best leaders take active steps to ensure conflicts are tackled early and not allowed to fester. Conflict resolution requires the leader to separate people and their emotions from the problem and focus instead on building mutual respect and understanding. Conflicts should be resolved in a united, cooperative way with all parties involved.

Stress in the workplace can have a debilitating effect on staff. Good leaders manage stress in the workplace by ensuring the consult their staff and include them in decisions that directly affect their role and duties. Set clear and realistic goals and objectives that do not include unrealistic timelines and give regular positive feedback and support. Deal with conflicts in a positive way and give rewards and incentives even if this is limited to praise and acknowledgement of a job well done.

Some leadership models which incorporate these leadership traits:

- **Transactional Leadership** in which the leader has a 'transaction' with the team in which the team agree to obey the leader and complete the task the way the leader defines, in exchange for reward, usually their pay. This is one of the least effective leadership models and tends to lead to low staff moral and high staff turnover

- **Situational Leadership** in which the leader adapts their leadership style to the given situation and the developmental needs or "maturity level" of the team. This style is suitable for new teams where a lot of coaching and training is required
- **Servant Leadership** in which the leader thinks less about themselves the leader and focuses instead on taking care of the team, freeing them up to achieve the goals set. This style works well when leading senior staff who know what they are doing and need help with roadblocks being cleared son they can get on with the job
- **Transformational Leadership** in which the leader has integrity and high emotional intelligence. They motivate the team with a shared vision of the future and they communicate well. Transformational leaders are self-aware, authentic, empathetic and humble. This style of leadership tends to be the most effective. Transformational leaders inspire their team to deliver their best

THE ROLE OF A CONSULTANT CLINICAL SCIENTIST IN H&I

The main role of a consultant clinical scientist in H&I is to be responsible and accountable for the managerial and scientific leadership for the clinical services provided by the laboratory and to provide advice to clinical teams on the interpretation of laboratory data.

On the management side this means recruiting, training and managing the scientific and administrative staff involved in the provision of the service and ensuring that the service is provided in a manner which meets with national and international standards, is compliant with the relevant legislation and with department of health policies and guidelines.

It also means managing the resources of the department in a manner which ensures the viability of the service and which meets the guidelines set out by the parent organisation. In addition, it also means being involved in the commissioning of services and in agreeing Service Level Agreements (SLA's) with users.

On the scientific side, the consultant clinical scientist is responsible for putting in place systems which ensure that the actual scientific tests undertaken and the results

generated are correct and that the interpretation of the results is correct. It also means being responsible for on-going service development within the department, staying abreast of new scientific, technical and other developments in the field and how they can be applied to the service to the benefit of patients.

In terms of advice to clinical teams, the consultant clinical scientist attends multi-disciplinary clinical meetings and provides advice to clinical teams on the appropriate tests to perform for individual patients, on the scientific and clinical interpretation of test results, on possible future investigations that may be required for particular patients, on transplant options open to patients and on donor selection for organ and stem cell patients. The consultant clinical scientist also liaises with other bodies involved in transplantation such as ODT and the Anthony Nolan for organ and stem cell transplantation and also contributes to the development of the field by contributing to BSHI, the Royal College and other guidelines development bodies, in consultation with other consultant clinical scientists.

MAINTAINING A PHYSICAL PRESENCE

It would be very easy for the head of a laboratory to become remote from their team, especially as they get pulled into a wider set of meetings and obligations in the parent organisation that the laboratory is a part of. It is crucial that active steps are taken to maintain a physical presence in the laboratory.

Different laboratory heads will take different approaches to this. One options, after checking urgent emails in the morning, is to spend maybe 30 mins or so walking around the lab talking to staff seeing if they want to raise any issues and generally making sure they are OK. You learn far more from these informal chats than you ever do in formal meetings. This is an opportunity to check to see who is in, if there are any sickness or on call absences and if there are any urgent issues to deal with.

LABORATORY IMPROVEMENT

There are many reasons why a laboratory could be in distress. As a director of a laboratory, the first concern should be for the safety and stability of the service, so there are 3 things to do straight away if a laboratory is failing:

- Rapidly review where the laboratory stands in terms of staff numbers, training, competence and experience to understand if there are any personnel issues that need to be addressed immediately
- An early meeting with clinicians on the solid organ as well as on the stem cell side is required to understand any concerns they may have and to reassure them about the service going forward
- An urgent review of the quality management system, NEQAS results and turnaround times is required to determine if there are any immediate causes for concern

What happens next depends to some extent on what is found. If there are issues around staff morale, training etc., these need to be addressed and a training plan put in place to make sure all staff are competent for the techniques in use.

If the QMS is not in place or is in place but is not fully developed, a quality plan needs to be developed that would move the laboratory from whatever the current position is to a place where all SOP's are fully catalogued and reviewed, all equipment put into a proper inventory, Planned Preventative Maintenance (PPM) schedules developed, service contracts put in place if not done already and calibrations done and reviewed. An internal audit schedule needs to be developed that would see all areas audited in some form at least once a year. The approach to recording and investigating quality incidents will also need to be reviewed to make sure this is in line with best practice.

In the medium term, the laboratory will need to work with clinicians to review the clinical service provided, looking at all policies including the typing, screening and listing policies as well as the donor selection policies to make sure they are in line with best practice and BSHI/BTS guidelines.

In the long term, one approach would be to organise a series of quality improvement (QI) events with the staff, looking in detail at each area of the service from sample reception to testing, analysis and reporting to look for improvements.

While doing all this, it is important to work very hard to foster a good team spirit, to bring the staff together so that the laboratory is on an improvement journey

together rather than the head of department imposing changes on the staff. Seek to mentor staff in good practices and make sure the door is always open. Set a clear direction of where the laboratory is going and communicate changes clearly and regularly, seeking input from the team.

FINANCE

BUDGET

A budget is an organisation's predetermined income and expenditure plan for a given future period. A budget involves income and expenditure. In the laboratory, income could come from payment for tests undertaken or from a fixed annual allocation from the parent organisation. Expenditure is typically be expressed as pay and non-pay. Pay is considered to be recurring. The non-pay budget is divided into recurring and non-recurring expenditure such as one-time capital equipment purchases.

The difference between income and expenditure is called the variance. A budget is in balance if income equals expenditure and therefore variance is zero.

ACTUAL

A budget actual is the real documented income and expenditure for an organisation in a given accounting period. A budget statement for instance would typically comprise data for the annual budget, the 'actual' for the given month, the 'budget' or planned income and expenditure for the month, together with the variance for the month. The statement would also include the 'actual' And 'budget' for the financial year to date together with the variance to date.

VARIANCE

A budget variance is a periodic measure to quantify the difference between budgeted and actual spend for a given accounting category. A favourable budget variance refers to positive variances or gains. An unfavourable budget variance describes negative variance, meaning losses and shortfalls.

Budget variances occur because it is not always possible to accurately predict the future. Budget variances can occur from controlled or uncontrollable factors. For instance, a poorly planned budget would count as a controllable factor. Uncontrollable factors are often external such as unexpected significant increases in workload.

PAY

The pay budget represents the actual amount spent by an organisation on pay in a given accounting period. In a budget statement, the pay actual is typically presented alongside the pay budget for that accounting period, along with a variance which shows if the pay budget is over or under spent. Pay is considered to be a recurring annual cost.

NON-PAY

The non-pay budget represents the actual amount spent by an organisation on non-pay in a given accounting period. In a laboratory, non-pay typically comprises the spend on reagents and consumables, on overheads and on equipment and maintenance contracts.

In a budget statement, the non-pay actual is typically presented alongside the non-pay budget for that accounting period, along with a variance which shows if the non-pay is over or under spent. Non-pay is made up of recurring annual costs such as reagents and consumables as well as non-recurring costs such as one-off equipment purchases.

ACCRUALS

Accruals refer to expenses incurred but not yet invoiced or income earned but not yet collected. Accountants use accruals to smooth out the budget to make sure there are no sudden spikes or troughs in the account.

COST SAVINGS

The two biggest laboratory costs are staff and reagents/consumables and so these are the keys areas to focus on to look for savings.

If the laboratory is currently carrying a vacancy and can continue to do so for a short while without affecting staff moral or putting the services, especially the on call service at risk, then a recruitment freeze for as long as possible will bring some savings. If the laboratory currently supports paid overtime, then this may need to be reconsidered. Reliance on expensive agency staff will also need to be reduced or even eliminated. However, if possible, the temptation to freeze training and development or conference attendance must be resisted as this will cost more in the long term if staff morale and therefore turnover becomes an issue.

On the reagents and consumables front there are multiple simultaneous steps that can be taken to reduce costs. First, look at which techniques are in use to see if a change would lower costs, for instance look at the alternative Luminex kit supplier to see if the laboratory is being offered the best deal. Most labs have introduced volume reduction in test kits used but if this has not yet been done then there may be some savings to be made. In addition, in house techniques for selected tests may need to be investigated to see if the benefit in cost reduction justifies the extra man hours it takes to maintain in house kits. One example for instance would be to look at in house PCR-SSP if your lab currently uses Luminex for B27 typing.

Finally, use of Quality Improvement (QI) techniques such as Lean/Listening into Action/Agile may help to optimise processes and procedures and to eliminate waste and therefore costs.

MANAGED SERVICE CONTRACTS

A Managed Service Contract (MSC) in pathology is one in which a single supplier is sourced who can provide all the equipment, services, reagents and consumables required for the entire pathology department of a Trust either directly or as a primary contractor who signs agreements with third parties. MSC contracts have a special tax structure which makes them free of VAT. This means that potentially entering into them can result in 20% savings.

One advantage of an MSC is that laboratories will only need to deal with a single supplier over the lifetime of the contract. This removes the need for annual negotiation with a large number of different companies for renewal of contracts for supply or reagents and/or service of equipment by providing a single point of contact. It also means all orders are placed with a single company, all requests for repairs, maintenance and calibration or equipment etc all go through a single company resulting in a streamlined supply chain. It also means a single supplier audit. This will significantly free up staff time spent on managing suppliers, allowing senior staff to focus on the clinical service.

In the absence of an MSC, departments tend to own all the equipment they use. This can result in equipment becoming old and obsolete as departments prolong their lifespans to save upgrade costs. Implementation of an MSC is usually accompanied by a significant refresh of all equipment as the MSC provider owns the equipment and charges for the use of reagents and consumables on those equipment. It is usually in the financial interest of the MSC supplier therefore to not be called out often for repairs. Clauses can be built into the MSC contract to ensure that the supplier upgrades the equipment as technology changes.

A potential disadvantage of the MSC approach, apart from the 'all eggs in one basket' scenario, is that laboratories may lose the close working relationship they currently enjoy with their suppliers and may lose access to short term deals on offer from suppliers. There is also the danger that being locked into a long-term commitment and not be able to take rapid advantage of changes in technology.

CONTINGENCY PLANNING

BUSINESS CONTINUITY PLAN (BCP)

Each laboratory or pathology service should have a detailed business continuity plan (BCP) which describes how the laboratory would function in times of disruption. The BCP plan should be accessible in hard copy format in case the disruption is to the IT systems and must be located in an area that the staff can have access to in case the disruption prevents access to the building where the laboratory is housed as in the case of a fire for instance.

As part of their BCP, the H&I Laboratory must have formal systems in place for another laboratory to provide their services should they not be in a position to do so. An SLA is useful this this.

Some disruptions are to specific services as a result of equipment failures for instance. While others may affect the whole laboratory e.g. power failure, fire, flooding or failure of ICT systems. Where a disruption is due to equipment failure, the BCP should include contingencies for the use of an alternative piece of equipment, perhaps in another department and for the service engineers to be called out. Where the disruption is more laboratory wide, consideration should be given to providing the service from another location or by another lab. Where disruption is due to a transport strike, accident or other issues outside of the organisation, the BCP should include plans to provide a reduced level of service, focusing on the critical, time sensitive tests only. Good communication with clinical users is key in such a situation.

Finally, disease outbreak such as an epidemic or pandemic may significantly affect the level of service that the laboratory may be able to provide. This may be because laboratory staff are directly affected, due to supply challenges or other general disruption withing the parent organisation. In such a situation, routine services may need to be suspended in consultation with clinical users. To reduce the risk of the whole laboratory being quarantined, staff may need to be divided into teams who work different rota's to reduce contact between team members.

ACTIVATING A LOCAL EMERGENCY

The laboratory must specify all critical services they provide and indicate the maximum downtime they can tolerate for each service offered before a local emergency is declared and the re-provisioning plans activated. For instance, the laboratory may be able to tolerate a downtime of 5 days in routine HLA typing but only a few hours in deceased donor typing or crossmatching.

There must be a formal system for assessing each disruption and documenting the decision to escalate or not. If a formal incident is declared, there must be a clear communication plan to communicate this to all stakeholders, including staff and clinical users.

The BCP must have the contact details of key laboratory personnel, stakeholders and third party providers so they can be alerted when a local emergency is declared

WHILE IN A LOCAL EMERGENCY

While in an emergency, it is vital that a full audit trail and proper records. Records to be kept must include as a minimum, a list of what was known at each decision stage, the decision that was taken with that knowledge, who made the decision, what action was then taken and what the outcome of the action was. Such a comprehensive log is vital for learning lessons for the future.

SERVICE RECOVERY

In addition to plans for going into a local emergency, there must be a clear, pre-defined exit strategy for the return to normal services. This must include a comprehensive debrief and review of all the logs kept during the incident. The debrief should focus on what went well, which areas could be improved and how they could be improved and what overall lessons can be learnt.

A list of all resources used during the incident must be collated for cost recovery where relevant.

The Business Continuity Plan (BCP) must be reviewed and updated with lessons learnt and a detailed incident report must be written and stored for future reference.

Finally there may be a need to organise counselling and support for members of staff who may have been affected by the incident.

CHAPTER 11 HUMAN RESOURCES

KEY LEGISLATION

EQUALITY & DIVERSITY

Equality is having equal opportunities and rights. It is being treated fairly. It also means being able and supported to reach your potential. Inequality is when people aren't given equal opportunities and rights. They are treated unfairly and experience discrimination. Discrimination is the unfair treatment of someone because of their protected characteristics, especially where that falls under the list of protected characteristics. Equity is ensuring equality of outcomes not just treating each person equally.

In the UK, equality of covered by the Equality Act of 2010 which came into force in 2010 and replaces a host of prior legislation including the Race Relations Act and Disability Discrimination Act. The Equality Act describes nine protected characteristics, discrimination against which is an offence under the act:

1. Age
2. Disability
3. Gender Reassignment
4. Marriage & Civil Partnership
5. Pregnancy and Maternity
6. Race
7. Religion or Beliefs
8. Sex
9. Sexual Orientation

A new requirement for annual assessments has come into force requiring large employers to carry out an annual workplace disability assessment.

EMPLOYMENT LAW

Employment law in the UK is enshrined in a series of statutes which together make up the United Kingdom Labour Law. The employment Acts of 2002 and 2008 make

further amendments to the employment law. Together, these laws regulate the relations between workers, employers and trade unions.

Employment laws cover requirements for contract of employment, security of employment and procedures for resolution of employment disputes. They also provide procedures for compensation for financial loss in cases of underemployment, under payment or unfair dismissal.

In addition, employment laws provide requirements for occupational pension, wage regulations including the minimum wage, the working time directive, maternity, paternity and adoption leave and pay, tribunal leave and pay such as when a person is doing jury service, requirements for equal pay and for flexible working.

Employment law also covers tax and national insurance requirements as well as H&S standards.

HEALTH & SAFETY (H&S)

In the UK, Health and Safety at work is governed by the Health and Safety at Work etc. Act 1974. This act lays down general principles for the management of health and safety at work and defines a set of duties for employers, employees, contractors, suppliers and manufacturers, those with responsibilities for premises, equipment, substances and emissions. The act also establishes public bodies, including the H&S Executive with enforcement powers.

The duties of employers under the act include the provision of a safe place and systems of work as far as is practicable without undue risk to the H&S of employees, visitors and contractors. The employers is also required to provide such training, instructions and supervision as may be necessary to ensure the H&S of employees, visitors and contractors.

Employers or those parties responsible for the provision and management of substances and/or emissions at work have a duty to ensure that such substances are

controlled, held, transported, stored and disposed of safely. They also have a duty to ensure employees or other who come into contact with such substances have the protective equipment and training and information required to use them safely.

Where premises, plant and equipment are maintained by a party other than the direct employer, they are required to also maintain a safe place and systems of work, including providing for safe entry and exit in case of emergency, otherwise these duties fall to the employer.

Employees have a duty under the act to take reasonable steps to ensure their own H&S and the H&S of other persons who may be affected by their actions or omissions. They also have a duty to cooperate with their employers to ensure their own H&S and those of others.

HUMAN TISSUE ACT (HTA)

The UK Human Tissue Act 2004, overseen by the Human Tissue Authority, replaced the Human Organ Transplant (HOT) Act and makes a number of provisions with respect to activities involving human tissue. The Human Tissue Authority license and inspect organisations that store and use human tissue by describing a series of 'scheduled purposes' which can only be undertaken under license.

The act affects H&I labs as it makes specific provision for transplantation from live as well as deceased donors. Some key aspects of the act include:

- The act specifies the level of consent required for donation
- It widens the pool of potential donors available to a patient by removing the requirement for proof of a 'genetic link' between individuals
- It introduces altruistic donation
- It introduces paired/pooled donations
- It makes provision for ethically approved research using human tissue or material derived from human tissue
- It clarifies which samples may be used for QC, validation and training

AHCS EQUIVALENCE

Equivalence is the process set up by Academy of Healthcare Sciences (AHCS) that enables experience and qualifications obtained outside of the modernising scientific careers (MSC) route to be assessed for equivalence. Equivalence is demonstrated by providing evidence of knowledge, skills and behaviours that are described in the relevant MSC training guide. Once submitted, the evidence is reviewed by a panel and if it is satisfactory, the applicant is awarded a Certificate of Equivalence which they can then use to join the relevant register.

From the H&I laboratory point of view, this potentially allows a suitably experienced and qualified member of staff to apply for state registration as a clinical scientist.

The exit from the HSST training programme is registration on the Higher Specialist Scientist Register, which then allows the registrants to apply for Consultant Clinical Scientists positions. The equivalence procedure for staff who have gone through the traditional RCPath route is not clear but they could in the future, be required to demonstrate equivalence with the HSST course. The key area that may require some work in that case would be the leadership modules.

WHISTLEBLOWING

Whistleblowing is the term used when a worker passes on information concerning wrongdoing typically in the workplace. One important concept is that the wrongdoing cannot be a personal grievance or complaint. To qualify as whistleblowing, the information must meet two critical tests:

1. The person passing the information must believe they are doing so in the public interest
2. The person passing the information must believe that the information passed shows criminal activity, cover up of criminal activity, injustice or that the information passed reveals danger to the health and well being of an individual such as a patient or to the environment

The law does not specifically require that employers have a whistleblowing policy but this is best practice. A typical whistleblowing policy will include recognition of the

role of workers in raising concerns about wrongdoing they may come across in the workplace, will highlight the protection the law provides for whistleblowing and ensure workers know their right not to be victimised should they raise concerns, will make provision for training of staff in the organisations whistleblowing policy, will describe the steps the organisation will take to investigate concerns raised under the whistleblowing policy and how long this might take and will highlight how the outcome of any investigation will be fed back to the person raising the concern.

Employees raising concerns under whistleblowing legislation should typically do so directly to the employers. Should they have reasonable concerns that they may be victimised for doing so they can in most instances find a Prescribed Person to raise the concern with. Examples of such Prescribed Persons include your local MP, the Royal Colleges, the Information Commissioner and the Care Quality Commission (CQC). In most circumstances, a whistle blower taking a concern directly to the press without first going to their employer or a Prescribed Person, will lose their protections under whistleblowing law.

If a whistle blower believes they have been unfairly treated as a result of blowing the whistle they can take their case to an employment tribunal. This may involve the use of the Advisory, Conciliation and Arbitration Service (ACAS) to resolve the issue in the first instance.

CALDICOTT

The Caldicott Principles were developed in 1997 following a review led by Dame Fiona Caldicott, into how patient information was managed by the NHS. The initial review identified six key principles and multiple recommendations which sow how organisations must respect patient information. Subsequent reviews led to the introduction of a seventh principle and the extension of all principles to cover social care alongside the NHS.

The seven Caldicot Principles are:

1. The holder of the information must be able to justify the **reason** why they hold patient confidential information

2. Use of patient identifiable information should only be in **exceptional** circumstances
3. Where use of patient identifiable information is unavoidable, you must use the **smallest** amount necessary
4. **Permission** to access to Patient identifiable data must be strictly controlled
5. All employee who have access to Patient identifiable information must be made aware of their **ethical** responsibilities
6. Use of Patient identifiable information must not be used for **criminal** purposes
7. Professionals with access to such information have a duty to **transfer** that information to other professional when it is in the best interest of patients to do so

Under Caldicott, employers have a responsibility to ensure there are adequate Information Governance (IG) arrangements in place across the organisation, including detailed IG policies and regular mandatory training of staff. Employers also have a responsibility to ensure there are sufficient data protection systems in place to prevent loss of data. All large organisations are required to appoint a Caldecott Guardian with responsibility for ensuring the origination complies with the Caldicott Principles.

RECRUITMENT

When recruiting new staff, the obvious first thing you are looking for is their ability to do the job for which you are recruiting so you are looking at their qualifications, recent experience and declared skills. Will they be someone that can hit the ground running or will they need significant further training or retraining. That doesn't necessarily preclude them but you would want to know.

The recruitment process starts even before you place the advert. When a vacancy arises or especially if you wish to create a new post is created, the parent organisation may require you to justify filling the post. The parent organisation may want you to consider any possible costs savings that can be generated before filling the post.

If permission is granted to fill the post then a suitable job description and person specification will need to be written or updated. For laboratory posts, these job descriptions and person specifications will need to be inline with the NHS Job Evaluation Handbook - https://www.nhsemployers.org/job-evaluation-handbook. This specifies the level of skill required in each of 16 categorise from communication to patient responsibilities in order to fit into the right NHS Agenda for Change pay band. The key is that the job description and person specification must have a clear set of mandatory and desirable requirements that can be objectively assessed either on the application form or at interview.

With the right job description and person specification in hand an advert can then be placed. This may be organised by the parent organisation or by the laboratory. If placed by the parent organisation, the laboratory may also want to consider posting the vacancy with BSHI to make it visible to a more targeted audience.

Once applications are received it is important that the shortlisting and interview are conducted by a team of at least 2-3 senior members of staff, at least one of whom must have undertaken some form of recruitment training. All must have read the organisations recruitment policy.

Depending on the role you are recruiting for, you would want to assess their technical skills, their leadership/management skills, their clinical skills or some combination of all of these. Some roles might require someone with very specific skills while others may be better suited to someone with wider skills. This partly also depends on the skill sets you already have in your lab. So, part of the art of recruiting, once you've identified candidates with the right skill set, is to assess which candidate best complements the skills you already have in your lab and would enhance the team.

The interview must be conducted fairly with all candidates asked the same core set of questions, though there may be a need to pursue specific questions with some candidates to better understand the skills they bring to the post. It is vital when doing this, that unconscious biases are not allowed to creep in. Some senior posts may require that an 'inclusion ambassador' be included in the panel to reduce the risk of this.

All band 8 posts should include an external person on the interview panel to help ensure that consistent standards are being maintained across the discipline.

The successful candidate should be informed as soon as possible and for senior posts, all unsuccessful candidates should be contacted directly and informed. It is also good practice to contact all internal candidates irrespective of the grade so that they hear directly from you rather than through an impersonal letter from HR.

Once a start date has been agreed the last stage is to organise the induction. The parent organisation may have a standard induction event that they organise covering core issues like fire safety, information governance, safeguarding, disciplinary policies etc. The laboratory needs to make sure they also organise a local induction to cover laboratory specific H&S issues as well as an orientation of the department and introduction to key personnel.

CHAPTER 12 PROFESSIONAL DEVELOPMENT

PROFESSIONAL BODIES AND ORGANISATIONS IN TRANSPLANTATION

BSHI

The British Society for Histocompatibility and Immunogenetics (BSHI) bring together professionals working in H&I laboratories in the UK. BSHI promotes collaboration between laboratories and holds an annual conference which allows the sharing of current research and new developments. BSHI takes the lead in the training of scientists in the H&I field in the UK and provides representation to government and other bodies on behalf of its members.

Website: https://bshi.org.uk/

EFI

The European Federation for Immunogenetics (EFI) is a pan European body which plays a vital role in ensuring professional standards are set and maintained. EFI promotes H&I as a specialised field within medicine. They help set standards for practice and contribute to national and pan European consultations that relate to transplantation and immunogenetics. They also provide a forum for exchange of ideas and scientific information through conferences and publications.

Website: https://efi-web.org/

HCPC

The Health and Care Professions Council (HCPC) are a regulator, responsible for regulating a number of healthcare professions in England including Biomedical Scientists, Clinical scientists and Physiotherapists. Each of the professions they regulate has one or more designated titles, which are protected by law. Anyone who uses one of these titles must be on their Register. A person who is not registered and uses a designated title is breaking the law and may be prosecuted.

To register and remain registered, professionals must meet the standards set by the HCPC. These include standards of professional and ethical conduct, standards of character and of health, standards of proficiency and continuing professional development (CPD) and standards of education and training.

The standards of education and training for Biomedical Scientists requires a Bachelor's degree with honours plus a certificate of competence awarded by the Institute of Biomedical Science (or equivalent). For Clinical Scientists, a Bachelor's degree is required along with a certificate of attainment awarded by the association of Clinical Scientists (or equivalent).

The CPD standard requires that registrants undertake regular CPD activities and keep a record of this training, carry out different kinds of learning activities, think about how CPD might improve practice and benefit users and take part in an audit if asked. Audits are carried out on a random sample of each profession at every renewal.

Continued registration also requires that the registrant maintains their fitness to practice. Fitness to practise is not just about professional performance but also includes acts by a registrant which may affect public protection or confidence in the profession.

The HCPC contribute to the delivery of a professional service by ensuring registrants maintain a high standard and give assurance to the public and to service users that the highest standards are being maintained.

Website: https://www.hcpc-uk.org/

NIHCE

The National Institute for Health and Care Excellence (NIHCE) provides national guidance and advice on the use of services and medicines to improve health and social care.

NIHCE develop and provide evidence-based guidelines and recommendations on a wide range of topics, from preventing and managing specific conditions, improving health and managing medicines in different settings, to providing social care to adults and children and planning broader services and interventions to improve the health of communities.

Many Clinical Commissioning Groups (CGC) and Trusts would often not fund a service or procedure if it is not in line with NIHCE guidelines.

NIHCE have a number of guideline for kidney and other organ transplantation, including recommendations for pre-emptive listing and for immunosuppressive regimes at the time of transplant as well as for biopsy when rejection is suspected.

Website: https://www.nice.org.uk/

CQC

The Care Quality Commission (CQC) are the independent regulator of health and adult social care in England. Their remit is to make sure health and social care services provide people with safe, effective, compassionate and high-quality care.

The CQC monitor, inspect and regulate health and social care providers to make sure they meet fundamental standards of quality and safety and they publish their findings, including performance ratings which range from Inadequate, through Requires Improvement to Good and Excellent. There are 12 items listed in the fundamental standards of care that the CQC expect all providers to be able to demonstrate that they meet:

1. That care is person centred
2. That care is provided with dignity and respect
3. That informed consent is always obtained
4. That the care is safe
5. That persons to whom care is provided are safeguarded
6. That care receivers are provided with sufficient food and drink
7. That the facilities and equipment must be clean, suitable and looked after properly

8. That there is a mechanism for complaints to be raised
9. That there must be effective governance and systems in place
10. That there must be enough suitably qualified, competent and experienced staff
11. That staff must be able to provide care and treatment appropriate to their role
12. That care providers must have a duty of candour

There is also a requirement that care providers must display their CQC ratings prominently.

The CQC hold providers to these standards by asking 5 key questions:

1. **Safe**: Is the service safe and are care receivers protected from abuse and avoidable harm
2. **Caring**: Is a caring service provide? Do staff involve users and treat them with compassion, kindness, dignity and respect
3. **Responsive**: Is the service organised so that it is responsive to the needs of the user?
4. **Effective**: Is the care effective? Does it achieve good outcomes?
5. **Well-Lead**: Is the service well-lead? Does the leadership, management and governance of the organisation make sure it's providing high-quality care?

Website: https://www.cqc.org.uk/

NHS IMPROVEMENT

NHS Improvement is the new umbrella organisation created to replace Monitor and other bodies and charged with the responsibility for overseeing NHS Foundation Trusts and NHS Trusts, as well as independent providers of NHS-funded care. NHS Improvement achieves its objectives by providing strategic leadership and practical help as well as holding providers to account and where necessary, directly intervening in the management of an NHS organisation under a series of 'Special Measures'.

NHS Improvement will often allow higher performing Trusts a greater degree of freedom that it does to those in special quality or financial measures. NHS Improvement operate under a 'single definition of success' which incorporates:

- Quality performance as defined by the CQC
- Financial Management
- Operational Performance as determined by A&E performance data, referral to treatment times, cancer treatment times, ambulance response times, access to mental health services and progress on implementation of seven-day services
- Leadership and
- Strategic Change measured against progress in implementing the five year forward view

Website: https://improvement.nhs.uk/

Since 1 April 2019, NHS Improvement and NHS England work together as a single organisation in the management of England's National Health Service.

Website: https://www.england.nhs.uk/

RCPATH

The Royal College of Pathologists (RCPath) is a professional membership organisation with charitable status, which supports the science and practice of pathology.

RCPath is a charity with over 11,000 fellows, affiliates and trainees worldwide, the majority of who are doctors and scientists working in hospitals and universities in the UK. The College oversees the training of pathologists and scientists working in 17 different specialties, which include Histocompatibility & Immunogenetics, Cellular Pathology, Haematology, Clinical Biochemistry and Medical microbiology. Although some pathologists work in laboratories, many work directly with patients in hospitals and the community. Together they are involved in over 80% of all diagnoses, as well as playing an important role in disease prevention, treatment and monitoring.

The College also promotes the work of pathologists, undertaking public engagement activities nationwide. Finally, the College represents its members interests to

government, responding to requests for information and advising government on policy development as they relate to the work of pathologists.

Website: https://www.rcpath.org/

MHRA

The Medicines and Healthcare products Regulatory Agency (MHRA) are an executive agency of the Department of Health whose key responsibilities include:

- Ensuring that medicines, medical devices and blood components for transfusion meet applicable standards of safety, quality and efficacy
- Promoting international standardisation and harmonisation to assure the effectiveness and safety of biological medicines
- Influencing UK, EU and international regulatory frameworks so that they are effective at protecting public health

The NHRA carry out inspections of organisation involved in the production and supply of medicines, medical devise and blood for transfusion, awarding licences for such organisations to carry out these activities. The MHRA also maintain a system for reporting of adverse incidents as they relate to the production and supply of medicines, medical devices and blood.

Website: https://www.gov.uk/government/organisations/medicines-and-healthcare-products-regulatory-agency

HTA

The Human Tissue Authority (HTA) is a regulator set up in the UK in 2005 under an act of Parliament, the Human Tissue Act of 2004, for the purpose of regulating and licensing organisations that remove, store and use human tissue and stem cell for research, medical treatment, post-mortem examination, education and training and for display in public. These activities are termed Scheduled Purposes and require that all such activities are only undertaken with appropriate levels of consent. Scheduled purposes include anatomical examination, post-mortem, storage, transplantation and public display.

The Human Tissue Act makes it a criminal offence to:

- Remove, store or use human tissue for Scheduled Purposes without appropriate consent
- Store or use human tissue donated for a Scheduled Purpose for another purpose
- Traffic in human tissue for transplantation purposes
- Carry out licensable activities without holding a licence from the HTA
- Have human tissue, including hair, nail, and gametes (i.e. cells connected with sexual reproduction), for DNA analysis without the consent of the person from whom the tissue came or of those close to them if they have died

Website: https://www.hta.gov.uk/

NHSBT-ODT

Organ Donation and Transplantation (ODT) is the division of NHSBT formally known as UK Transplant. NHSBT is a special health authority supporting organ transplantation across the UK and blood transfusion in England and Wales. NHSBT is funded by and accountable to the four Departments of Health in the UK government and devolved administrations.

ODT maintains the transplant waiting list for all patient in the UK waiting for an organ. ODT receives referrals of potential organ donors, coordinates the matching and offering of transplants to potential recipients, commissions the National Organ Retrieval Service and collects data on transplant outcomes for the National Transplant Database.

ODT develops policies for transplantation through a series of organ specific advisory groups who consult with the wider transplant communities for those specific organs. Existing advisory groups include the Kidney Advisory Group (KAG), the Pancreas Advisory Group (PAG), the Cardiothoracic Advisory Group (CAG), the Liver Advisory Group (LAG) and the Multi-visceral and Composite Tissue Advisory Group (MCTAG).

ODT also publish as number of strategic objectives which guide transplantation services in the UK. The current plan is called 'Taking Organ Transplantation to 2020' and outlines a number of strategic aims which seeks to increase the pool of people who can and do donate their organs after death. It strives to ensure that clinical practice throughout the NHS makes organ donation happen for every potential donor where donation is appropriate. It also seeks to ensure that when consent has been given donation, it will happen and that all suitable organs are transplanted and survive as long as possible, delivering the greatest benefit for the greatest number of patients.

NHSBT Website: https://www.nhsbt.nhs.uk/

ODT Website: https://www.odt.nhs.uk/

CONTINUING PROFESSIONAL DEVELOPMENT (CPD)

Continuing Professional Development (CPD) is the intentional systematic development and maintenance of the knowledge and skills needed to perform in a particular professional context. The ability to demonstrate ongoing CPD is increasingly a mandatory requirement of many professions. CPD has benefits both for the employee and for the employer.

For the employee, CPD provides a systematic way to demonstrate that knowledge and skills are being kept up-to-date and that the professional standard of qualifications and registrations are being maintained. CPD helps employees to showcase their achievements thus helping with career progression and advancement.

For employers, the primary benefit of CPD is ensuring that high and consistent standards are maintained across the service. CPD, when used as a tool to ensure staff are getting the training opportunities they need to develop, can help promote greater work engagement from the workforce and general commitment to job roles. CPD also provides a useful benchmark for annual appraisals.

Steps that could be taken to ensure staff are getting the CPD training they require include making sure they have opportunities for attendance and presentation at national and international conferences and for attendance at internal training course put on by the parent organisation such as leadership course, time management skills,

interview training etc. Regular journal club meetings should also be organised which staff either attend or lead. Staff can also gain CPD points by leading internal audits and by taking the lead on testing or analysing EQA schemes, by training in new areas of the laboratory and by contributing to and even leading the implementation of new techniques or equipment and writing SOPs. We also make sure the chair and minute taker of lab meetings are rotated so all staff gain experience managing meetings.

Senior staff carry out a review of CPD as part of the annual appraisal and once a year, as the head of department I review all CPD points to make sure all staff are gaining the requisite number of points and that their training covers all the required sections of professional, managerial, educational and competence. As part of this also review the annual schedule of for staff taking the lead on EQA testing and analysis.

STAFF ANNUAL APPRAISALS

All H&I laboratories have strategic plans, based on the Trust's strategic plan, covering a defined future period, usually a year but can be 3 or even 5 years, which sets out the main priorities for that period. In high performing teams, each member of staff knows their roles and responsibilities in delivering the strategic plan.

Appraisals are relevant to the provision of high-quality H&I services. The annual appraisal is a tool which sets out clear objectives for each member of staff, linked to the overall strategic aims of the laboratory which help the member of staff not just to help the laboratory achieve its goals but also help that member of staff to achieve personal growth and development.

The annual appraisal should be the formalisation of an ongoing discussion between management and each member of staff, not a once off annual conversation which is then promptly forgotten.

The key word in the annual appraisal is therefore 'conversation'. In its most basic form, the appraisal is a conversation between and manager and a member of staff which formally reviews the member of staff's effort over the last 12 months against a set of previously agreed objectives and provides feedback and recognition while agreeing sup-ort and further training that may be needed. The appraisal goes on to

set objectives for the coming year which will include objective that directly support the laboratory aims and objectives as well as objectives for personal growth and development which ultimately also contribute significantly to the laboratory.

When done well therefore, a system of appraisals benefits both the member of staff and the laboratory. An appraisee should prepare for the annual appraisal by agreeing a date and time with their appraiser and documenting their achievements against the objectives set the year before, listing any objectives not achieved together with 'blockers' or difficulties, document how they have demonstrated their commitment to the Trust values, list any personal issues they wish to discuss and any learning and development they wish to undertake.

The appraiser should prepare by agreeing a date and time with their appraisee, critically review the appraisees performance against the objectives set the year before, give praise and constructive feedback as required, check on the health and wellbeing of the appraisee and how they feel about their work and the working environment, agree objectives that supp-ort the laboratory aims as well as objective for the personal growth and development of the appraisee.

Wherever possible, objectives should be SMART, i.e. they should be Specific, Measurable, Assignable, Realistic and Time bound.

Both the Appraisee and Appraiser need to the happy with the appraisal conversation and should have an opportunity to review the final appraisal document before it is signed.

GOOD SCIENTIFIC PRACTICE

"Professional scientists need leadership skills if they are to head up clinical research"

There are 5 domains of Good Scientific Practice. These are:

1. Professional Practice
2. Scientific Practice

3. Clinical Practice
4. Research, Development and Innovation
5. Clinical Leadership

Professional Practice

This domain covers the requirement for high levels of professional practice, probity and ethical standards. It requires scientists to exercise a duty of care, to work within their scope of competence, to keep skills and knowledge up to date, to work constructively with colleagues and other professionals and to engage in evidence based practice. There is also a requirement to help train the next generation of scientists.

The professional practice domain also includes a requirement to treat each patient as an individual, respecting their dignity and confidentiality and upholding their rights.

Scientific Practice

The Scientific Practice domain requires scientists to evaluate and/or develop and undertake investigations that take account the relevant clinical scenario, to provide results and reports of those investigations and to critically evaluate the results of those investigations to draw conclusions and provide technical, scientific and clinical advice as may be needed.

There is also a requirement to set and maintain quality standards and to regularly audit all investigations undertaken, to make judgments about the effectiveness of those investigations and to make regular improvements as needed.

Clinical Practice

The Clinical Practice domain requires that scientists are able to demonstrate expertise in the clinical situations that apply to their field of practice and are therefore able to plan investigations that optimize diagnosis, treatment and treatment monitoring.

Scientists are required to be able to provide expert interpretation of complex and/or specialists data to aid clinical decision making.

Finally, last but not least, this domain covers the requirement for scientists to ensure that patients give informed and freely given consent and that their confidentiality of their data is assured.

Research, Development and Innovation

This domain requires all scientists to regularly review and critically appraise the scientific literature in their field and to participate and research and development or innovative approaches that further knowledge in the field. This also includes a need to publish research findings and to take part in meetings and conferences where knowledge is shared with colleagues.

Clinical Leadership

The key requirement in this domain is that a senior scientist must retain overall responsibility for the services provided by their department even when specific duties are delegated. Furthermore, scientists are required to show respect for the contribution of colleagues and to treat them fairly. To make sure systems are in place for colleagues to raise concerns and to take steps to correct deficiencies in the team.

Clinical Leadership also requires that scientists refer colleagues and patients to other professionals as appropriate.

PATIENT CENTRED CARE

Today it is more correct to talk of person-centred care rather than patient centred care as the later takes donors into account. It also takes into account the changing nature of the NHS into an organisation that is also engaged with disease prevention rather than just with cure.

The concept of patient or person-centred care is all about putting the patient at the centre of their own treatment plan, ensuring they are listed to and that they are fully informed about the options open to them and are involved in the decisions about their care. This ensures that the patient is cared for and treated in a way that is meaningful and valuable to them.

Some key actions healthcare professionals can take to ensure the patient/person is at the centre of their own care include:

- Show respect for the patients values and belief systems
- Inform and educate the patient
- Ensure the patient has the right physical and emotional support as needed
- If it is the wish of the patient, ensure the next of kin or family and friends are involved and informed as appropriate
- Ensure care is as integrated as possible so that patients are not faced with multiple visits to the hospital to see different healthcare professionals
- Ensure the patient has access to care when needed or can care for themselves in their own home if required

SOCIAL ISSUES IMPACTING ON TRANSPLANTATION

The NHS system in the UK means that the cost of transplantation is not in itself a social barrier to transplantation. However financial considerations to present a complex social barrier disproportionately affecting patients from lower socio-economic backgrounds. Social barriers to care may be more common in certain groups, such as members of racial and ethnic minorities and those living in rural areas.

Some typical social barriers to transplantation include:

- Financial
    - Inability to afford time off work
    - Inadequate housing
    - Food insecurity
    - An inability to obtain transportation
- Lack of adequate Support

    - A lack of a care partner
    - A chaotic social environment including many stressors such as family issues
    - Difficulty in obtaining child care
- Education
    - A low educational level
    - A language barrier

PUBLIC ENGEGEMENT

Scientists have a responsibility to engage with and explain their work to the public. Public engagement can be described as a system of events and activities through which the work and benefits of science and research can be shared with the public. A key element of public engagement is deriving feedback and input from the public in a two-way process with the goal of generating mutual benefit to scientists and the public. BSHI and EFI plays a key role in this. Some of the aims of public engagement in science are to:

- Increase public awareness, understanding and interest in science
- To support the teaching and learning of science in schools
- To support and encourage scientists to become involved in science engagement activities with their local communities

A commitment to public engagement is of strategic importance to science as it helps with maintain the publics' trust in the work of scientists and it helps scientists to maintain accountability for their work, ensuring their research and development activities fit with the expectations of wider society.

CHAPTER 13 SERVICE DEVELOPMENT

A CHANGING LANDSCAPE

There are a large number of significant challenges facing H&I services. These include financial pressures, there are significant on-going changes in the environment in which laboratories operate and there are challenges to staffing. All that before even consider the clinical and technical challenges.

On the financial side, laboratories are all being challenged to do more with less. Workloads have increased not just in number but also in complexity. In some hospitals, laboratories are required to achieve 5% savings year on year. Techniques such as lean are helping labs to improve business processes and reduce costs but these can only go so far. It is doubly difficult the laboratory is not in a position to recover full costs from service users. Funding for deceased donor typing is a current topic and it is not yet clear exactly what proposals ODT will come up.

On the environment side of things, labs are facing a significant increase in the regulatory requirements with the introduction of ISO15189 and for labs inspected by the MHRA, there has been a significant ramping up of the data integrity and security requirement.

There are also various reviews of H&I and other pathology services going on, such s the Carter review, which may potentially lead to consolidation of services. There is increasing competition for the provision of H&I services from the charitable and private sectors.

Perhaps one of the biggest of all in this category is the recent DOH push for a 7 day NHS which will present its own challenges.

On the staffing side, laboratories are all finding it difficult to man the on call rotas, so options like extended day and 7 day NHS will significantly affect the way labs provide services. Especially so as trained/state registered staff are almost at the premium at

the moment. Until recently, the introduction of the STP training system had the unintended consequence of reducing the number of trained staff being produced and those that are completing the training need significant further support before they are ready to go on the on call rota. This has however improved.

There is also talk of a shortage of staff ready to operate at consultant level with concerns about succession planning, though this too has improved.

Finally, there are a number of clinical and technical challenges that H&I laboratories face. NHSBT is partway through their 20/20 transplant strategy which they hope will result in more transplants which is having an impact on the H&I labs. Welcome programmes such as antibody incompatible transplants has resulted in a significant amount of work for each transplant. On the stem cell side, H&I laboratories are seeing more transplants in older patients. Many of the techniques used to widen access to transplantation leads to more work in the lab.

On the technical side H&I laboratories are beginning to see more widespread use of newer techniques and technologies, NGS and qPCR being the prime examples. These are already making a difference to the way H&I laboratories work.

In addition, all these changes will require significant upgrades of IT systems to better support electronic transfer of data from analysers to LIMS and between LIMS and external partners such as ODT and the Nolan. ODT for example have a big program to introduce a transplant IT HUB and H&I labs would want to make sure their systems can plug into this.

BREXIT

Brexit has had and will continue to have a significant impact on laboratory service provision. Three key impacts are on:

1. Supply chain for reagent and consumables
2. Impact on staff of EU origin and
3. Impact on organs and stems cells that cross EU borders to reach the UK

Many of the reagents and consumables that are used in H&I laboratories are supplied from manufacturing sites in the EU and can potentially be impacted by delays in crossing the border and in increasing tariff costs. Steps that can be taken to mitigate the impact including pre-ordering and stock piling where possible or agreeing future purchases with suppliers so that they can stock pile and hold items for you in the UK.

Staff of EU origin are significantly impacted by Brexit and while there is a process of applying for settled status it does still leave these staff feeling undervalued. Many staff of EU origin have already returned home and been lost to the NHS as a result of Brexit. H&I laboratories managers have a duty of care to their staff and need to ensure they know that they are valued and help them where required with their application for settled status.

Movement of stem cells and solid organs across EU borders has till now been straightforward under UE legislation. Brexit means that the Anthony Nolan for instance now has to put individual agreements, including custom declarations, in place with multiple registries across the EU to ensure confirmatory typing samples and actual stem cells and cord blood can move across the borders without delay. Similarly, organs, especially for paediatric patients, frequently move across the EU-UK border. NHSBT have put agreements in as well to ensure these are not significantly impacts.

Finally, there are a number of other potential issues which may not affect the H&I laboratory directly but may potentially impact the transplant process. These include potential shortages of supplies such as drugs, including immunosuppressive drugs. Other impacts include how EU legislation such as GDPR and CE marking affects laboratory services.

THE CARTER REPORT

The original Carter report into NHS pathology services was written back in 2008. It made a number of recommendations covering all areas from quality and accreditation, IT systems and connectivity, to tariffs for test and to personnel and qualifications. Interestingly, the recommendations included a proposal that specialists

laboratories be consolidated and other laboratories come together in networks to offer their services and single providers to a number of hospitals.

The key drivers for change were seen as the need to redesign services to be more responsive to users, especially phlebotomy services, to embrace a plurality of suppliers including the private and voluntary sectors, to raise standards to those of the best performing providers and to reduce costs.

Barriers to change include the lack of accurate information that could be compared between hospitals on tests offered, turnaround times and costs of those tests and the fact that the finances of pathology services are often integrated into the hospital finances in a way that potentially makes it difficult to separate out pathology.

More recently, Lord Carter has produced a much more detailed report into NHS pathology services, having collected data from a number of hospitals. These new proposals include specific details for creating networks of pathology providers. Since the report, details have been published of which hospital pathology services will be merged into single hub and spoke type networks, with most of the high volume, non-specialists tests being moved to the hub and automated in a way that makes optimal use of equipment and staff.

To facilitate this, a number of process mapping exercises are currently ongoing in a select set of pathology providers to determine how they currently work and how the services could potentially be improved. Consolidation into the networks may well follow on very soon after this.

NHS SUSTAINABILITY AND TRANSFORMATION PLANS (STPS)

The NHS Sustainability and Transformation plans (STPs) are plans drawn up on a regional basis by NHS organisations and local authorities in different parts of England to come together to develop region specific plans for the future of health and care services that would put these services on a long-term sustainable footing. An initial total of 44 regions have been identified across England in which NHS providers, Clinical Commissioning Groups, Social Care providers and Local Authorities have

been encouraged/required to develop joint STP plans. STP plans are five-year plans covering all aspects of NHS spending.

Development of STPs represents a shift in the way NHS services are provided. Whilst previous changes have encouraged competition between providers, STPs require collaboration.

The key themes for STP plans were:

- Strengthening preventative services
- Improving mental health services
- Improving productivity
- Workforce planning
- Developing enabling services such as IT and estates
- Developing integrated management structures
- Reconfiguration of acute and specialist services
- Redesign of primary care

The initial development process for STP was not encouraging. Frontline clinical staff and patients had very little involvement and Local Authorities have not been as engaged as originally envisaged. One of the easy wins that some STPs have identified is the reconfiguration of Pathology services along the lines proposed in the Carter review of Pathology services.

In the North East London (NEL) STP for instance in which Barts Health NHS Trust, the largest NHS Trust in the UK falls, the Pathology plans include take steps to reduce unnecessary test requests (identified as up to 25% of total test requests). There is also discussion of consolidation of pathology services provided for Barts Health NHS Trust, the Barking Havering and Redbridge University Trust (BHRUT) and the Homerton. Not all these hospitals have H&I services but for STP regions with more than one H&I laboratory, this could lead to consolidation of services.

As part of the earlier NHS five year forward view and the new NHS long-term plan, new models of care, based on new ways of joining up and delivering health and social care are being developed and tested.

As part of the initial 5 year plan, selected groups of NHS providers to were asked to develop and test new models of care which could then serve as inspiration for other providers. Fifty vanguard providers were identified and asked to develop New Models of Care for specific services ranging from enhanced health in care homes, to viable small hospitals, to urgent and emergency care networks and Multispecialty community providers. A further 25 providers were selected to develop and test new and different ways of joining up health and social care services across England. A number of other New Models of Care were developed around prescription for instance and GP federations.

The new long-term plan focuses on:

- Boosting out of hospital care
- Reducing pressure on emergency services
- Giving people more control over their own care
- A digital upgrade across the NHS
- More focus on prevention and reducing health inequalities
- Improving outcomes and
- Giving more backing and support to NHS staff
- Maximising the effects of investments in the NHS

From an H&I perspective, there are no direct New Models and Care centred specifically around transplantation and transfusion. However, the bringing together of various providers to jointly commission services may well result in fewer commissioners of H&I services and potentially eventually lead to fewer laboratories.

PROJECT MANAGEMENT

Many projects, both complex and straightforward, often fail for multiple reasons. These include:

1. Poor conception and no clear link to strategic goals

2. Lack of leadership and insufficient human and other resources to complete the project
3. Excessive complexity and insufficient definition of the required outcomes
4. Lack of or poor communication within the project team and with key stakeholders
5. Inadequate planning and oversight as the project progresses

Project management methodologies, tools and techniques aim to address these issues and help projects to succeed. There are a large and diverse number of project management methodologies. Some work best in specific environments such as construction, while others are more adaptable to multiple situations. Broadly, project management methodologies can be classified as traditional or agile.

Traditional project management techniques include:

- PRINCE2 - A process-based approach which focuses on organization and control over the entire project from start to finish, with up front planning and structuring of all details
- Waterfall – Similar in many ways to PRINCE2 but with very little overlap in stages. Each stage must be completed and signed off before the next stage starts
- Critical Path Method – Similar to Waterfall but does allow some tasks to proceed simultaneously. However, a number of key tasks are classified as being on the critical path. For these, each prior step must be completed and signed off before the next critical path step commences

The traditional methodologies suffer from the fact that they are not sufficiently open to change. As the whole project is pre planned, any changes disrupt the whole chain and often lead to project failures. The agile methodologies address this issue. They are designed upfront to be accommodating of change. When agile methodologies were first being developed there was some concern that they may only be applicable to small projects. However, this has proven to be incorrect and agile methodologies are currently applied to projects of any scale. Agile methodologies include:

- Agile – An approach which values a working solution over detailed upfront planning and is based on constant feedback from the eventual user/customer as the project develops

- Scrum – A system in which the team delivers aspects of the project in short cycles, typically of 2 weeks, with review and re-planning after each cycle
- Kanban – A visual approach to managing projects which does not reply on detailed checks but clear visual indicators of when things need to happen. An example is a re-order level so that raw material in automatically re-ordered when that level is reached, without the need to repeated stock checks
- Lean – An approached focused of optimising the project process by eliminating waste. This could for example be by removing stages from the project or process that do not add value
- Six Sigma – This is a statistical process control approach which is based on eliminating errors (to the 6 standard deviations) in each stage of a process

DEVELOPING A BUSINESS PLAN FOR H&I

All laboratories should be planning for th long term, even as they face and meet the day to day challenges. One approach is to develop 3-5-year business plans.

A three-year business plan should take account of the short, medium and long-term changes taking place in the healthcare provision environment and in the wider political context. In the short to medium term, there are challenges around Brexit, potential workload increases due to the introduction of the new kidney allocation scheme and due to deemed consent and more demand for additional laboratory services such as non-HLA testing. Seven-day working has gone a little quiet but may not have gone away.

In the long term, there is an increase in vascularised composite tissue allotransplantation (VCA) such as hand, face and uterus taking place and more hospitals may become involved in this and require laboratory support. Other additional services include pharmacogenetic testing as more drug reactions and/or interactions are shown to have a genetic and even HLA component, CAR-T cells and other emerging therapies.

All of these factors need to be take into account when defining the **strategic intent** of the department over that time period. The strategic intent need only be a single sentence or paragraph, which describes at a high level where the department would like to be at the end of the 3-year period. For example:

"To be a highly productive, modern, digitally and technologically enabled laboratory, offering the full range of H&I services to our users, with a highly skilled workforce who have access to development opportunities"

From such a strategic intent flows a number of short, medium and long term goals which all have resource, technology and service configuration implications.

In the short to medium term the laboratory will have to critically review staff resource requirements. Transplant services in regions such as London which have large proportion of BAME patients on their waiting lists are likely to see an increase in workload. In addition, ICT technology will have to become 100% transcription free, from electronic request, through all testing and reporting stages, to electronic reporting.

In the medium to long term, staff development will have to be stepped up to take into account the new services such as VCA become more widespread.

MAKING A CAPITAL BID

A business case would need to be developed which makes the case for the capital spend. The business case would need to include:

- Details of the clinical case being made for the introduction of the new equipment including how use of the equipment fits in with changes in the clinical/regulatory landscape
- Details of any improvements to patient safety that the new equipment brings e.g. higher resolution results
- Details of the financial case being made e.g. would the introduction of the new equipment, when serving and kits costs are taken into account, improve operational efficiency and reduce costs?
- A description of how the new equipment use aligns with the overall strategic direction of the parent organisation

Other items which can potentially be included if available include:

- Competitive quotes from different supplier
- Benchmark of overall whole lifetime cost of the new equipment compared to alternatives on the market

TENDERING FOR NEW SERVICES

The first step is to assess whether or not the team has the capacity to actually undertake the proposed work. It may be that the current team could undertake the work but the size of the team may need to grow and perhaps particular skill sets may need to be brought in. If there is scope for this type of expansion that that is acceptable.

The key elements to include in a proposal would be a description of the team, including an organogram the CV's of senior staff highlighting their experience in providing the required service.

The next key item is a description of the services currently provided by the laboratory, showing the experience in providing the required service highlighting any areas where the laboratory has a unique advantage over other potential providers and noting any areas of complementarity between the lab and the service requester. This would then lead on to how the laboratory proposes to meet the needs of the service requester. This can include things like transport of samples, the tests that would be offered, the turnaround times, how results and reports will be dispatched, the availability of senior staff for consultation and to attend clinical meetings etc. It can also in this section other clinical services on offer such as stem cell donor searching and selection.

Another key aspect that needs to be included is a description of the laboratory's QMS system and how this will help provide assurance about the quality of the service on offer. Copies of UKAS and EFI accreditation certificates can be included at this stage.

Finally, the costing of the service needs to be included. The service needs to be costed to recover expenses in order to make it viable.

INTRODUCING NEW SERVICES

CHANGE MANAGEMENT

Introduction of a new service or item of equipment requires a clear change control process with detailed validation. However, change can be difficult. There are a number of common barriers to change. These include:

- Insufficient understanding of the change itself with no clear vision, direction and/or priorities
- The change envisioned is too complex and the team leading the change does not have the resources or experience to deliver the change
- Lack of leadership needed to inspire and engage people in the change resulting in lack of engagement and buy in from key stakeholders
- Lack of good project management of the change with no clear accountabilities and definition of roles and quick wins are not identified
- The changes are not communicated sufficiently
- People practices are not reviewed and re-aligned - this is needed to ensure the change is sustained and to enable people to operate in a new way
- Successes are not recognised and/or celebrated resulting in a fall in enthusiasm and commitment to the change
- Progress is not measured and the learning is not reviewed resulting in changes not being sustained

The first step in any change control process is to define the changes desired and make the case for the change both of clinical and nowadays on financial grounds. Some initial investigation may then be carried out to provide more information for those that need to approve the potential change.

Once the initiation of the change is approved, a change team would have to be put together and a detailed change plan developed. Key aspects of the change control plan include but are not limited to:

- Carrying out a risk assessment of the proposed change
- Identification of who is affected by the change so they can be brought in as potential stakeholders
- Establishing the level of validation required for the change

- Establishing the acceptance criteria for the validation e.g. simple verification or full scale validation involving IQ, OQ and PQ stages
- Identifying which processes and SOPs need to be reviewed and updated
- Identifying which staff need to trained or retrained
- Agreeing a phasing for changing over from the old to the new process or procedure
- Establishing a communications plan for communicating the change to the laboratory's users

Once the change plan is developed and agreed then it's a case of executing against the plan and reviewing each stage. A Consultant Clinical Scientist or quality lead may need to review each stage to give approval to proceed to the next stage. The final stage is reviewing the whole change process to agree whether to go live or not and then reviewing post go live as part of the PQ stage. One useful step which often gets missed is to review the change planning itself to see what worked well and what didn't and what can be changed in time for the next big change.

INTRODUCTION OF NGS INTO THE LABORATORY

Use of NGS revolutionises HLA typing by increasing the speed at which laboratories can go from sample to allele level or high resolution HLA type. Currently, for some labs Sanger Sequencing is now a real bottleneck in their stem cell services potentially leading to delays in getting high resolution HLA types and completing donor searches for stem cell patients. Use of NGS could be a real option for such lab. If an NGS platform is already available then on kit costs, the use of NGS would be cheaper than the current standard of Luminex for low to intermediate resolution typing followed by Sanger sequencing.

In addition to being faster and cheaper than low resolution typing followed by Sanger sequencing, NGS also has the advantage of yielding far fewer ambiguities than Sanger sequencing. One of the current problems with Sanger sequencing is the additional requirement for GSSP's to resolve most types, adding to the cost and turnaround times. NGS has a significant read depth compared to the single forward and reverse of Sanger sequencing, helping with the allele assignment.

Finally, the fact that NGS gives fewer ambiguities than Sanger sequencing, is an advantage for stem cell donor selection. Certainly, the availability of NGS typed donors on the stem cell registries is having a significant impact on donor selection.

The first step when deciding whether to introduce NGS is to review the strategic context to see if NGS is a good fit for your service. This may require that a business case is developed, especially if funding is required. The business case needs to clearly define the available options and give weights or scores to those options. Potential options for an NGS business case are to retain the status quo, to partially replace Luminex plus SBT with NGS or to replace all typing with NGS. These options need to be scores in terms of risk, financial benefits or costs and impact on staff. If the business case is approved, then a change control process needs to be initiated.

As there are multiple platforms and kits available for NGS the first step in the change control could be to evaluate a range of systems. This may be limited to a specific platform if one is already available to the department. Suppliers such as Illumina, GenDX and Omoxin provide kits for the MiSeq platform for instance and all three should be evaluated if the lab has access to a MiSeq. If buying a new platform then a tender process may be required.

An evaluation using the same 12 - 24 samples on different platforms will be sufficient to give the department information on the pros and cons of each system. The evaluation needs to look at the supplementary equipment requirements alongside the platform such as DNA quantification systems, amount of manual versus automation of the system, the ease of use of the kit, the reliability and reproducibility of results, the speed of the system, the user friendliness of the software, the computer specification and data storage requirements of the system, the overall cost and the level of support from the suppliers.

Once a final system is selected, any remaining equipment such as faster computers and additional extra-large hard drives or servers as well as laboratory equipment will need to be sourced. Specifically, NGS does tend to require good quality DNA as the PCR tends to be over long DNA sequences. A good DNA extraction system/robot would be required plus a means of accurately assessing DNA concentration and quality. In addition, a couple of calibrated thermocyclers with adjustable ramp speeds would be required so that the program can be set up exactly as specified by the

suppliers. The library prep stage can be consumables hungry depending on the kit used. A good set of automated multi-channel pipettes will be required. A means of quantitation and size selection of the post PCR product may be required. Options for this include a qPCR machine, a Pippin Prep or magnetic beads.

With those in place, the change control can proceed to the installation or IQ stage. This will involve suppliers coming in to install the different items of equipment, tests that they are installed correctly and provide installation certificates. The suppliers will also deliver some basic training to the change control team.

When the IQ stage is signed off the change control can proceed to the main validation or OQ stage. This will require sufficient samples to be tested to cover a range of HLA types, including null alleles and homozygous types, as well as a range of DNA quality to establish the robustness of the technique. The OQ stage usually requires that a change plan is drawn up to show in detail, the tests that will be carried out and the acceptance criteria that will be used to judge success.

The change control team will write the SOPs and complete staff training during the OQ stage. A go live date can then be agreed and users informed of the pending changes.

Once validation is completed and signed off, the lab can go live with NGS and the change control moves into the monitoring or PQ stage. For go live, final decisions will need to be made about workflow and types of cases for NGS. These options will have been reviewed during the tender if one was required or else when the business case was being put together. The PQ stage involves monitoring performance closely over a agreed number of runs or over an agreed period of time. Any lesson learnt during the PQ stage will be applied and changes made to the SOPs and workflow if required. The PQ stage can then be signed off and the change control closed.

INTRODUCING CARDIOTHORACIC SERVICES INTO YOUR LABORATORY

The provision of laboratory services in support of cardiothoracic transplantation is one of the services provided by H&I laboratories, though not all labs are involved in

this service provision. Should a laboratory need to become involved in the provision of these services, perhaps because they have inherited this from another lab or because their Trust has started a cardiothoracic transplant programme, there are a number of requirement which will need to be taken into consideration.

Depending on the trigger for introduction of cardiothoracic transplant services, the laboratory may need to develop a business case, especially if funding is required. The business case needs to clearly define the available options and give weights or scores to those options. Potential options for a cardiothoracic transplant service business case include providing all in and out of hours services from the lab or contracting out some of the service, particularly the out of hours services, to an external lab. These options need to be scored in terms of risk, financial benefits or costs and impact on staff. If the business case is approved, then a change control process needs to be initiated.

There are a number of staffing, technical and clinical requirements which will need to be taken into account. On the staffing side, support of a cardiothoracic transplant service means staff need to be available to man an 24/7 on call rota to provide some services though a prospective crossmatch is often not required for cardiothoracic transplantation. As the staff will mainly be lone working out of hours, this means that the laboratory needs enough state registered staff to safely cover the rota while still being able to provide the routine service. Staff may need extra training in deceased donor HLA typing, in Luminex single antigen bead testing and in CDC and flowcytometric crossmatching, depending on the techniques in use in the laboratory.

The technical requirements include a means of extracting DNA and assessing the quality and quantity. Equipment such as a Nanodrop or Qubit can be used for this. In addition, an intermediate resolution HLA typing technique is required to undertake rapid initial HLA typing of patients, and donors for full HLA-A, B, C, DRB1, DRB3, 4 and 5, DQA and DQB and DPA and DPB. This intermediate resolution typing is often performed by Luminex, though SSP can also be used (more labour intensive). This of course requires that the laboratory have enough PCR thermocyclers to do the amplification and other steps that require a heated block and also have access to a Luminex platform. A means of doing gel electrophoresis and imaging gels may be useful to check amplification but is not mandatory.

In addition, the laboratory needs facilities to be able to provide a full range of serological services in support of cardiothoracic transplantation. These include HLA antibody screening and identification as well as crossmatching. HLA antibody testing techniques needs to include a solid phase technique such as Luminex. Laboratories may choose to source their Luminex kits from a single supplier or from both the main suppliers in order to have options. Whichever set of kits are used needs to include the screening as well as single antigen bead kits. It is advisable that an H&I laboratory providing support for a cardiothoracic programme be able to provide CDC screening technique to aid in identifying complement fixing antibodies as well as IgM antibodies. This allows the laboratory to identify risk levels for HLA antibodies detected and be able to carry out an immunological risk assessment at the time of a donor offer.

Although routine antibody screening and identification may have a turnaround time of several days, the laboratory needs to be able to provide same day turnaround for scenarios such as investigation of possible donor specific antibodies in post-transplant patients.

As a minimum, a laboratory needs to be able to offer flowcytometric crossmatching in and out of hours. This means that the laboratory needs to have 24/7 access to a flowcytometer allowing for a potential prospective out of hours crossmatching though in the majority cases of cardiothoracic transplantation, crossmatching is performed retrospectively. In addition, some laboratories may choose to offer CDC crossmatching.

On the clinical side, senior staff need to have training and experience to carry out the immunological risk assessment at the time of a donor offer as well as carry out interpretation of crossmatching results and being able to confidently give advice to the cardiothoracic surgeons and the rest of the team. They must also be available to attend MDT meetings.

INTRODUCING KIDNEY TRANSPLANT SERVICES INTO YOUR LABORATORY

The provision of laboratory services in support of kidney transplantation is one of the core duties of an H&I laboratory, though not all labs are involved in this service

provision. Should a laboratory need to become involved in the provision of these services, perhaps because they have inherited this from another lab or because their Trust has started a transplant programme, there are a number of requirement which will need to be taken into consideration.

Depending on the trigger for introduction of kidney transplant services, the laboratory may need to develop a business case, especially if funding is required. The business case needs to clearly define the available options and give weights or scores to those options. Potential options for a kidney transplant service business case include providing all in and out of hours services from the lab or contracting out some of the service, particularly the out of hours services, to an external lab. These options need to be scored in terms of risk, financial benefits or costs and impact on staff. If the business case is approved, then a change control process needs to be initiated.

There are a number of staffing, technical and clinical requirements which will need to be taken into account. On the staffing side, support of a kidney transplant service means staff need to be available to man an 24/7 on call rota. As the staff will mainly be lone working out of hours, this means that the laboratory needs enough state registered staff to safely cover the rota while still being able to provide the routine service. Staff may need extra training in deceased donor HLA typing, in Luminex single antigen bead testing and in CDC and flowcytometric crossmatching, depending on the techniques in use in the laboratory.

The technical requirements include a means of extracting DNA and assessing the quality and quantity. Equipment such as a Nanodrop or Qubit can be used for this. In addition, an intermediate resolution HLA typing technique is required to undertake rapid initial HLA typing of patients, and donors for full HLA-A, B, C, DRB1, DRB3, 4 and 5, DQA and DQB and DPA and DPB. This intermediate resolution typing is often performed by Luminex, though SSP can also be used (more labour intensive). This of course requires that the laboratory have enough PCR thermocyclers to do the amplification and other steps that require a heated block and also have access to a Luminex platform. A means of doing gel electrophoresis and imaging gels may be useful to check amplification but is not mandatory.

In addition, the laboratory needs facilities to be able to provide a full range of serological services in support of kidney transplantation. These include HLA antibody screening and identification as well as crossmatching. HLA antibody testing techniques needs to include a solid phase technique such as Luminex. Laboratories may choose to source their Luminex kits from a single supplier or from both the main suppliers in order to have options. Whichever set of kits are used needs to include the screening as well as single antigen bead kits. Some laboratories may also choose to maintain a CDC screening technique to aid in identifying complement fixing antibodies as well as IgM antibodies. This allows the laboratory to identify risk levels for HLA antibodies detected and be able to carry out an virtual crossmatch at the time of a donor offer.

Although routine antibody screening and identification may have a turnaround time of several days, the laboratory needs to be able to provide same day turnaround for scenarios such as investigation of possible donor specific antibodies in post-transplant patients or of pre and post plasmapheresis samples where a patient is following a desensitisation protocol.

As a minimum, a laboratory needs to be able to offer flowcytometric crossmatching in and out of hours. This means that the laboratory needs to have 24/7 access to a flowcytometer. In addition, some laboratories may choose to offer CDC crossmatching

On the clinical side, senior staff need to have training and experience in interpreting crossmatching results and being able to confidently give advice to the renal surgeons and the rest of the renal team. They must also be available to attend MDT meeting, including live donor work up meetings.

INTRODUCING STEM CELL SERVICES INTO YOUR LABORATORY

The provision of stem cell services in support of HSCT programmes is one of the core duties of an H&I laboratory, though not all labs are involved in this service provision. Should a laboratory need to become involved in the provision of these services, perhaps because they have inherited this from another lab or because their Trust has started a stem cell transplant service, there are a number of requirement which will need to be taken into consideration.

Depending on the trigger for introduction of stem cell services, the laboratory may need to develop a business case, especially if funding is required. The business case needs to clearly define the available options and give weights or scores to those options. Potential options for a stem cell service introduction business case include developing the services inhouse or outsourcing to another laboratory for all or part of the service. These options need to be scored in terms of risk, financial benefits or costs and impact on staff. If the business case is approved, then a change control process needs to be initiated.

If a lab were to decide to develop stem cell services completely inhouse, there are a number of technical and clinical requirements which if not available will need to be sourced, validated and introduced. The technical requirement include a means of extracting DNA that yields high quality samples with long unbroken fragments to facilitate long reads. This can be a manual extraction process but given workloads and to make the best use of staff time, it is preferable if this is automated/semi automated. A means of quantitating the DNA and checking its quality is also needed. Equipment such as a Nanodrop or Qubit can be used for this.

In addition, an intermediate resolution HLA typing technique is required to undertake rapid initial HLA typing of patients, family members and potential unrelated donors for full HLA-A,B,C,DR, DQ and potentially DP typing. This intermediate resolution technique needs to give results that meet the EFI criteria for stem cell HLA typing. These include being able to assign HLA class I types to serological equivalents, must keep heterozygous ambiguities to a minimum and be able to assign 4 haplotypes wherever possible. This intermediate resolution typing is often performed by Luminex though SSP can also be used (more labour intensive). This of course requires that the laboratory have enough PCR thermocyclers to do the amplification and other steps that require a heated block and also have access to a Luminex platform. A means of doing gel electrophoresis and imaging gels may be useful to check amplification but is not mandatory.

The laboratory must also be able to undertake HLA class I and II typing to high resolution. This is often performed using Sanger Based Sequencing (SBT) but increasingly, laboratories are introducing Next Generation Sequencing (NGS),

allowing them to bypass th initial intermediate resolution typing and proceed straight to high resolution typing. For SBT, a sequencer such as an ABI3500 will be required.

In addition, to provide a full stem cell service that supports mismatched transplants such as haplo, 11/12 and cord blood, a means of testing for HLA antibodies, such as Luminex single antigen bead testing, is required.

On the clinical side, the laboratory must have staff trained and experienced in the provision of stem cell services, including optimal donor selection and interaction with registries. Staff must have the ability to contribute to MDT meetings with clinicians and provide advice on donor selection strategies.

INTRODUCING SMALL BOWEL SERVICES INTO YOUR LABORATORY

The number of small bowel transplants undertaken in much smaller than that of other organs such as kidneys and it therefore makes sense typically for such services to be concentrated into a small number of laboratories. Things to consider if your hospital is considering introducing small bowel services include:

- Bowel transplantation is an option for patients who are no longer able to tolerate intravenous feeding perhaps due to infection/sepsis or lose of central venous access
- Short gut and functional bowel problems are the major indicators for bowel transplant
- Transplantation may also be indicated in patients with intestinal malignancies or abdominal surgery where the amount of viscera that is to be removed means that multi viscera transplantation is required
- Only four transplant centres in the UK currently undertake small bowel transplantation, two 2 and 2 paediatric
- Isolated intestinal transplantation common in adults
- Liver plus intestinal transplantation more common in children
- Recently abdominal wall transplants have taken place
- Intestinal mucosa is sensitive to ischemic injury, making DCD donors less attractive for bowel transplantation
- In addition, the infectious-related mortality was higher and the absorptive function lower with DCD donors

- Intestinal grafts are very susceptible to rejection due to high concentration of lymphoid tissue, which may contribute to high incidence of solid organ graft *versus* host disease (GVHD)
- Use of lymphocyte depleting agents for induction
- A high level of immunosuppression is required to prevent rejection, which can lead to serious and life-threatening sepsis and potentially to post-transplant lymphoproliferative disease (PTLD)
- Tacrolimus plus steroids has proved effective for long term immunosuppression
- The vast majority of PTLDs in small bowel transplantation are EBV driven and arise either as a consequence of the reactivation of latent infection or more commonly, infection of the host by latent virus from donor B cells. Screening for EBV pre- transplant is vital to avoid PTLD
- Small bowel graft can be removed in cases of graft-related problems without serious impact on the recipient's well-being
- Preformed DSA may represent a barrier to isolated intestinal transplantation
- A virtual crossmatch is done at the time of a donor offer
- Presence of DSA is used inform the immunosuppression strategy
- AMR remains the most serious challenge in post-transplant patients
- Post-transplant monitoring is required to test for the development of de novo HLA antibodies
- Presence of complement fixing pre-formed and de novo DSA post-transplant correlates with acute rejection episodes
- IVIg, Rituximab and Bortezomib have been used for DSA reduction
- Intestinal transplantation can reduce renal function by as much as 45% in some patients. Therefore, for patients with poor renal function or those for whom intestinal transplantation would mean a loss of venous access for dialysis, simultaneous intestinal and kidney transplantation is an option

CHAPTER 14 QUALITY MANAGEMENT SYSTEM

CLINICAL GOVERNANCE

Clinical governance is an umbrella term. It covers those elements and activities that help sustain and improve high standards of patient care. It is part of the determination of a 'Well Led' organisation taken into account by external assessors such as the Care Quality Commission in the UK.

Elements of a good clinical governance framework for an organisation include:

- Visible and engaged leadership
- Working to a clear set of standards
- Mature issues, risks and complaints management system
- Continuous team development, education and training
- Clinical effectiveness, with integrated care pathways, monitored through clinical audits
- Openness, emphasis on a duty of candour and learning from mistakes
- Secure information management systems

THE QUALITY MANAGEMENT SYSTEM (QMS)

The 'quality' of a product or service has been defined as a measure of how well the inherent characteristics of that product or service match all the requirements of it. When all the inherent characteristics meet the requirements the quality is said to be high but if the requirements are not meet quality is said to be low. Quality therefore depends not just on the inherent characteristics of a product or service but it also critically depends on the requirements and expectations of the product or service. Those requirements may be driven by customer or user expectation and/or they may be driven by legislation and regulatory requirements. The Quality Management System (QMS) describes the sum total of processes put in place by an Organisation to manage Quality. Key aspects of a Quality management system usually include a Quality Manual, Quality Policy, a set of Quality Objectives and a Quality Plan to achieve those objects, provision for sufficient Personnel, Document Control processes, provision of adequate Facilities, control of the Examination processes, control of Clinical Materials and Quality Review processes.

QUALITY MANUAL

A quality manual documents a laboratory's quality management system and includes the quality policy and arrangements for its implementation. It can be in paper manual or an electronic form. A quality manual should define the scope of the quality management system, i.e. list what is included and what is specifically excluded. Certain external suppliers may for instance be excluded from the scope of the quality management system. Where exclusions are listed, they must be justified. The processes and procedures that are included e.g. document control, management review etc, must be listed or referenced if described elsewhere together with a description of how the processes interrelate. The quality manual must describe the human and other resources necessary to support the operations of the laboratory and to monitor the laboratory processes. The quality manual must also crucially describe the actions necessary to achieve planned results and continual process improvements. The ISO 9001 standard specifies a set of standard requirements for a quality management system for organisations and provides a good basis for a quality manual though care must be taken not to simply reproduce the standards.

QUALITY POLICY

The quality manual starts with a definition of the 'quality policy' of the laboratory or more likely, its parent organisation. This should be a clear statement of the organisations commitment to quality. The quality policy must be based on the principles of leadership from top management, a focus on users which in the context of H&I laboratories include patients, donors, clinicians and other colleagues, a commitment to involve staff in the maintenance of quality, a standard approach to processes and procedures and a commitment to continuous improvement.

QUALITY OBJECTIVES

This provides the basis for the laboratories to develop quality objectives. Quality objectives must be measurable and consistent with the quality policy of the parent organisation. They must meet or exceed user expectations and may even be developed with user input. Quality objectives must be updated periodically.

QUALITY PLAN

One means of itemising the quality objectives and monitoring progress to meet those objectives is to describe them in a 'quality plan'. A quality plan specifies the objectives and describes the procedures and resources that will be needed to meet those objectives, including who will do what and when. It also describes the criteria by which success in achieving each of the objectives will be measured and provides a basis for regular review of the implementation of the quality management system including annual review of quality.

PERSONNEL

Another key feature of a quality management system is a description of the organisational structure and staffing levels, including staff training, required to meet the quality objectives. In the H&I laboratory context this means that the laboratory must be directed by an individual with the qualifications and experience to fulfil the responsibilities of the 'Director' of the laboratory. In the UK, the Director is usually a Consultant Clinical Scientist who holds the FRCPath (i.e. is a Fellow of the Royal College of Pathology).These, according to the current EFI standards, include an earned doctorial degree in a biological science or a medical qualification and in addition must have 4 – 5 years experience in H&I or Immunology (two or which must be in H&I in the case of the later). The laboratory must have an organogram describing its organisation and management and the place of the laboratory in any parent organisation. The quality management system must also specify the responsibilities, authority and interrelationships of all staff. A quality management system should require that staff undertaking any procedures which affect the quality of the service must be fully trained in those procedures. The quality management system should list or provide a reference to documentation which lists the competencies required by staff to undertake any processes and provide for training to take place for staff to achieve those competencies.

DOCUMENT CONTROL

'Document control' is an essential part of the quality management system. A good quality management system requires that all key documents, including procedure descriptions and process documentation must be controlled. In the H&I laboratory context, these documents include amongst other, clinical policies, management process descriptions, standard operating procedures, information and datasheets,

forms, records of processes and certain print outs from analysers. The quality management system must include the requirement that all documents be approved for use by authorised personnel prior to issue; that there must be a readily accessible master list of all documents which identifies the current revision status and distribution of all documents in order to prevent the use of invalid and/or obsolete documents; that all documents must contain a title, unique identifier, a review date and/or date of issue and/or revision version, the total number of pages and the name of the authoriser; that legible, readily identifiable documents must be available at points of use; and that all documents shall be regularly reviewed and updated as required. In addition, the quality management system must provide for the identification, secure storage for the legislated retention period, protection, retrieval and disposal of process documentation, test records, result reports and quality documentation.

FACILITIES

A quality management system must provide for adequate premises, equipment and other resources to ensure that the work of the laboratory is performed safely and efficiently. This requires that the premises has sufficient space for separation of incompatible activities such as pre and post PCR activities and dedicated facilities for sample reception for instance. It also requires that the premises be secure from access by unauthorised personnel. There must be adequate storage for personal items away from laboratory items and sufficient storage for samples, reagents and records.

EXAMINATIONS

CPA and EFI define standards for examination processes such as sample reception, quality control of examinations, post examination processes such s handling reports and dealing with telephone requests for results. In the laboratory, the examination processes and their control forms a key aspect of the QMS.

CLINICAL MATERIALS

In the H&I laboratory context, another key feature of a quality management system is the control of clinical materials. Clinical materials include all blood and tissue samples. The quality management system must describe how clinical material is

uniquely labelled to allow identification and must describe how it is securely stored and retrieved as required and disposed of when no longer stored. These must be in compliance with current legislation, regulations and guidelines. Retained material must be stored in a manner which ensures the validity of a repeat examination.

QUALITY REVIEW

Finally, another key feature of a quality management system is provision for process improvement including regular 'management quality reviews'. Ongoing evaluation and improvement processes are essential to ensure that the laboratory continues to meet the needs and requirements of its users. Methods for undertaking such evaluations include user surveys, vertical, horizontal and examination audits, monitoring the speed of dealing with non compliances from audits, monitoring the speed of dealing with complaints, monitoring of key performance indicators such as turnaround times, monitoring the status of preventative and corrective actions and monitoring the speed of follow up of actions from quality management reviews. All of these must be reviewed at regular intervals, no less than on an annual basis to ensure the continued suitability of the quality management system and to change and improve the system as required. The output from a management quality review must include decisions on what improvements can be made to the quality management system, what improvements can be made to the service and what resources are needed by the laboratory to continue to provide a high quality service that meets the needs of its users. The laboratory must have an identified individual whose role includes direct responsibility for quality. This individual has a responsibility to ensure that the quality management system is fully implemented and maintained and to report this back to the management of the laboratory.

H&I laboratories are required to maintain Clinical Pathology Accreditation (CPA) in order to continue providing their services. In addition, must H&I laboratories choose to obtain and maintain European Federation for Immunogenetics (EFI) accreditation. In addition, depending on the parent organisation the H&I laboratory is a part of, the laboratory may need to obtain and maintain the Medicines and Healthcare Regulatory Authority (MHRA) accreditation. Accreditation through all of these schemes requires that the laboratory meet the highest standards of quality, something which is facilitated by the implementation of a quality management system.

A quality management system contribute to H&I laboratory service provision by helping the lab to standardise and document its processes and train all staff to operate to that standard procedure thereby helping to improve process control. This ensures that the right tests are done on the right samples, using the right processes and the reports are sent to the right recipient. A quality management system facilitates staff training and helps reduce repeats and waste thereby lowering costs. Implementation of a quality management system helps reduce errors and potentially reduces the risk of litigation.

TRAINING AND COMPETENCE

TODO: Training and competence

EQUIPMENT MANAGEMENT

TODO: Equipment management

AMR

TODO: AMR

AUDITS

It is important and is a requirement of a number of accreditation bodies that the laboratory undertake a number of internal and external audits. Maintaining accreditation requires a certain number of external audits from the accrediting bodies. One way of ensuring internal audits are completed is to maintain an annual schedule of internal audits. These should include as a minimum, one examination audit per year of each key test, one horizontal audit a year and one vertical audit in each of the main sections of the laboratory. Other key audits which need to be done annually include an equipment audits, H&S audits and external audits such as supplier audits and user audits.

It is important that suppliers who provide the key reagents and services to the laboratory are periodically audited to ensure that they themselves are maintaining any required accreditations and that they are complying with QMS procedures.

User audits are a useful check on the view service users have of the service being provided and can be a useful source of improvement ideas.

Key steps when investigating a poor performance in an internal or external audit include the following:

- Log the audit on your audit tracking system and raise quality incidents (CAPA's) for each finding in the audit
- For each finding, conduct an immediately investigation to make sure the issues which gave rise to the poor performance are not affecting patient care and safety. This might even require the temporary halt of a particular test service
- Once that initial investigation is over the next step would be to do a root cause investigation into what went wrong. This will be informed to some extent by the initial investigation but you would need to look deeper
- The root cause investigation would then inform the next step, the definition of the corrective action to put right any procedures or processes that may need to be updated/corrected, followed by the preventative actions, to stop the issues arising in the future
- If the issue directly affects patient care, a clinical risk assessment would be required
- Finally, any lessons that can be learnt need to be noted and shared with other similar departments in the organisation

IMPORTANCE OF CONSENT

Consent in medicine refers to the principle that a person, with full capacity to do so, must give voluntary, fully informed consent, before they receive treatment, including tests and examinations. So, three important principles of consent are capacity, being fully informed and consent being voluntary.

Capacity refers to the fact that the person must be capable of giving consent, which means that they must understand the information they are provided with. Examples of situations where a person may not have the capacity to give consent include where there might be language barriers or where there may be learning difficulties.

Consent being voluntary refers to the fact that the consent must not be coerced by family, friends or medical staff.

Finally, informed consent refers to the fact that the person must be given all the information they need to make a decision, including the benefits and risks, reasonable alternatives and what will happen if treatment does not go ahead.

H&I laboratories must obtain consent or assure themselves that clinicians who request laboratory tests have obtained consent. This is important both for legal as well as ethical reasons. The Human Tissue Act of 2004 places a legal requirement on all service providers who remove, store and use human tissue and stem cell for research, medical treatment, post-mortem examination, education and training and for display in public to be licensed and to obtain fully informed consent for carry out those activities. Under certain legislation, touching a patient or carry out tests or procedures on them or their samples may be legally classified as battery.

From the ethical point of view, patient autonomy and basic human rights are a key consideration. Patients have the freedom to decide what should or should not happen to them and to gather information before undergoing a test or procedure. No one else has the right to coerce the patient to act in a particular way.

The H&I laboratory must take reasonable steps to obtain consent. One method of doing so is to put a disclaimer on the test request form advising the person requesting the test to obtain fully informed consent. Typically, the request form would state that the laboratory assumes that consent has been obtain by the test requester.

MANAGING RISKS

TODO: Risk Management

MANAGING ISSUES

COMMON SOURCES OF ERROR IN THE LABORATORY

There are a large number of potential sources of error both in and out of the laboratory. These errors can occur at the preanalytical, the analytical and the post analytical phases.

Errors that can happen in the preanalytical phase include, inappropriate test request, misidentification of patient, inappropriate sample collected, improperly labelled samples, inadequate sample storage and transportation, sample mix up during registration, wrong tests booked and/or labelling errors during sample preparation.

In the analytical phase, errors that can occur include equipment malfunction, sample mix-ups, interference, undetected failure in quality control and/or procedure not followed.

In the post analytical phase, errors that can occur include transcription errors, failure in reporting, erroneous validation of analytical data, improper data entry and/or excessive turnaround time.

Of these, perhaps the three most frequent are sample mix up, transcription errors in data entry and equipment malfunction. Laboratories can take steps to reduce sample mix up by having second person checks where appropriate in any manual process to ensure samples are being set up in the correct order. Better still, manual processes can be replaced with automated, fully barcoded and tracked processes. To reduce transcription errors, laboratories need to reduce manual transcription as much as possible and have electronic transfer of data. Where manual transcription is unavoidable, second person checks can be used to try to ensure errors are spotted. Equipment malfunctions which lead to error can be potentially reduced by upgrading equipment whenever possible, to ensure adequate PPM is in place for all equipment.

QUALITY INCIDENT MANAGEMENT

The first step is to do an immediate investigation to determine if the CT is the correct result or if the original type was correct and the CT is wrong. Possible reasons for a wrong initial type or CT include sample mix-up, wrong blood in tube, data transfer errors or typographical errors amongst other things. To rule out sample mix up you may need to need to re-test all the samples on the same run as your CT. If the original sample tube is still available, you may need to go back to that source if possible to re-test. You may need to ask for a new sample to determine the correct result. If this investigation shows that your CT is correct and your original type was wrong then you need to send out an amended report and may need to write to the referring clinicians, informing them of the error.

Once that initial investigation is over the next step would be to do a root cause investigation into what went wrong. This will be informed to some extent by the initial investigation but you would need to look deeper. The could for instance be training issues or a gap in you review and authorization process which allowed an incorrect result to be reported.

Once the root cause has been identified the finding would inform your corrective and preventative actions. Your corrective action could for instance involve some retraining. Your preventative action might involve some technical changes or at least changes in your review process.

One key thing is that lesson should be learnt. You should also share those lessons with other similar departments in your organisation. You should also periodically review the effectiveness of your CAPA's to make sure the same issues do not arise again.

MANAGING COMPLAINTS

A complaint is a statement made by a user that something is unsatisfactory or unacceptable. In raising a complaint, a user brings a problem to the attention of the provider and expects some redress, probably over and above simply supplying the original service that was the cause of the complaint. Laboratories should view

complaints as an opportunity to improve. Some users may not complain but instead not will not renew an SLA when it falls due.

Most complaints raised against a laboratory are usually centred around poor turnaround times for results and/or reports. Some complaints related to social care are reportable under local authority and NHS regulations.

Complaints can be made in person or on the phone and therefore not in writing, as well as by email or letter. Laboratories should make complaints procedures clear in their user guides.

Complaints are often used by regulators as one measure of the success of the organisation's service. NHS Trusts and all healthcare providers are required to have a detailed documented Complaints Policy that shows that they are committed to delivering a safe service, that concerns and complaints from users are welcome, that complainants are listened to and not treated differently as a result of having made a complaint and that the issues complainant raises will be investigated vigorously.

As well as having a detailed complaints policy, there are a number of other strategic steps laboratories must take in order to have a robust complaints handling procedure. These include training of all staff in handling complaint and setting up processes to log and analyse all complaints and share learning once complaints are resolved. Laboratories and NHS organisations are under a further duty of candour to provide service users and other relevant persons all relevant information in the event of a reportable patient safety incident.

Key actions required when a complaint is made include:

- Immediately acknowledge the complaint and record it in the laboratory complaints management system such as Datix
- Review and risk assess the complaint and if serious enough, for example if reportable, escalate it according to the Trust procedures
- A senior member of the laboratory staff to contact the complainant early in the review process to discuss options and the complainant's preference for

managing and responding to their concerns. This is also a good initial opportunity to offer and apology

- The senior member of staff may lead the investigation or this may be led by an appointed investigator from the laboratory
- The lead investigator will determine the root cause of the issue which led to the complaint, will identify corrective actions that can be taken and will identify preventative steps that can be taken to prevent the same issue happening again
- Where a complaint relates to a specific member of staff, senior managers should not forget their duty of care for the welfare of that member of staff during the investigation
- The investigation team will draft a response, which subject to Trust review procedures, will be sent to the complainant

Some complaints are occasionally raised as the first step leading towards litigation. Trusts are under an obligation, even when potential litigation is cited, to continue to try to resolve the issue that gave rise t the complaint.

There are also occasions where complaints are raised in unreasonable circumstances or in a persistent manner. Laboratory staff are not expected to tolerate abusive or threatening behaviour and can trigger the Trust procedure relating to abusive users.

EXTERNAL QUALITY ASSURANCE (EQA) SCHEMES

NEQAS

External quality assurance (EQA) testing schemes are a tool that allows participants to monitor, evaluate and improve their own performance. Participation involves receiving samples at regular intervals from an external body, testing them by the same routine methods used for normal referrals and reporting the results to the EQA organising centre. In the UK, H&I laboratories participate in the UK National External Quality Assessment Service scheme for H&I (UK NEQAS for H&I) for all services they offer. This is a requirement of CPA and EFI accreditation. They may also optionally take part in the Terasaki HLA typing schemes.

The UK NEQAS for H&I includes scheme 1A - HLA phenotyping by serology, scheme 1B - HLA B27 typing, scheme 2A - cytotoxic crossmatching, scheme 2B - crossmatching by flowcytometry, scheme 3 - HLA antibody specificity analysis, scheme 4A - HLA typing by DNA based methods, scheme 4B - ABO grouping by DNA based methods, scheme 5A - HFE typing, scheme 5B – HFE interpretation, scheme 6 - HLA antibody detection and scheme 7 - HLA B57 typing and an educational scheme. Each laboratory receives an analysis of their individual results both for current specimens and over a period of time allowing assessment of performance. They also receive anonymised results from all other laboratories allowing them to assess their performance relative to other laboratories.

Participation in these schemes are an important aspect of quality management in an H&I laboratory. The benefits of participation are that it helps demonstrate to colleagues and to customers/Users the laboratories commitment to quality. It also provides the laboratory management with an insight into the performance of the laboratory. Participation in NEQAS acts as a stimulus for improvement helping to drive up standards in the laboratory. It may help reveal unsuspected areas of difficulty and act as a check on the efficacy of internal quality control procedures.

The benefits of participation in an EQA scheme are that it helps demonstrate to colleagues and to customers/Users the laboratories commitment to quality. It also provides the laboratory management with an insight into the performance of the laboratory. Participation in an EQA scheme acts as a stimulus for improvement helping to drive up standards in the laboratory. It may help reveal unsuspected areas of difficulty and act as a check on the efficacy of internal quality control procedures. In addition, it can be used as a tool for competence assessment of laboratory staff. Ultimately participation in EQA schemes are a requirement of CPA and EFI accreditation.

RESPONSE TO POOR PERFORMANCE

A poor performance in a NEQAS may indicate underlying problems with the clinical service. My first action would be to immediately investigate the nature of the unacceptable performance and make a decision as to whether or not the clinical service should be temporarily halted. For instance, the poor performance could be as a result of a recent change in test kit or technique or personnel. If a temporary

halt is needed, then provisioning may be required so that would need to be looked into.

Once that initial investigation is over the next step would be to do a root cause investigation into what went wrong. This will be informed to some extent by the initial investigation but you would need to look deeper. If for instance there was a problem with your test kits, you would have to ask why your validation did not pick this up.

The root cause investigation would then inform you corrective and preventative actions. Your corrective action would involve a look back to see if/how clinical samples were affected and could involve retesting or at the very least re-analysis of some results. This would be part of your clinical risk assessment. If any clinical samples are found to have been reported incorrectly then your duty of candour requires that you write to the referring clinicians to inform them of the error. Your preventative action might involve re-training, it might involve changes to validation procedures, it might involve technical changes or more.

One key thing is that lesson should be learnt. You should also share those lessons with other similar departments in your organisation. You should also periodically review the effectiveness of your CAPA's to make sure the same issues do not arise again.

ACCREDITATION

Accreditation is the procedure by which an authoritative body gives formal recognition that a body or person is competent to carry out specific tasks. In the laboratory, whilst accreditation is no guarantee of acceptable performance at every level, it does serves as confirmation that a laboratory is performing to a given set of standards and provides assurance to the users of the laboratory's commitment to quality.

As part of the Modernisation of Pathology Strategy, the Department of Health in England requires that all medical laboratories enrol with an accreditation programme. This is as has been recommended by the latest Carter report. All UK

laboratories, including those in the independent and private sector, are now required to the registered with the Care Quality Commission (CQC) who are the new regulators of all Health and Social services. The CQC are working with Clinical Pathology Accreditation (CPA) who currently accredits medical laboratories in the UK.

EFI

Accreditation of Histocompatibility and Immunogenetics laboratories in the UK usually means accreditation by both CPA and by the European Federation for Immunogenetics (EFI). CPA ltd. is a 'not-for-profit' organisation, whose main shareholders are the major pathology professional bodies, including the Royal College of Pathologists. CPA assesses pathology laboratories against the 'CPA standard for the medical laboratory' which incorporates ISO 15189. CPA is in turn owned by United Kingdom Accreditation Services (UKAS) which is the sole National Accreditation Body for the UK under EU regulations and carries out accreditation activities beyond the medical laboratory including in business. Whilst CPA is UK based, EFI is pan European (and beyond). The aims of EFI include the development of Histocompatibility and Immunogenetics in Europe as a discipline of medicine and the development of standards of techniques, quality control and criteria for accreditation in the provision of Histocompatibility and Immunogenetics services. Stem cell transplant services who seek accreditation to Joint Accreditation Committee of the ISCT & EBMT (JACIE) standards must use an EFI accredited H&I laboratory for their H&I services.

The benefits of accreditation by CPA or EFI include helping to ensure staff are fully trained and supported to carry out all activities for the services offered by the laboratory, helping to ensure that procedures are in place and are followed, helping to ensure good documentation of all processes and procedures and helping to ensure a continuous cycle of improvement in the services offered.

To become accredited by CPA or EFI, a laboratory must be able to demonstrate compliance with the standards published by those accreditation bodies. The processes involved in attaining laboratory accreditation in Histocompatibility and Immunogenetics include gathering of information, preparing and submitting the application including payment of any fees, preparing for and undergoing an the

inspection, receiving the report and putting in place any corrective actions identified and receiving the accreditation.

The first process is to gather the relevant information required for accreditation. This includes information on the laboratory, its personnel, test repertoire and workload, evidence of participation in external quality assurance (EQA) schemes, information on SOPs and evidence of having undertaken internal audits. Laboratory information includes its address and contact numbers its relationship to any parent organisation and. Information on personnel includes covers non technical and technical staff, including the Director of the laboratory and may be presented as an organogram showing the levels of staff within the laboratory. Details required on Personnel include qualifications, duties and responsibilities. For senior staff a CV may be required. Evidence of participation of staff in continued professional development (CPD) may also be required. Information that should be gathered also includes the types of services provided by the laboratory, its test repertoire and annual workload. His may need to be presented both as number of requests for various investigations as well as number of actual tests. In addition, evidence will be needed on the laboratories participation in external quality assurance (EQA) schemes covering all services offered by the laboratory. The evidence will constitute results obtained in EQA schemes and evidence that completion of the tests is rotated between staff. Accreditation will require proof that the laboratory has documented its processes in standard operating procedures (SOPs) and will require that a master list of SOPs be provided on application. Some SOPs may also need to be provided on application for accreditation. Another key piece of information which will need to be gathered for accreditation is evidence of participation in a program of internal audits.

Once all the required information is gathered, the next process is the completion of the application form. CPA has a dedicated 'Application for Accreditation' form that needs to be filled. A new application form is submitted every four years. EFI have a 'Packet A' form for first time accreditation application, a 'Packet B' for self inspection at the end of years 1 and 2 of an accredited laboratory and 'Packet C' for re-inspection in the third year. Several additional documents need to be provided with the application. For CPA, this includes the Quality Manual. For EFI, this includes copies of CV's for senior staff, evidence of participation in CPD, lists of SOPs and copies of SOPs used for internal quality control, print outs from analysers, copies of laboratory worksheets and reports and printouts of some computer screens.

Once an application is processed and accepted, an inspection is scheduled. CPA use two UK based inspectors. One is a professional inspector and the other is a senior scientist working in the laboratory being inspected. EFI also use two inspectors but both are scientists working in the H&I field. One is from the same country as the laboratory being inspected and the other is from a laboratory in a different country which has a similar repertoire to the laboratory being inspected. CPA and EFI inspect to their own standards. The CPA standards have a number of clauses which 'Shall' be complied with, meaning they are mandatory and a number of clauses which 'Should' be complied with which means they are non mandatory recommendations. The EFI standards have a number of clauses which 'Must' be complied with, meaning they are mandatory and a number of clauses which 'Should' be complied with which means they are non mandatory recommendations. The inspection starts with an opening meeting in which the schedule of the inspection is discussed. Key personnel such as the Director and quality manager or deputies must be present for the inspection. The inspection reviews laboratory practices and does include questioning of laboratory staff about the laboratory processes. CPA inspection includes meetings with senior management of the parent organisation of the laboratory as well as meetings with clinical user groups.

The inspection concludes with a closing meeting in which the inspectors present key findings to the senior laboratory staff. There is an opportunity to discuss these findings in this meeting and some items may be closed as a result of the meeting.

The next process is for the inspectors to prepare a report for the accrediting body. The report includes general comments on the inspection together with a list of non conformances with mandatory requirements and observations as well as in the case of EFI, suggested corrective actions. CPA has two categories of non conformances, critical for systematic non conformances and non critical for other non conformances. Where non conformances are raised, the applicant would be given time to correct them. This would require evidence of corrective action to be submitted. Depending on the nature of the non conformance, a re-inspection may be required. If there are no corrective actions required or where the corrective actions have been completed, accreditation can be granted. The nature of the accreditation granted could be full accreditation, provisional accreditation, awaiting accreditation of accreditation withheld. EFI may grant accreditation but with reduced categories than were either applied for or was previously held.

CPA and EFI have similarities in their large areas of similarities in their accreditation requirements but do have significant differences which reflect the heritage of the two bodies. CPA accreditation standards are generic and can be applied to all UK pathology laboratories irrespective of discipline. CPA accreditation is based on a Quality Management Systems centred approach which does not set standards for individual tests and techniques. A Quality Management System is a set of such interrelated and interacting elements that organisations can use to direct and control how quality policies are implemented and quality objectives are achieved. A good quality management system helps organisations to consistently meet the needs and requirements of their users. EFI accreditation standards are however very specific for H&I laboratories. Like CPA accreditation, EFI accreditation does also require most elements of a good quality management system, with requirements for personnel, facilities, quality assurance, handling of process and quality records and use of SOPs. Unlike CPA, however, EFI accreditation does include H&I specific service and technical requirements.

Both schemes require that the laboratory be directed by a qualified individual who takes responsibility for the clinical, scientific, consultative, advisory, organisational, administrative and educational activities of the laboratory. However whilst CPA are not prescriptive about the qualifications of the laboratory Director, EFI do require the person to hold a PhD, MD or equivalent and have 4 or 5 year laboratory experience two of which must have been in H&I. Both schemes similarly require that the laboratory be sufficiently staffed for the services provided. Again EFI is prescriptive in requiring supervisors to have a minimum qualification of BSc or equivalent plus three to five years experience.

Both CPA and EFI have similarities in the quality management system needs of their accreditation requirements. Both require that the premises are adequate for the activities undertaken, with sufficient storage of material, reagents and documents and that there be adequate separation of incompatible activities. EFI specifically mentions pre and post PCR activities in this regard. Both schemes require that laboratory procedures be documented in SOPs and that personnel are trained against those SOPs before undertaking any of those activities. Both schemes require that process and quality records are collected and stored and that result reports are controlled and stored securely. Both schemes are similar in requiring that laboratories participate in external proficiency testing programmes.

Both CPA and EFI have similarities in accreditation requirements for specimen handling and labelling pre test, for quality control during testing and for handling of results and reports post testing. EFI has specific requirements for the actual tests used and how the results are reported. In addition, both CPA and EFI require that the needs of the users be taken into account and that processes are in place that allow for continuous evaluation and improvement.

The key difference between the accreditation requirements of CPA and EFI is the emphasis CPA places on the quality management system itself versus the emphasis EFI places on standards for services and techniques. CPA uses a system of vertical, horizontal and examination audits to assess compliance with these quality management system requirements without being prescription about the techniques. EFI on the other hand defines standards for the various services offered by H&I laboratories including organ transplantation, haematopoietic progenitor cell transplantation, disease association and transfusion as well as for various techniques including serological and DNA based typing, antibody screening and identification and crossmatching. These service and techniques are not covered by CPA accreditation requirements.

RELEVANCE OF EFI ACCREDITATION FOR H&I LABORATORIES

EFI is designed specifically for the provision of H&I services and so while it does have clauses that cover general quality management system requirements, it in addition has specific clauses covering specific areas of H&I in great detail.

For instance, while other accreditation standards discuss personnel and their responsibilities, the EFI standard also includes specific requirements for the qualification of laboratory personnel.

In addition, while other accreditation standards talk in general terms of pre examination, examination and post examination procedures, EFI has specific standards for the actual tests carried out in H&I laboratories including nomenclature requirements, standards for HLA typing, antibody screening and crossmatching and standards for validation of reagents and controls. There are a number of technical standards covering nucleic acid amplification, flowcytometry and serological techniques, including ELISA and solid phase assays.

Finally there are also standards specifically covering various clinical areas, including renal and other solid organ transplantation, stem cell transplantation, transfusion and disease association.

UKAS ISO15189

ISO15189 represents a significant increase in the regulatory requirement on H&I labs as it has new compliance requirements not found in the old CPA standards. Some labs who are already inspected to MHRA or FACT-NETCORD standards might find the transition slightly easier but even when you do a gap analysis and include those standards there are new requirements in ISO15189 not in any of those standards or with slightly different emphasis.

If you do a gap analysis from CPA to ISO15189, the differences are:

- Measurement of uncertainty needed for key tests
- Need to have service agreements in place which may not have been explicitly stated in the old CPA standards but which all labs already have in place
- Need to have change controls in place for all existing tests even if introduced a long time ago. This may require some retrospective change controls in place for the older techniques in the lab
- Need to undertake supplier audits
- Need to have risk assessments for all test and what the risks are if a test fails. E.g. could an incorrect result be sent out? What are the risks if this was to happen?
- Need to regularly review and document the performance of equipment and tests
- Need to have a system for managing and validating reagents and consumables
- Need to have policies and procedures for CAPAs
- A much greater level of detail seems to be required for compliance with UKAS standards even where they match previous CPA standards

The most significant impact on H&I services is the much greater level of detail required for compliance with ISO15189 compared to CPA. A much greater amount of evidence seems to be required to demonstrate compliance with each of the ISO

standards. This will require dedicated resources and more time from the quality lead and more training in all aspects of quality for all staff. On the other hand, this level of scrutiny does help to raise standards and give confidence to the lab, users and patients about the level of quality in the department.

DIFFERENCES BETWEEN CPA AND UKAS ISO15189

CPA standards provided a comprehensive QMS systems for the provision of laboratory services but ISO15189 represents a significant increase in the regulatory requirement both in terms of new standards and an increase in the evidence required to demonstrate compliance with existing standards.

A gap analysis from CPA to ISO15189 shows the following new standards:

- Measurement of uncertainty needed for key tests
- Need to have service agreements in place which is not explicitly stated in the old CPA standards
- Need to have change controls in place for all existing tests even if introduced a long time ago
- Need to undertake supplier audits
- Need to have risk assessments for all test and what the risks are if a test fails
- Need to regularly review and document the performance of equipment and tests
- Need to have a system for managing and validating reagents and consumables
- Need to have policies and procedures for CAPAs

For existing standards, a much greater level of detail seems to be required to demonstrate compliance with UKAS standards compared CPA standards. For example, CPA requires validation of new tests but CPA inspectors have not historically asked for validation of pre-existing tests. However, ISO15189 requires that tests that pre-date the laboratory validation system have retrospective validation completed.

This will require dedicated resources and more time from the quality lead and more training in all aspects of quality for all staff. On the other hand, this level of scrutiny

does help to raise standards and give confidence to the lab, users and patients about the level of quality in the department.

DIFFERENCES BETWEEN EFI AND UKAS ISO15189

TODO: DIFFERENCES BETWEEN EFI AND UKAS ISO15189

TURNAROUND TIMES

Maintaining turnaround times is very important for the service we provide for patients. If turnaround times are not being met the first thing to do is place this on the organisations risk register so that there is visibility of the issue and perhaps some help from the wider organisation. Also write to our stem cell and solid organ clinical teams to raise the issue and assure them of the steps we are taking to address the situation. Also offer to meet with them to discuss ways we can work together to improve the situation.

One immediate step is to put in place a short-term system to prioritise cases so for instance stem cell transplant cases and renal cases would be prioritised over some DA cases such as B27 testing.

Then to truly address a poor performance issue with turnaround times need to get to the root cause of the failure. Usually there are multiple interrelated issues that need to be addressed. There may be staffing issues both with the number of staff and with training and competence. There may be a need to recruit new staff and/or organise additional training for existing staff. There may be problems with insufficient equipment. There may also be an overreliance on time consuming manual techniques like CDC or PCR-SSP over and above use of techniques such as Luminex for instance.

MEASUREMENT OF UNCERTAINTY

UKAS ISO15189 has introduced the concept of Measurement of Uncertainty (MoU) into pathology services. ISO15189 requires that all laboratories determine the measurement uncertainty of each measurement procedure in the examination phase that goes towards producing a report.

In general, all measurements have a degree of uncertainty associated with them and to truly and accurately report a measure, it must be associated with some indication of the uncertainty in it. Uncertainty arises from the measuring instrument itself, from the item being measured and from the measuring process itself. It also arises from the skill of the operator or from the environment in which the measurement is taking place. Repeated measurements of the same item can and do give different results, with the average giving an indication or estimate of the true measure and the dispersion of the values giving an indication of how well the measurement has been made.

To determine the uncertainty in a measure such as MCS or RMF in a flowcytometric crossmatch or MFI in a Luminex single antigen bead test, the uncertainty in each of the inputs would need to be calculated from repeated measurements such as 10 repeats of a pipette measurement for instance to get the average and standard deviation, or else the uncertainty would need to be estimated such as by assigning a score to the skill of the user.

The overall uncertainty in the result can then be calculated from all the uncertainties in the inputs. In reality, the uncertainty in many of the inputs are mitigated in some way, through regular calibration for instance or through training and competency assessments. In practice therefore, many H&I laboratories carry out a risk assessment for the uncertainty associated with non-numeric reports such as Luminex HLA typing, SBT, NGS, SSP typing and qPCR. In this RA they must demonstrate that they have considered all the potential input sources of uncertainty and how these are mitigated. For example pipettes are regularly calibrated, staff are trained and competency assessed, the environment is controlled, with temperature and sometime humidity monitoring and the sample, i.e. DNA, are controlled for quality and quantity.

These same items are also controlled in tests such as Chimerism, Luminex SAB and Flowcytometric crossmatching but as these techniques yield numeric results, it is

possible to determine the dispersion of the results and report this alongside the MSC/RMF, MFI etc.

For Luminex SAB, Neg control or a QC control can be added to the same run 20 times, the mean and standard deviation (SD) of the MFI values obtained can then be used to calculate the SU as the SD divided by the square root of the number of runs (20 in this case) and this could be reported alongside the MFI.

For flowcytometric crossmatching, one option is to run a single cell against the same Negative control 20 times, taking the mean and SD of this to determine the SU. The SU value can then be reported value alongside the MCS/RMF.

In Chimerism, a confidence interval is required so the laboratory can report their result with say 95% confidence interval. For this, a slightly different approach is required. The laboratory will have to calculate the SU when the chimerism test is repeated say on 20 different occasions on sample known to have mixed chimerism and also calculate the SU when the chimerism test is repeated in the same run 20 times. The two resulting SU's are then combined and multiplied by a conversion factor of 2, which roughly equates to 95% confidence interval. This can be reported with the chimerism results.

An interesting new test to consider is next generation sequencing (NGS). Like SBT it does not report a numeric result as such but it does provide quality parameters which go into whether or not the result can be accepted and it may be possible to determine the MoU of these quality value.

The laboratory should regularly repeat and update each MoU for all tests where this is reported.

CHANGE CONTROL

A change control process represents the sum total of procedures undertaken to ensure that a change is fully validated and risk assessed before being put into routine

use in the laboratory. Both CPA and EFI require that new processes, procedures and equipment must be validated before being implemented. In this context, validation is defined as the production of objective evidence to demonstrate that a process of procedure meets its intended purpose.

A change control process typically starts with a definition of the required change and the development of a change control plan. Key aspects of the change control plan include identification of who is affected by the change, establishing the level of validation is required for the change, identifying which processes and SOPs need to be reviewed and updated, which staff need to trained or retrained and establishing a communications plan for communicating the change to the laboratory's users.

VALIDATION

CPA and EFI both require that any new laboratory techniques, procedures, equipment or reagents be validated before being put into use. CPA defines validation as the confirmation through the provision of objective evidence, that the requirements for a specific intended use have been fulfilled. Validating a new laboratory technique involves answering the question 'Is there evidence that this new technique does the job that it is intended to do' and then producing the evidence to demonstrate this.

New laboratory techniques which involve the use of CE marked products and which are used as indicated by the suppliers do not need full validation as this is already undertaken by the supplier. The laboratory need only verify that the level of performance claimed by the supplier is achievable in the laboratory. In house techniques and techniques which make use of modified CE marked products need to have full validation.

Where an existing technique is being replaced, validation involves undertaking a systematic comparative evaluation of results obtained in parallel with the new and the existing standard techniques. In general, the new technique must yield equivalent results of equal or higher resolution or detail to be considered an adequate replacement. For the introduction of new techniques which are not replacing existing ones, validation involves testing the new techniques against know reference ranges. For quantitative techniques, this involves establishing the accuracy (closeness

to reference value) and precision (reproducibility of results) of the new technique. For quantitative techniques this may be achieved by challenging the technique with known complex samples. An example in a new H&I laboratory would be challenging a HLA typing technique with rare types for instance.

Validation should always be undertaken in accordance with a document validation plan which describes the laboratory's requirements of the new technique as well as lists what will be done and who will be involved. It also describes the responsibilities of all involved. The validation plan usually consists of clearly identifiable stages called the Installation Qualification (IQ), the Operational Qualification (OQ) and the Process Qualification (PQ). The IQ is usually provided by a supplier when they install a new piece of equipment and it verifies that the installation has taken place according to the manufacturer's specifications. In the OQ stage the validation exercise demonstrates that the new technique operates as expected in the laboratory. Finally, in the PQ stage the validation seeks to demonstrate that the new technique performs satisfactorily in the laboratories processes.

QUALITY IMPROVEMENT

A good quality management system underpins the laboratory service and if it falls below requirements can have a direct effect on the clinical service. The first step to improving quality would be to review the existing quality management system (QMS) and how it is applied within the department. Areas to look at include how documents such as SOPs are controlled and reviewed, how equipment are managed, including how often maintenance and calibration are undertaken and reviewed, how often internal audits are undertaken and how any findings are catalogued and acted upon and how quality incidents are recorded and managed. A vertical and horizontal audits may be undertaken to help provide insight into the scale of any issues that need to be addressed.

Having completed all this, the next step would be to draw up a quality improvement plan (QIP) that would describe in detail how to get from where the department is currently, to where it needs to be. A typical QIP includes a description of the aims and objectives of the QIP and a detailed description of the activities that would be undertaken to meet those aims and objectives. The plan would need to include a descriptions of what success looks like and how it will be measured as well as the training and support that will be provided to all those helping to deliver than plan.

Finally a communication plan for the QIP needs to be developed that identifies all stakeholders and describes how their input will be obtained and how they will be kept informed of progress. A post implementation step would be to review the effectiveness of the QIP so that lessons can be learnt for the future.

For a laboratory QMS, an improvement plan would need to encompass:

- A systematic review of all controlled documents to make sure they reflect current practice
- An audit schedule for the next 12 months which will ensure at least one examination audit of each test technique in use, one horizontal audit for the lab and one vertical audit for each section in the lab
- A review of all equipment in the lab, making sure there is a service contract in place for each item of equipment that needs one and making sure there has been maintenance carried out in the last 12 months. For items of equipment that need calibration, ensure the calibration has been carried out and the calibration certificate check and signed as acceptable and any return to service checks are completed
- A review of the approach taken to reagents and kits validation to make sure all required validations are being carried out, all records are being completed fully and all reagents and kits are being stored at the correct temperatures
- A review of all in use tests, techniques and equipment to make sure there are original or retrospective validation/verification records in place
- A review of staff training, competence and continued professional development (CPD) records to make sure all required competences are covered by all staff. This may require a new training schedule be drawn up to cover the next 12 months
- These changes need to be undertaken by involving all staff in the department to ensure that a culture of quality is developed to ensure standards do not fall again

CHAPTER 15 LABORATORY HEALTH & SAFETY

INTRODUCTION TO LABORATORY H&S

All laboratories must have written H&S policies and procedures which guide how the laboratory ensures a safe working environment for staff and visitors. The documents should describe who has direct and delegated responsibility for policy making, for planning H&S activities, for implementing H&S initiates and for monitoring H&S. Ideally, this should be displayed in an organisational chart.

These documents should be backed up by an annual H&S plan which describes the key H&S priorities for the laboratory for the year and outlines H&S audits and reviews that are planned for the year. The H&S plan should also describe how H&S related information will be communicated to staff and visitors. As a minimum, the H&S plan should include the H&S objectives of the laboratory for the year. This could for example include a plan to replace a particular reagent with a less harmful one during the year. The H&S plan should include a timetable for review of general, display screen, COSHH, fire safety, manual handling, lone working and other risk assessments. The H&S plan should allow for regular self inspections or audits.

Ideally the laboratory should appoint a H&S lead who monitors performance against the H&S plan, who meets regularly with the organisations H&S advisors and with other H&S leads from other departments. Finally, the H&S system itself must be regularly reviewed and improved.

Where a H&S inspection or audit has identified a risk of harm from a particular procedure, steps should immediately be taken to mitigate the risk. The audit or inspection would have highlighted the nature of the risk. The first priority should be to eliminate the risk by changing the procedure, perhaps introduce a safer reagent or change to a different technique. Where the risk cannot be eliminated, other mitigating steps must be taken. These include use of correct personal protective equipment (PPE), training in safely carry out the procedure, moving the procedure to a different part of the laboratory or to a different time of the day where interruptions are less likely.

LABORATORY RISK ASSESSMENT

As a general rule, all risk assessments should include a title, the date the risk assessment (RA) was carried out and the author of the RA. The RA must also include a detailed description of the activities for which the RA is being carried out, a list of hazards associated with those activities and the likelihood and nature of any potential harm if no mitigating steps are taken.

The RA should include detailed control measures that have been put in place to reduce the likelihood of harm together with the risk score with those control measures in place. The risk score is calculated by multiplying the consequences of injury on a scale of 1-5, by the likelihood of injury also on a scale of 1-5. In general, a score less than or equal to 6 is considered low, 7-12 is considered intermediate and 13 and above is considered high.

Where the risk score remains high despite mitigation, the RA should outline the steps being taken to find alternative ways of working that reduce the overall risk. Finally, the RA should include the next review date.

GENERAL RISK ASSESSMENT

A general risk assessment (RA) should include all the items described in the introduction to this section. For use of freezers, the specific hazards include risk of burns from exposure to low temperatures, potential manual handling risks from lifting and manoeuvring heavy trays, racks and other items out of freezers and injury risks from edges and corners of freezers doors and trays, especially if working in restricted spaces. The likelihood of harm from these hazards is generally low but increases as the temperature of the freezers decreases. A -150°C freezer for instance has causes more harm from cold burns than a -20°C.

Control measures which can be put in place to reduce the risk of harm from freezers include good laboratory practices, use of insulated gloves, use of lab coats and other CE marked PPE and training in manual handling techniques. Additional control measures such as always having a second person present may be required when working with ultra-low temperature freezers.

With these measures in places, the consequences of injury, on a scale of 1-5, with 1 being minimal injury requiring no treatment and 5 being death or permanent injury, is 2 minor injury requiring minor intervention. The likelihood of harm, on a scale of 1-5, with 1 being rare and 5 being almost certain, is 2 for unlikely. This gives an overall risk score of 4 which is a low risk.

The RA should be reviewed every year.

A COSHH RISK ASSESSMENT

A COSHH RA for gel electrophoresis should include all the items described in the introduction to this section. In addition, a COSHH RA should include a review of all the chemicals associated with a procedure to identify all hazards. For gel electrophoresis, the specific hazards include potential risk of electrocution, a potential manual handling risk when toping up the tanks, risk of exposure to TBE and risk of exposure to ethidium bromide if that is in use. Exposure to these risks is likely to be daily.

Control measures which can be put in place to reduce the risk of harm during gel electrophoresis freezers include annual PET testing for electrical safety, good laboratory practices, use of lab coats, gloves, goggles and other CE marked PPE and training in manual handling techniques. A review of the MSDS associated with all reagents and chemicals, particularly TBE and EB should be carried out and staff warned of all hazards, potential routes of expose and steps to take if exposed. Potential routes of exposure include through the eyes, through inhalation or ingestion or through skin contact.

Warning signs should be prominently displayed and safe storage arrangements made for all chemicals and reagents, especially if poisonous. Where a substance poses a fire risk, it should be stored in a fire safe cabinet. Control measures should also outline steps to take in case of spillage of chemicals or reagents and safe disposal steps for all chemicals and reagents.

Where EB is in use, pregnant members of staff should be removed from all gel electrophoresis duties.

Hazard Symbols (to be used in pictograms for substances of the particular class)		
		
FLAME OVER CIRCLE—USED FOR THESE CLASSES : ▪ Oxidizers	**FLAME—USED FOR THESE CLASSES:** ▪ Flammables ▪ Self Reactives ▪ Pyrophorics ▪ Self-Heating ▪ Emits Flammable Gas ▪ Organic Peroxides	**EXPLODING BOMB—USED FOR THESE CLASSES:** ▪ Explosives ▪ Self Reactives ▪ Organic Peroxides
		
SKULL & CROSSBONES—USED FOR THESE CLASSES: ▪ Acute toxicity (severe)	**CORROSION—USED FOR THESE CLASSES:** ▪ Corrosives	**GAS CYLINDER—USED FOR THESE CLASSES:** ▪ Gases Under Pressure
		
HEALTH HAZARD—USED FOR THESE CLASSES: ▪ Carcinogen ▪ Respiratory Sensitizer ▪ Reproductive Toxicity ▪ Target Organ Toxicity ▪ Mutagenicity ▪ Aspiration Toxicity	**ENVIRONMENTAL HAZARD—USED FOR THESE CLASSES:** ▪ Environmental Toxicity	**EXCLAMATION MARK—USED FOR THESE CLASSES:** ▪ Irritant ▪ Dermal Sensitizer ▪ Acute toxicity (harmful) ▪ Narcotic Effects ▪ Respiratory Tract Irritation

Figure 6 - Hazard Warnings

With these measures in places, the consequences of injury, on a scale of 1-5, with 1 being minimal injury requiring no treatment and 5 being death or permanent injury,

is 1. The likelihood of harm, on a scale of 1-5, with 1 being rare and 5 being almost certain, is 2 for unlikely. This gives an overall risk score of 2 which is a low risk.

The COSHH RA should be reviewed every year.

MANUAL HANDLING RISK ASSESSMENT

A manual handling risk assessment (RA) should include all the items described in the introduction to this section. In addition, a number of specific questions need to be asked. Do the activities for instance involve twisting, holding loads away from the body or over long distances, are they repetitive, frequent, strenuous, do they involve reaching above shoulder height or large vertical movements of loads and is there scope for variation or breaks. Additional questions include whether the activities require specialist training and/or equipment. Finally questions must be asked about the environment in which the activities are taking place. Is space restricted, is the floor uneven, unstable or slippery, is there adequate lighting, ventilation, extreme hot or cold conditions?

The answers to these questions help to identify the specific hazards associated with a given manual handling procedure.

Control measures which can be put in place to reduce the risk of harm from manual handling start with specific and regular training and refresher training in manual handling techniques. Manual handling training needs to include specific training on handing loads, on bending the knees and an understanding of posture. Access to porters who have specialist training and equipment is also a vital control measure. Other control measures include decluttering the laboratory environment, making sure manual handling tasks which require two persons are not attempted by a single person and generally reducing the size and bulk of laboratory supplies in use.

The manual handling RA should be reviewed every year.

LONE WORKING RISK ASSESSMENT

A lone working risk assessment (RA) should include all the items described in the introduction to this section. In a transplantation laboratory staff often work alone at night and at weekends in support of deceased donor HLA typing and of crossmatching. The specific hazards associated with working include potential security risks accessing or egressing the premises, risk of harm if the lone worker were to fall ill or get injured and not be able to raise the alarm and other risks associated with working in a laboratory environment.

Control measures which can be put in place to reduce the risk of harm from for lone workers include ensuring someone else always knows when a member of staff is attending the laboratory for lone working. In addition, there is always someone else elsewhere in the building, including other laboratory staff and security who can render assistance if needed. An additional potential measure is to give lone workers panic alarms or monitors which can raise an alarm if the wearer is inactive for a given period.

Other control measures include good laboratory practices, use of lab coats and other CE marked PPE and training and competence in safe laboratory working.

With these measures in places, the consequences of injury, on a scale of 1-5, with 1 being minimal injury requiring no treatment and 5 being death or permanent injury, is 2 minor injury requiring minor intervention. The likelihood of harm, on a scale of 1-5, with 1 being rare and 5 being almost certain, is 2 for unlikely. This gives an overall risk score of 4 which is a low risk.

The lone working RA should be reviewed every year.

RESPONDING TO ACCIDENTS

The laboratory risk assessments should already include an assessment of the risk of injury to visitors to the department including engineers. If an injury occurs, the first step is to call a trained first aider to attend and offer help. The first aider would decide if ambulance attendance is required. In the case of an obvious serious injury the emergency services should be called immediately even while waiting for a first aider to attend.

The first aider would assess the injury and offer assistance. Depending on the nature of the injury, they may take to injured person to hospital or call an ambulance.

The next step, which needs to start as soon as it safe to do so, is to secure the area where the injury took place and make it safe for staff. This may require spills to be cleaned up for instance or other potential hazards removed.

An investigation would then need to be undertaken to understand why the accident happened and how it can be prevented in the future. As part of this, most organisations would have an accident reporting system and this would need to be activated. Depending on the nature of the injury or near miss even, there may be a need to report outside of the organisation to statutory bodies such as RIDDOR reporting to the Health and Safety Executive in the UK.

The outcome of the investigation should include steps to be taken ton reduce the risk of the accident happening again.

CHAPTER 16 TRAINING

KEY BODIES IN UK HEALTHCARE EDUCATION

The role of HEE is to ensure that the NHS has sufficient staff with the right skills at all levels. They provide funding at the moment for a number of training routes for laboratory staff including the STP and HSST as well as some leadership courses. The Academy of Healthcare Sciences is the single overarching body for healthcare scientists in the UK. The AHSC is responsible for developing regulations for the healthcare workforce and for assessing equivalence of existing qualifications and issuing certificate of attainment as scientists complete their training.

An important aspect of developing a training system in the laboratory is putting in place a comprehensive training policy that identifies key staff involved in delivering training and how their skills are kept up to date, a system of training needs analysis for all staff grades, a clear process and timetable for training new staff in the systems and procedures used in the lab including comprehensive induction, a process for continuous training and development of existing staff, a process for ensuring that statutory and mandatory training such as fire safety and safeguarding and completed on time and a system of annual appraisal. Finally, the training system and policy should be regularly reviewed and audited. Where possible, H&I laboratories should seek to be accredited by HEE as training centres.

To develop a training system for a laboratory, steps that could be taken include, draft a long-term training plan for the lab which would address training at all levels and would be based on the principle of trying to always have staff ready to step up to the next senior role above theirs which becomes vacant. The training plan would therefore start with laboratory support grade staff and creating opportunities for some of them to undertake the BSHI certificate of competence. Some of these staff may go on to follow the full BSHI Diploma course. Also apply for STP funding to have at least one member of staff being trained as a future band 7. If there are BMS staff, create opportunities for them to pursue the specialist portfolio and also apply for HSST funding for one or two of the band 7/8 staff who wish to do so to train towards the college exams. Finally, apply for funding for senior leadership training on schemes such as the Mary Secombe leadership course.

All this would of course require funding, some of which may be available from HEE but would also require significant support from the parent organisation. Other potential sources of funding include charities and the royal colleges.

SUCCESSION PLANING

The key is to encourage as many on of your team as possible to pursue higher training courses and to provide opportunities for those who want to, to do so. These days that could mean applying for funding for in place HSST training positions so you have at least one but preferably two members of staff going staff going through that route. Other members of staff not on the HSST could still go through the college exams the old way by doing the part 1, a PhD or case book while that route is still an option. This would require significant support, including study leave as well as helping to review essays.

For even longer term planning the lab could look to have staff at all levels taking part in some sort of training scheme to create a pipeline of staff ready to step up to the next level. Options include the BSHI certificate of competence, the STP training route, the specialist portfolio for some staff all the way to leadership training for more experienced scientific staff who can move into or take on more management roles. This could also contribute to demonstrating equivalence to the leadership training received by HSST trainees.

STATUTORY AND MANDATORY TRAINING

In the UK, NHS employers are obligated to provide a number of statutory and mandatory training programmes for staff. Employers normally provide the initial training as part of their standard staff induction process.

NHS staff are required to undertake these training sessions at the start of their employment within the NHS and at regular intervals depending on the course. Recently, the NHS in the UK have provided the capability for staff to carry their mandatory training records from one employer to the next thus reducing the need to repetitive training sessions.

Examples of statutory and mandatory training include:

- Health & Safety, including manual handling and fire safety
- Information Governance
- Safeguarding for children and vulnerable adults
- Infection control
- Diversity and Inclusion
- Conflict resolution
- Risk management

COMPETENCY SYSTEM

UKAS ISO15189, in common with a number of other accreditation schemes, has a requirement that laboratories are able to produce documentary evidence that their personnel are competent to carry out the tasks they undertake. There is a requirement not just to demonstrate initial competence but also on going competence or re-validation over time.

Competence encompasses the theoretical knowledge, skills and attitude required to perform a given set of tasks to the level required for the staff grade of that member of staff. The design of a competency assessment system for a laboratory must therefore cover all these factors for all personnel in the laboratory across all tests and services offered by the laboratory and must include details of the assessment criteria that will be used to judge if a member of staff can be deemed competent in each task they undertake.

In the context of an H&I laboratory, a competency assessment scheme must include sample reception and preparation, antibody screening and identification, HLA typing by all techniques offered by the laboratory and to all resolutions at which the laboratory reports results, all crossmatching techniques, all solid organs, transfusion or stem cell services offered by the laboratory and all administration functions.

The structure of each individual competency assessment for different techniques may vary as required but as a minimum needs to include the theoretical knowledge required and the list of SOP's which need to be read, any H&S instructions with the member of staff needs to be familiar, details of the reagents, consumables and

equipment with which the member of staff needs to be familiar to be considered competent, the actual techniques the member of staff needs to be familiar with including details of the number of runs/tests that need to be completed satisfactorily to be considered competent, for those staff grades involved in results analysis and interpretation or review and authorisation, the number of runs/cases they need to complete to be considered competent.

Finally, the scheme needs to include details of how often a particular test or procedure needs to be carried out for the member of staff to remain competent. This re-validation may also include details of routine laboratory activities which may contribute to revalidation without the need for a specific set of re-validation assessments. These could include successful completion of an EQA scheme or completing a confirmatory tests which is in concordance with the initial test or vice versa.

CHAPTER 17 LABORATORY INFORMATICS

GENERAL DATA PROTECTION REGULATION (GDPR)

Maintenance of confidentiality of patients, donors and healthcare professionals is important for legal, financial and moral reasons.

The new EU General Data Protection Regulation (GDPR) is incorporated into UK law from May 2018 and replaces the old Data Protection Act. One of the main changes is that those holding personal data about individuals need to keep a record of when and how consent was obtained to hold that data. Often this means that those holding such personal data need to seek such consent if they do not have records to show that they already have consent.

Under GSPR, personal data is defined as information that relates to an identified or identifiable individual. GDPR has 7 principles for holing personal data:

1. The data must have been obtained lawfully, with **consent** and be used fairly and transparently for lawful purposes
2. The purpose for which the data is **held** must be clear and the use must be limited to this purpose
3. The **extent** of the data held must be related to the purpose for which it was collected and not be excessive
4. The data must be **accurate**
5. The data must be held for a **time limited** period
6. The data must be held securely and confidentially
7. The person or body holding the data must be **accountable** for how the data is used

Loss of healthcare data can cause reputational damage and may open the organisation to significant costs both in terms of fines from Information Commissioners but also in legal costs. In addition, a breach in confidentiality can lead to patients losing the trust in their healthcare providers, with a direct effect therefore on the care of those patients.

Under GDPR, individuals have a right to be informed about the collection and use of their personal data, they have the right to access that data, to have inaccuracies corrected and under certain circumstances, have the right to have that data deleted.

To ensure data is held securely, data must be held on secure IT systems with strong password protection, with only authenticated users allowed access and remember to remove access from employees who have left. The physical IT infrastructure also must be secure from access by unauthorised users and security systems and anti-virus software must be kept up to data.

To prevent data loss, systems must be regularly backed up with restore from backup regularly tested.

Information must only ever be transmitted over secure networks which use encryption to prevent hacking. Use of portable devices for transmitting data must be kept to a minimum and must be encrusted if use is unavoidable. Avoid carrying hard copies of confidential data out of secure premises, especially if using public transport.

Where there is a breach, the relevant authorities in the organisation must be informed immediately. Depending on the nature of the breach the UK Information Commissioner may need to be informed.

ICT CONTINGENCY PLANNING

Part of your contingency planning should always include making sure you have the ability to work manually offline, even if only for short periods such as a couple of days. This means having paper copies of some key patient data and of forms and other records required for test set up.

Depending on how severe the outage is and the estimated time it would take for the LIMS to be back, contingency plans may need to be activated with manual processing of samples. As this would likely cause a delay in testing and report generation, key service users may need to be informed.

The manual process would allow samples to be received, assigned sample Id's and catalogued ready to book in when the IT system is back up and running. Tests can be assigned and testing started safely but would require more checks than perhaps are normally undertaken is manual worklists are being created instead of electronic ones from your LIMS. It is also use if hardcopies of test history of patients are available. Where this is not the case, there would need to be ready access to an up to date backup of the LIMS for looking up patient history. This is why it is important to regularly test your back-up and restore systems to make sure they are reliable.

Manual reports can be generated if the LIMS is still not restored but would require careful crosschecking to avoid errors.

Once the LIMS is restored, it would first need to be carefully checked to ensure data integrity has not been affected. After that the backlogged cases can be entered. A quality incident should be logged so that lessons can be learnt and shared.

ROLE OF SOCIAL MEDIA

The Department of Health has published new guidelines on the use of instant messaging apps for communication of healthcare information. They have not banned apps such as WhatsApp but have set out a number of principles which healthcare professionals need to be mindful of. These include the need to maintain patient confidentiality and abide by data protection rules at all times.

These principles include:

- Instant messaging apps are only to be used if the employer does not provide a suitable alternative
- When an instant messaging app is used it must
    - Only be used on a password protected device
    - Meet NHS end to end data encryption standards
    - Be password protected
    - Have a way of verifying that the person receiving the messaging is who they say they are

    - Allow automatic deletion after a set period of non-use or remote deletion should a device be lost
- Do not use the same groups for clinical and social contributions
- When using instant messaging, patient identifiable information should be kept to a minimum
- Remember that instant messaging does not reduce the obligation for correct documentation of clinical encounters
- Message notification of the lock screen must be disabled so casual observers cannot see these messages when the phone is locked
- Review the links from the app to other apps or services and disconnect wherever possible
- Switch on additional security features such as two factor authentication if available
- Remember that instant messaging data can be subject to FOI requests

BIOINFORMATICS TECHNIQUES USED IN GENETICS

There are a number of categories of Bioinformatics tools and techniques used in genetics which H&I scientists can make use of and which impact the way the service are provided. These include:

- A number of online curated databases and data mining tools such:
    - the Immuno Polymorphism Database (HLA types, KIRs etc.) at https://www.ebi.ac.uk/ipd/index.html
    - the citations database PubMed https://www.ncbi.nlm.nih.gov/pubmed
    - Allele frequencies http://allelefrequencies.net/
    - HLA Nomenclature http://hla.alleles.org/nomenclature/committee.html

- HLA matching and sequence alignment tools such as:
    - WMDA https://www.wmda.info/
    - BLAST https://blast.ncbi.nlm.nih.gov/Blast.cgi

- Online calculators including:
    - The HLA-DPB1 T Cell Epitope Predictor https://www.ebi.ac.uk/ipd/imgt/hla/dpb_v1.html
    - The KIR ligand calculator https://www.ebi.ac.uk/ipd/kir/ligand.html

SPECIALIST H&I WEBSITES AND DATABASES

ALLELE FREQUENCIES

http://allelefrequencies.net/

Set up by Derek Middleton, this website curate data from publications about the frequencies of HLA types in different populations.

HLA-DPB1 T CELL EPITOPE PREDICTOR

https://www.ebi.ac.uk/ipd/imgt/hla/dpb_v1.html

A web resource for classification of HLA-DPB1 mismatches based on T-cell-epitope groups has been shown to identify permissive mismatches and non-permissive mismatches.

HLA NOMENCLATURE

http://hla.alleles.org/nomenclature/committee.html

A central repository for updates to the HLA nomenclature.

IMMUNO POLYMORPHISM DATABASE

https://www.ebi.ac.uk/ipd/index.html

A centralised system for the study of polymorphism in genes of the immune system, primarily, MHC, KIR and HPA

PUBMED

https://www.ncbi.nlm.nih.gov/pubmed

Histocompatibility & Immunogenetics

A website comprises more than 29 million citations for biomedical literature from MEDLINE, life science journals, and online books. Citations may include links to full-text content from PubMed Central and publisher web sites.

WMDA

https://www.wmda.info/

The World Marrow Donor Association (WMDA) took over the management of World Marrow Donor Association (WMDA) and the NetCord Foundation in 2017 to form a single organisation that promots global collaboration and best practices for the benefit of stem cell donors and transplant patients.

HISTOCOMPATIBILITY & IMMUNOGENETICS

https://histocompatibilityandimmunogenetics.com/

Set up by Delordson Kallon, this website contains brief primer notes for H&I staff and others who need a refresher on HLA, tissue typing and the associated laboratory services

CHAPTER 18 RESEARCH AND DEVELOPMENT

RESEARCH FUNDING

Funding for research is becoming harder to obtain and many funding bodies now require applications to come from multi-disciplinary teams working in collaboration. Potential sources of funding include:

- The hospital or parent organisation may have internal funding opportunities
- Medical research charities, including the welcome trust
- Research councils and the Royal Society
- Some government departments, including the department for health

- International bodies such as the European Council and the NIH in America

A research grant application will typically contain the following headline: The title of the research, the name of the organisation where the grant will be held, the CV's of the principal investigator (PI) and other investigators, the aims and objectives of the research, an abstract of the proposed research, a plain English summary of the proposed research, references to any prior work that informs the proposed project, a description of the suggested benefits of the research detailing who will benefit and how, a proposed start date and duration of the project, a detailed description of the resources, human, financial and any others required for the research and finally the technical and ethical considerations for the project.

RANKING SCIENTIFIC EVIDENCE

There are various types of scientific evidence all with differing levels of validity. These include:

- **Systematic review and meta-analysis** - especially of randomized clinical trials
- **Multi centre studies** – Studies involving data from more than one site designed to reduce centre bias
- **Randomized control trials** - Participants in the trial are randomly allocated to either the group receiving the treatment or to a group receiving standard treatment as the control
- **Non-randomized control trials** - studies in which participants are allocated to different interventions or treatment using methods that are not random
- **Observational studies (Cohort, case control, cross sectional and case series)** - studies that draw inferences from a sample to a population where the independent variable is not under the control of the researcher because of ethical concerns or logistical constraints
    - **Cohort study** - study in which a defined group of people (the cohort) is followed over time, to examine associations between different interventions received and subsequent outcomes
    - **Case control** - study that compares people with a specific outcome of interest ('cases') with people from the same source population but without that outcome ('controls')

    - **Cross sectional** - study that collects information on interventions (past or present) and current health outcomes for a group of people at a particular point in time
    - **Case series** - Observations are made on a series of individuals, usually all receiving the same intervention before and after an intervention but with no control group
- **Retrospective studies** - a study in which patient groups are separated non-randomly by exposure or treatment, with exposure occurring before the initiation of the study
- **Interpretive studies** - the subjective viewpoints or experiences of the individual and how they have a bearing on outcomes that are being measured
- **Before and after studies** - study in which observations are made before and after the implementation of an intervention or treatment, both in a group that receives the intervention and in a control group
- **Descriptive Studies** – describe the characteristics of a population or phenomenon being studied without answering questions about how/when/why the characteristics occurred
- **Expert Opinion** - evidence about a scientific, technical, or professional issue given by a person qualified to testify because of familiarity with the subject or special training in the field

These different forms of scientific evidence can be divided into categories of Excellent, Good, Fair and Poor depending on the validity and trustworthiness of the research methodology.

- **Excellent** - Systematic review and meta-analysis and Multi centre studies
- **Good** - Non-randomized control trials, Retrospective studies, some Observational studies and some Interpretive studies
- **Fair** - Non-randomized control trials, Before and after studies and Descriptive Studies
- **Poor** – Case studies and expert opinion

A number of standards have emerged for assessing the quality of the scientific manuscripts. For instance, in diagnostic accuracy publications, the STARD statement (Standards for Reporting of Diagnostic Accuracy Studies) has been developed to improve the completeness and transparency of reports of diagnostic accuracy studies. Other standards include the Consolidated Standards for the Reporting of

Trials (CONSORT). These standards require manuscripts to meet a number of defined criteria, including:

- Clearly defined scientific and clinical background, including the intended use and clinical role as well as aims and objectives
- Eligibility criteria for any participants and/or exclusion criteria as well as baseline demographic and clinical characteristics of participants
- Definition of and rationale for test positivity cut-offs or result categories
- Statistical methods applied, how sample size was determined and randomization protocol if applicable
- Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed as well as results of any other analyses performed, including subgroup analyses and adjusted analyses
- Interpretation of the results, balancing benefits and harms, and considering other relevant evidence and implications for clinical practice
- Study limitations, including sources of potential bias, statistical uncertainty, and generalisability

These standards are used in H&I when developing guidelines. The BSHI/BTS guidelines for the detection and characterisation of clinically relevant antibodies in allotransplantation for instance lists three levels of evidence assigned to each of the recommendations, level I being based on publication in peer reviewed journals, level II being consensus opinion and level III being expert authors opinion.

IMPORTANCE OF PUBLISHING

Publication in peer reviewed journals is one of the ways a H&I scientist can help share and spread knowledge as well as develop their own scientific research skills. Writing up your research to publish it often helps the writer fully understand the significance of their own work and how it fits in with the body of scientific knowledge. Publishing this in peer review journals provides an opportunity to get review and comment from other researcher in the field and will not only improve the work being published, it can provide ideas for new potential areas of research. Publishing also makes the scientist a part of their research community and can lead to interesting collaborations.

Publishing is also the key outcome used to demonstrate to those funding the research, be they the parent organisation or an external body, that their money has been put to good and legitimate use.

Many national and international journals have their own specific requirements for scientific manuscripts submitted to them for publication but these generally follow the same broad outlines. These include:

- The journal will specify the language the manuscript must be submitted in and would often require that the writing style be in the direct and active voice
- The journal will also specify formatting requirements such as single side of A4 paper, double spaced with wide margins
- Only one author must submit for all authors and with the permission of those authors together with address details for all authors
- The material submitted must be accurate, original and unique
- The manuscript must not have been published anywhere else before
- Some of the higher impact journals will also require that the manuscript not be under consideration for publication anywhere else
- The journal will also specify referencing requirements such as:
    - The Vancouver style: References are numbered in the order in which they appear in the text and at the end of the manuscript, the reference list is produced showing for each numbered reference, the authors, followed by the title of the article, the abbreviation of the journal name, the year of publication, the volume number and the first to last page numbers
    - The Harvard styles: This is an author-date style in which the author and year of publication are cited in the text and at the end of the manuscript, the reference list is produced showing for each author-date combination, the authors, year of publication, followed by the title of the article, the journal name, the volume, issue or number and the first to last page numbers. If the article has been viewed online, then the date last accessed and the URL
- The journal will also have rules for tables and images, such as whether they need to be included inline in the t3ext or submitted as separate files. There will also normally be a limit to the number of tables and images
- Finally the journal will have requirements for conflict of interest declarations and for ethical approval statements, including statements on consent

RESEARCH ETHICS

Research Ethics is a set of principles governing the way any research involving interaction between the researcher and other humans and/or animals, human tissue or data relating to humans, is designed, managed and conducted. These principles are designed to ensure the dignity, rights, safety and well-being of human participants or animals are at all times considered, respected and safeguarded.

The employing organisations Research Ethics policy must be read in conjunction with legislation such as the GDPR and other data protection laws, animal welfare legislation.

Ethics is often misunderstood by researchers as hindering the scientific progress, however a crucial outcome of rigorous ethical review of proposed research is that it can protect the researcher by providing clear guidelines and a system of oversight.

All research undertaken must be reviewed and approved by the relevant Ethical Review body of the employing organisation. The Ethical Review body will want to assure themselves that certain universal standards are met. These include ensuring that:

- The proposed research is justified based on whether it is likely to add to the existing knowledge base, is of sufficient standard and the proposed researchers are qualified to carry out the roles proposed in the research proposal. All of the researchers involved and participants must be fully informed of the nature of the research being undertaken
- Harm to participants is avoided and the risks posed to participants is outweighed by the potential benefits of the research. Participants must be informed of what their participation entails and must give their consent voluntarily, free from any coercion
- The research is carried out transparently and impartially and any potential conflicts of interest declared and the research complies with all statutory and other guidance, including those relating to data protection
- Any data and information gathered as part of the research must be treated confidentially and the anonymity of participants respected

- An important consideration is whether or not the financial arrangements in support of the proposed research appear sound. It could be considered unethical to start research that may not be completed because insufficient funds were available
- Finally, last but not least researchers should give cognisance to any cultural, religious, gender or other variances in a research population

These principles helps support a fair balance between a researcher's right to unrestricted academic enquiry whilst ensuring adherence to appropriate and robust ethical standards to ensure the protection of all those participating in research studies, including the protection of the employing organisation from legal and other penalties if there are breaches.

INTELLECTUAL PROPERTY

Intellectual Property (IP) refers to tangible unique items created by a person or persons. This means that an idea on it's own does not count as IP but anything created from that idea can potentially be consider IP, provided it has not already been protected by someone else.

Some IP rights apply automatically to your creations. These include copyright which covers writing and literary works, art, photography, films, TV, music, web content, sound recordings and Design rights which cover the shape of items i.e. how different parts of a design are arranged together.

Other types of IP protection have to be applied for. These include Trade Marks such as product names, logos and jingles, Registered designs including the shape, packaging, patterns, colours, and decoration of an item and Patents.

When reviewing research for aspects that may have some IP worth protecting, it is best to seek expert advice at the research planning stage and especially at the publication stage as publication may have some implications for what can then be protected. Some IP considered to already be in the public domain cannot then be protected.

STATISTICAL TOOLS

RELATIVE RISK

The relative risk (RR), also known as the Risk Ratio, is the ratio of the probability of an outcome in an exposed group to the probability of an outcome in a non-exposed group. It is typically used to determine association in Cohort Studies where a study starts with exposure for example to a drug or presence of a particular genetic mutation and goes forward in time looking at development of the disease or condition amongst the exposed and non-exposed groups. It helps with assessing the likelihood that an association representing a causal relationship.

Relative Risk is a ratio of two probabilities whilst Odds ratio is a ratio of two odds.

In H&I, RR is often used in HLA disease association studies or to compare outcomes of two treatments, for instance acute rejection episodes in renal transplant patients treated with Tacrolimus versus those treated with Cyclosporin.

Relative risk is calculated by dividing the risk of developing the disease or condition in the exposed group by the risk of developing a disease or condition in the non-exposed group.

- A RR = 1 means that there of no difference between the exposed and non-exposed groups
- A RR < 1 means that the risk of the outcome is decreased by the exposure
- A RR > 1 means that the risk of the outcome is increased by the exposure

When interpreting the RR it is important to take the 95% confidence interval (CI) into account. For the RR to be significant, the CI must not include 1 as that implies no effect. e.g. If RR = 1.2 and 95% CI = 0.8 – 1.6, this would not be considered significant as the CI include the number 1.

Consider a disease association study in which 10/24 people who tested Positive for HLA-B27 had Ankylosing Spondylitis (AS) and 6/216 people who tested Negative for HLA-B27 also had AS.

Table 8 - Relative Risk Calculation

	AS Pos.	AS Neg.	Total	AS Pos. Freq.
B27 Pos.	10	14	24	10/24 = 0.417
B27 Neg.	6	210	216	6/216 = 0.028
				RR = 0.417/0.028 = **15**

This means that:

- The frequency of AS positive in the B27 positive people was 10/24 = 0.417
- The frequency of AS positive in the B27 negative people was 6/216 = 0.028

The RR of susceptibility to AS conferred by B27 is calculated as the ratio of the probability of AS positive in B27 positive individuals (0.417) divided by the probability of AS positive in B27 negative individuals (0.028):

i.e. RR = 0.417/0.028 = 15

ODDS RATIO

Case control studies start with the condition and go backwards in time looking for exposures. Case control studies use Odds Ratios to measure association. Odds ratios are the ratio of the odds of developing a condition having been exposed versus the odds of developing a condition not having been exposed.

Odds ratio is a ratio of two odds whilst Relative Risk is a ratio of two probabilities.

Consider a disease association study in which 10/16 people with Ankylosing Spondylitis (AS) tested Positive for HLA-B27 and 14/224 people without AS also tested Positive for HLA-B27 for AS.

Table 9 - Relative Risk Calculation

	AS Pos.	AS Neg.	AS Pos. Odds
B27 Pos.	10	14	10/14 = **0.714**
B27 Neg.	6	210	6/210 = **0.029**
			OR = 0.714/0.029 = **25**

This means that:

- The Odds of a person with AS versus a person without AS having B27 positive is 10/14 = 0.714
- The Odds of a person with AS versus a person without AS having B27 negative is 6/210 = 0.029

The Odds Ratio (OR) is the ratios of the odds of a person with AS versus a person without AS having B27 positive (0.714) divided by the odds of a person with AS versus a person without AS having B27 negative (0.029):

i.e. OR = 0.714/0.029 = 25

T-TEST

A t-test (also known as the Student's t-Test), compares two means to assess whether the groups from which the means are drawn are similar or different from each other. Where they are different, the t-test also gives an indication of how significant the differences are i.e.it gives a p-value.

Note that there are a number of assumptions about the underlying data that must be satisfied for the t-test to be valid, one of the key ones of which is that the populations are normally distributed. Where the data is not normally distributed, the Mann–Whitney test should be used instead.

A t-test can be used to:

- Compare the means for two groups. This is called an independent t-test
- Compares means from the same group at different times. This is called a paired t-test
- Compare the mean of a single group against a known mean

The t-test yields a t-value and a p-value. The t-value is a derived measure which says how similar the two-population means are. A t-value of 0 means that the means are the same. The larger the negative or positive t-value, the greater the difference between the two mean. However, to determine if any difference is significant, the p-value is required. By convention, a p value or less than 5% or 0.05 is considered significant.

In H&I, the t-test is often used to validate new reagents before they are put into use. Consider a situation in which a new AB Serum is being considered for use as a negative control for flowcytometric crossmatching. One potential validation step would be to run the existing and new controls several times and compare use a t-test to compare the mean channel shifts or relative mean fluorescence to determine if there is a difference or not between the two controls.

CHI SQUARE TEST

The chi-squared test is used to determine whether there is a significant difference between the expected frequencies and the observed frequencies in one or more categories.

In H&I, Chi Square calculations are often used in disease association studies. Consider a South East Asian population in which HLA-B54 was seen anecdotally often in patients with a chronic obstructive pulmonary disease (COPD). A disease

association study could be set up to determine if the association is significant. Suppose 28/76 patients were B54 Pos. and 16/110 controls were also B54 Pos.

Table 10 - Chi Square Calculations - Observed

Observed	COPD Pos.	COPD Neg	Total
B54 Pos.	28	16	44
B54 Neg	48	94	142
Total	76	110	186

The Chi Square values could be calculated as follows:

1. Using the observed data, calculate the expected value in each cell by multiplying the row and column totals that cell and dividing by the grand total, e.g. for COPD Pos. B54 Pos. = 44*76/186 = 18

Table 11 - Chi Square Calculations - Expected

Expected	COPD Pos.	COPD Neg	Total
B54 Pos.	18	26	44
B54 Neg	58	84	142
Total	76	110	186

2. For each cell, calculate the square of the difference between the observed and expected values, divided by the expected value, e.g. for COPD Pos. B54 Pos. = $(28\text{-}18)^2/18 = 5.59$

Table 12 - Chi Square Calculations

Expected	COPD Pos.	COPD Neg	Total

B54 Pos.	5.59	3.86	9.45
B54 Neg	1.73	1.20	2.93
Total	7.32	5.06	12.37

3. The Chi Square value is the grand total of all the cells = 12.37
4. To determine if this Chi Square value is significant, the value must be compared to the appropriate critical values based on the number of degress of freedom (df) e.g. https://www.medcalc.org/manual/chi-square-table.php
5. For a 2x2 chi square table the df is (2-1)*(2-1)=1
6. For 1 degree of freedom the critical value for p=0.05 is 3.841
7. As our Chi Square value (12.37) is greater than 3.841, we assume the difference between our observed data and the expected data is significant
8. This means that in this population, there is a statistically significant association between HLA-B54 and COPD

One restriction on the Chi Square test is that all the expected frequencies in each cell (of a 2x2 table) should be at least five and sample size must not be small for the calculation to be reliable. When the Chi-squared test is used for small sample sizes, Yates's correction may need to be used to make adjustments for continuity. Yates correction does not however remove the need for each cell to be at least 5. If any of the values are less than 5 then use of Fisher's Exact Test is recommended.

FISHERS EXACT TEST

Fisher's exact test is a statistical significance test used in the analysis of contingency tables. It is used mainly in place of the Chi Squared test when any of the values are less than 5 and/or when the overall sample size is small. Technically though, Fisher's exact test is valid for all sample sizes. The result of a Fisher's test is usually more or less the same as the result of the Chi-squared test with Yates's correction.

The Fisher's exact test also uses the 2x2 contingency table. However, the calculation is based on the observed row and column totals and does not include expected totals. To calculate Fisher's Exact score, all possible 2x2 contingency tables which will give the same row and column totals as the observed data are constructed. For each table, probability for such data to arise if the null hypothesis is true is

calculated. To get the overall probability, the probability of the observed data and all other probabilities for alternative 2x2 tables equal or more extreme than that of the observed data are added up.

Like the Chi Squared test, Fisher's exact test is used in H&I mostly for disease association studies.

ROC CURVE

A Receiver Operating Characteristic (ROC) Curve is a graphical plot that illustrates the diagnostic ability of different tests. It is a way of comparing diagnostic tests. It is a plot of the true positive rate against the false positive rate.

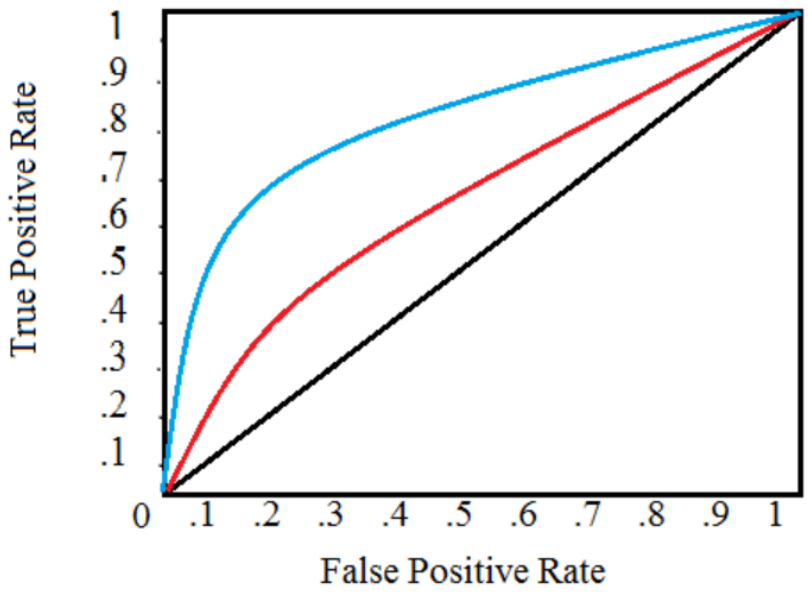


Figure 7 - ROC Curve

The closer the graph is to the top and left-hand borders, the more accurate the test is. On the other hand, the closer the graph to the diagonal, the less accurate the test. A perfect test would go straight from zero up the top-left corner and then straight across the horizontal. A perfectly random test would follow the diagonal.

The accuracy of a test is based on the area under the curve. The greater the area under the curve, the more accurate the test. A perfect test would have an area under the ROC curve of 1. A perfectly random test would have an area under the curve (represented by the diagonal) of 0.5, i.e. half of the area of the graph. IN the example above, the test with the blue line has a greater area under the ROC curve and is therefore a better test than the one represented by the red curve.

KAPLAN-MEIER SURVIVAL ANALYSIS

Kaplan-Meier survival analysis is used to measure the fraction of subjects living for a certain amount of time after a given intervention or treatment. In other words, Kaplan-Meier is used for 'survival analysis'.

Survival analysis comprises of measuring the time from a treatment or intervention to the occurrence of a given event, such as relapse or death. One of the challenges in undertaking survival analysis is that participants in the study may become uncooperative or refuse to remain in the study, researchers may simply lose touch with the participants midway through the study or the participants may not experience the event or death before the end of the study, although they would potentially have experienced the event or died if observation continued. On the other hand, some participants may have joined the study at some after the initial start and so will have shorter follow up periods. These situations, taken all together, are called censored observations. Kaplan-Meier survival analysis is one of the simplest ways of computing the survival over time despite all these difficulties.

The Kaplan-Meier survival curve is defined as the probability of subjects surviving in each period of time between observations, while considering periods of time in many small intervals. There are three assumptions used in this analysis:

1. At any time, patients who are censored have the same survival prospects as those who continue to be followed
2. The survival probabilities are the same for subjects recruited early and late in the study
3. The event happens at the time specified

The estimate is more accurate with smaller intervals between observations.

For each time interval, survival probability is calculated as the number of subjects surviving divided by the number of patients 'at risk'. Subjects who have died, dropped out or move out are not counted as 'at risk'. The total probability of survival till any given time interval is calculated by multiplying all the probabilities of survival at all time intervals preceding that time.

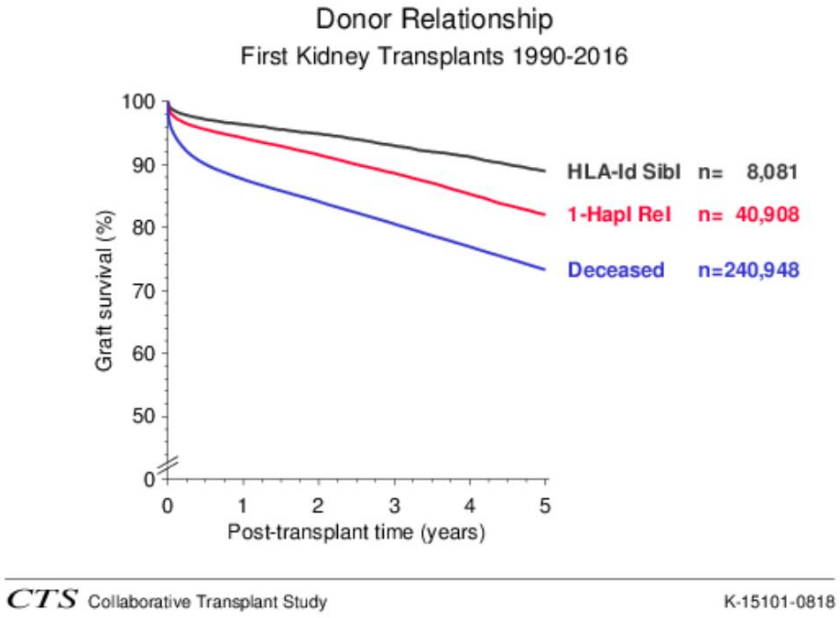


Figure 8 - Kaplan-Meier Plot

COX

Cox regression (also known as proportional hazards regression) is used to analyse the effect of several risk factors on survival. Whereas Kaplan-Meier curves and log rank tests are used to assess survival according to one factor, ignoring the impact of any other factors, the Cox regression model extends survival analysis methods to assess simultaneously the effect of several risk factors on survival time. In addition, Kaplan-Meier and log rank tests are useful only when the predictor variable is categorical (e.g. treatment A vs. treatment B), whereas Cox regression can be used with quantitative values such as weight and age.

The Cox regression effect of each risk factor on survival is expressed as the hazard ratio (HR) Note that the HR is not the same as relative risk (RR). While RR is the risk of an event over the whole time period, HR is an instantaneous risk of the event. The risk at a given point in time.

- A HR = 1 means no effect
- A HR < 1 means reduction in the hazard
- A HR > 1 means increase in the hazard

When interpreting the HR it is important to take the 95% confidence interval (CI) into account. For the HR to be significant, the CI must not include 1 as that implies no effect. e.g. If HR = 1.2 and 95% CI = 0.8 – 1.6, this would not be considered significant as the CI include the number 1.

An important assumption in Cox proportional hazard calculations is that the ratio of the hazards remains constant over time. I.e. the HR is independent of time. If a patient has a HR of dying of 2.0 compared to a control, that risk remains the same over time.

EXPECTATION MAXIMISATION

The Expectation-Maximization (EM) algorithm is a way to find maximum-likelihood estimates for model parameters when your data is incomplete or has missing data points. It is an iterative way to approximate the maximum likelihood function.

While maximum likelihood estimation can find the "best fit" model for a set of data, it doesn't work particularly well for incomplete data sets. The EM algorithm can find model parameters even if you have missing data. It works by choosing random values for the missing data points and using those guesses to estimate a second set of data. The new values are used to create a better guess for the first set and the process continues iteratively until the algorithm converges on a fixed point.

IN H&I, EM is used to calculate haplotype frequencies. The EM algorithm leads to maximum-likelihood estimates of the molecular haplotype frequencies under the assumption of Hardy-Weinberg proportions.

POSITIVE AND NEGATIVE PREDICTIVE VALUE

The Positive Predictive Value (PPV) of a test for a disease or condition is the proportion of positive tests results that represent true positives for the disease or condition. It is the ratio of true positives divided by the sum of all positives, true and false.

Consider a study in which we test 100 patients known to have a condition and 100 controls known not to have the condition with a brand-new test. If for instance 80/100 of the patients known to have the condition tested positive and 5/100 of the patients known not to have the condition also tested positive, the new test can be said to have a PPV of 80/(80+5) = 80/85 = 94.12%. The test can also be said to have a false positive rate of 5/(80+5) = 5/85 = 5.88%.

Table 13 - PPV and NPV Calculations

	Disease	No Disease	
Positive Test	80	5	**PPV**: 80/(80+5) = **94.12%** False Pos: 5/(80+5) = 5.88%.
Negative Test	20	95	**NPV**: 95/(20+95) = **82.61%** **False Neg**: 20/(20+95) = **17.39%**
	100	100	200

A PPV of 100% for the association of the HLA-B*57:01 tests with abacavir hypersensitivity means that there are no false positives. One hundred percent of those patients who test positive for the HLA-B*57:01 gene will be hypersensitive to abacavir.

The Negative Predictive Value (NPV) of a test for a disease or condition is the proportion of negative tests results that represent true negatives for the disease or condition. It is the ratio of true negatives divided by the sum of all negatives, true and false. Consider a study in which we test 100 patients known to have a condition and 100 controls known not to have the condition with a brand-new test. If for instance 80/100 of the patients known to have the condition tested positive and 5/100 of the patients known not to have the condition also tested positive, this would mean that 20/100 patients known to have the condition have nevertheless tested negative. It would also mean that 95/100 patients known not to have the condition have tested negative. The new test can be said to have a NPV of 95/(95+20) = 95/115 = 82.61%. The test can also be said to have a false negative rate of 20/(95+20) = 20/115 = 17.39%.

A NPV of 97% for the association of the HLA-B*57:01 tests with abacavir hypersensitivity means that 97/100 patients who test negative for the HLA-B57:01 allele are not hypersensitive to abacavir and that there are three out of every 100 patients who tests negative for the HLA-B57:01 allele who are nevertheless hypersensitive to abacavir.

POWER CALCULATIONS

We would perhaps calculate the Relative Risk or hazard ratio and use power calculations to determine the sample size require.

Statistical power is the probability of rejecting the null hypothesis assuming the alternative hypothesis is true. e.g. the probability of finding a difference between the test and control arms of a study when a difference truly exists. A well-designed study should have a high statistical power, at least 80%. There are two techniques for increasing the power of a study:

1. Increase the size of the effect or difference we are trying to measure
2. Increase the sample size. Increasing the sample size decreases the standard error, which results in higher statistical power

Three things are therefore required for sample size calculations. The p value required (typically 0.05), the sample size and the size of the effect or difference being measured. This could be informed by pilot data or previous studies.

PRECISION AND ACCURACY

Accuracy is a measure of how close a given measurement is to the actual answer while Precision, on the other hand, refers to the reproducibility of this measurement irrespective of how close it is to the actual answer.

During calibration of pipettes, several measurements are made, typically 5 or 10 at a given pipette setting and the measurements obtained are compared to the setting of the pipette.

The accuracy of the pipette relates to how close the average of the measurements taken is to the expected results. The precision of the pipette relates to how close the 5 or 10 measurements are to each other. It is possible for a pipette to be accurate but not precise, that is to say the average of the measurements is close to the expected value but the measurements are all far from each other.

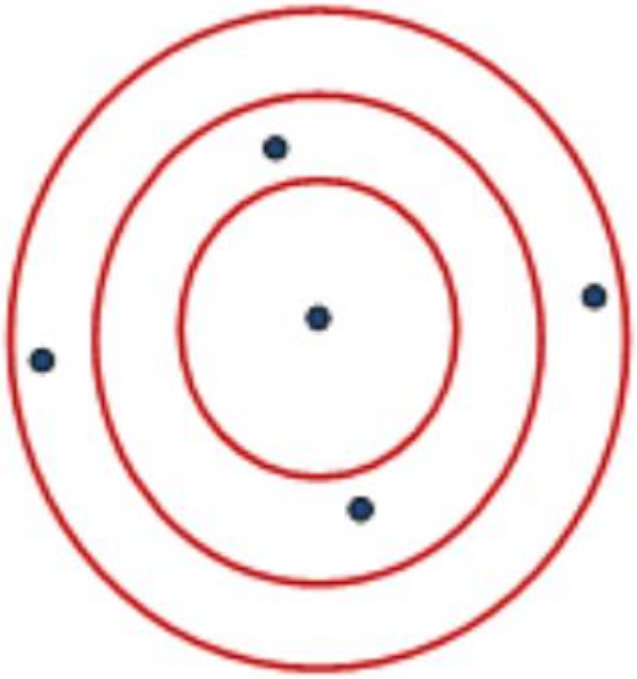


Figure 9 - Accurate but not Precise

The precision of the pipette relates to how close the 5 or 10 measurements are to each other. The accuracy of the pipette relates to how close the average of the measurements taken is to the expected results. It is possible for a pipette to be precise but not accurate, that is to say the measurements are all close to each other but not close to the expected value.

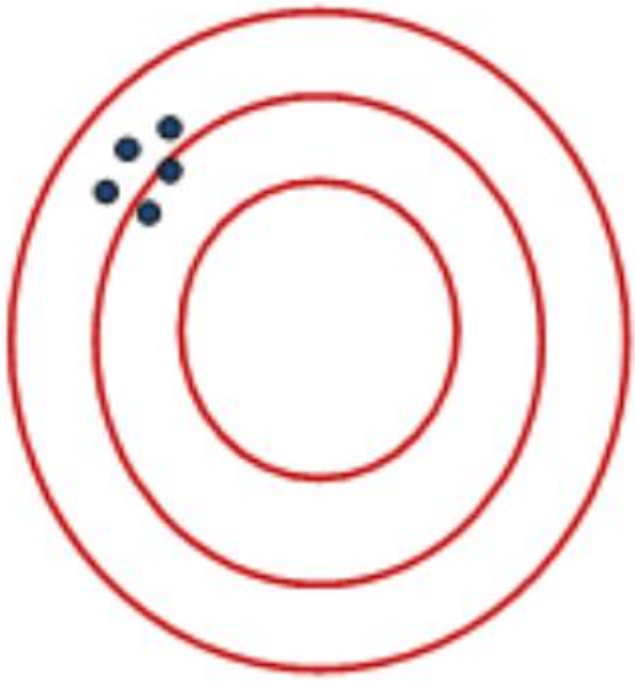


Figure 10 - Precise but not Accurate

Ideally, the pipette calibration measurements should be both accurate and precise.

Figure 11 - Accurate and Precise

When pipettes fail their calibration, they should ideally be taken out of use and sent for repair or if not possible to repair, should be replaced. A CAPA should also be raised and a look back undertaken to understand how tests carried out with those pipettes may have been affected. The look back should cover the period since the last satisfactory calibration.

Occasionally it may be possible to continue to use a pipette if it fails accuracy and/or precision tests at its high or low point by specifying that the pipette should not be used at that range.

TREND ANALYSIS

Trend analysis simply means the process of collecting data and usually expressing it graphically over time to detect a pattern. It is used in many industries and forms a system of statistical process control to alert users to a process that is varying from the norm. In some systems of statistical process control, a waring line is set at one

standard deviation above and below the mean to advice users when to take action and at two standard deviations to alert users to a significant shift where urgent action is required.

In the H&I laboratory there are a number of situations in which such trend analysis is useful. Where a control sample is routinely added to a test that gives quantitative results, such as a Luminex antibody screen, it may be possible to plot the MFI values obtained between runs to observe the trend and alert the laboratory if there is a drift or sudden change in the results.

In the direct clinical context, plotting the MFI levels of Luminex single antigen bead detected HLA antibodies is useful both in HLA incompatible transplants where a desensitisation protocol may be in use and in standard post-transplant DSA level monitoring, especially is graft dysfunction is detected.

Another clinical area where trend monitoring is very useful is in chimerism monitoring post HSCT where changes in donor chimerism levels over time are just as useful to know as the absolute levels of donor chimerism.

On the management side of H&I laboratory service provision there are a large number of activities that are very useful to trend and monitoring, including monthly non-pay spend, number of quality incidents, SOP reviews, audits and audit findings etc.

SENSITIVITY AND SPECIFICITY

Ideally a test should be able to deliver results with 100% sensitivity and 100% specificity but in a practice, this can be difficult to be achieved. In most cases, a trade-off between the two is required. The difference between the two is that:

- Sensitivity measures the probability of something being tested being a true positive
- Specificity measures the probability of something being tested being a true negative

Sensitivity is also often called the recall rate. It is a measure of the probability of actual positives. Sensitivity is more focused on identifying the sample members who are true positives. For instance, how often does the new assay detect a positive screen when the sample is known to be positive from other historical tests.

Sensitivity can be shown by a simple equation:

- Sensitivity = Number of true positives (correctly identified)/[Number of true positives (correctly identified) + Number of false negatives (incorrectly rejected)]

Specificity is also known as true negative rate. It measures the probability of actual negatives. Specificity is more focused on finding out the sample members who are true negatives.

Specificity can also be easily put to an equation:

- Specificity = Number of true negatives (correctly rejected)/ Number of true negatives (correctly rejected) + Number of false positives (incorrectly identified)

Consider a study in which we test 100 samples known to be HLA antibody positive and 100 samples known to be antibody negative using a new screening method. If 80/100 of the known positive sample tested positive and 5/100 of the known negative samples also tested positive, the new test can be said to have sensitivity of 80/(80+20)*100 = 80%. If 5/100 of the known negative samples tested positive and 95/100 of the known negative samples tested negative, the new test can be said to have specificity of 95/(5+95)*100 = 95%.

Table 14 – Sensitivity & Specificity Calculations

	Known Pos.	Known Neg

Positive Test	80 (true positive)	5 (false positives)
Negative Test	20 (false negatives)	95 (true negatives)
	Sensitivity: 80/(80+20)*100 = **80%**	**Specificity**: 95/(5+95)*100 = **95%**

Specificity is very important in medical testing. In medical testing confirming that a person does not have the disease can be more important than detecting a person has it. Because when the positive response is taken into consideration there is no assurance on the degree of disease, for it simple states the person is positive. But knowing a person has not got a disease can be a more decided and strong result. It is same for biochemical testing, where finding that certain substances are present is a weak result compared to finding its absence. Both these statistical properties are important and it is crucial to decide which should be traded off for which.

KEY CHART TYPES

COLUMN CHART

A column chart shows data in vertical columns with spaces in between them. Each column represents a category and the height of the column represents the data value for that category. A column chart should be used when making comparisons between two or more discrete (i.e. non-continuous) data items, especially if the data labels (names) of each category are small/short e.g. kidney patient waiting list size for a given centre by ABO blood group.

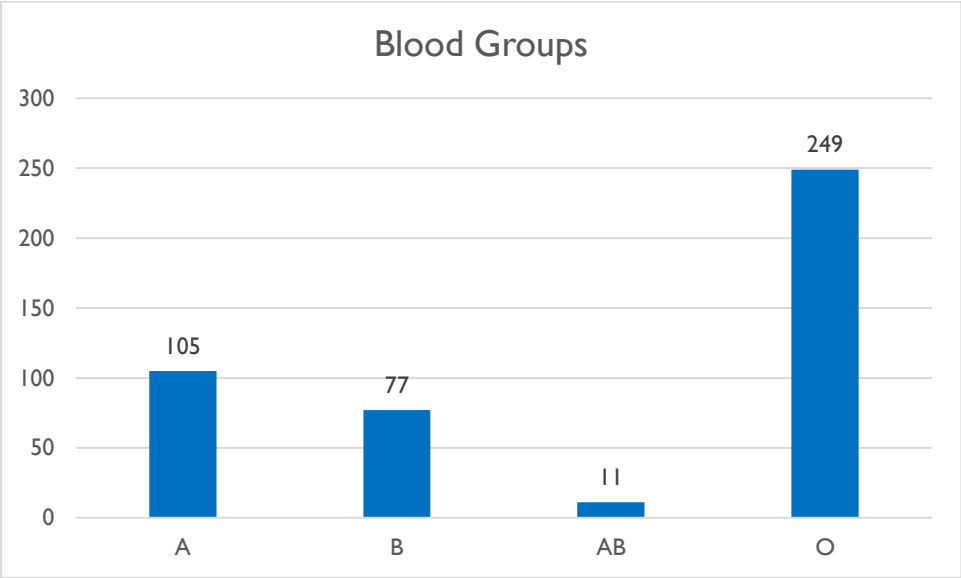


Figure 12 - Column Chart

One main advantage of a column chart is that it shows the scale of the categories. A column chart is also useful as negative values below the horizontal axis.

One disadvantage is that depending on the order of the categories, it is possible to wrongly draw the wrong conclusion, assuming a trend for instance where one might not exist. A column chart is also not always very useful if the names of the data categories are long e.g. different diagnosis, though it is possible to orient the data label vertically.

BAR CHART

A bar chart shows data in horizontal bars with spaces in between them. Each bar represents a category and the length of the bar represents the data value for that category. A bar chart should be used when making comparisons between two or more discrete (i.e. non-continuous) data items, especially when the names of each category are too long to the squeezed into the space below each column in a column chart for instance e.g. Active kidney patient waiting list size by transplant centre.

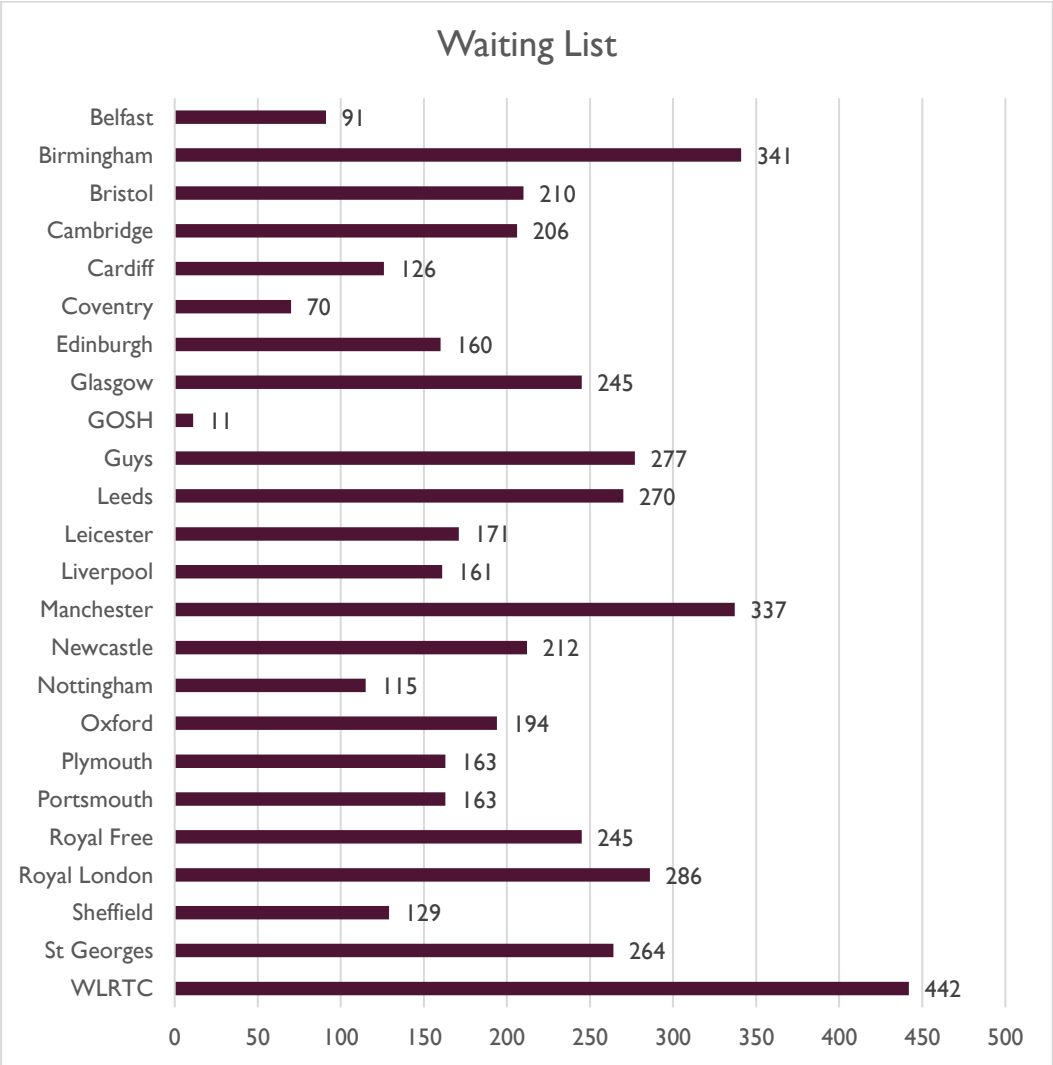


Figure 13 - Bar Chart

One main advantage of a bar chart is that it shows the scale of the categories. Bar charts are easier to read when there are many categories compared to column charts.

One disadvantage is that depending on the order of the categories, it is possible to wrongly draw the wrong conclusion, assuming a trend for instance where one might not exist.

HISTOGRAM

A histogram chart shows continuous data usually in vertical bars with no spaces in between them. Each bar represents a category and the length of the bar represents the data value for that category. A histogram should be used to show data where there are no gaps between the categories, weeks since transplant for instance. e.g. Active kidney patient waiting list size by transplant centre.

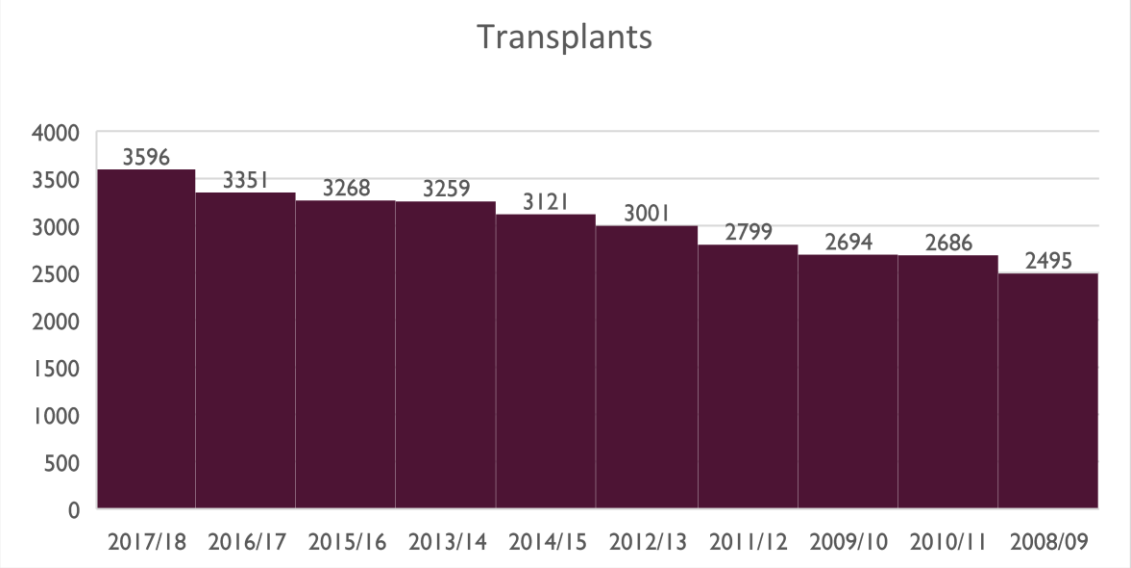


Figure 14 - Histogram

One main advantage of a histogram is that it shows the continuity of the data categories.

A disadvantage is that because the data is grouped together, it can be difficult sometimes to see the individual data.

BOX AND WHISKER PLOTS

A box and whisker plots show the median and range of a group of data with the size of each box representing the spread of the data from the first or lower quartile (Q1 - the middle number between the lowest number and the median) to the third or upper quartile (Q3 - the middle number between the median and the largest number) and a bar across the box representing the median or second quartile (Q2).

Whiskers on both ends of the boxes show the variability outside the upper and lower quartiles. This is measured in different ways in different plots so should be checked when reading data. It could for instance be the min and max of all data but it could also represent the standard deviation above and below the mean.

Outliers are shown as individual data points, usually a dot on the chart.

A box and whisker plot should be used for data that has a spread e.g. waiting times for patients on the kidney transplant waiting list by blood group.

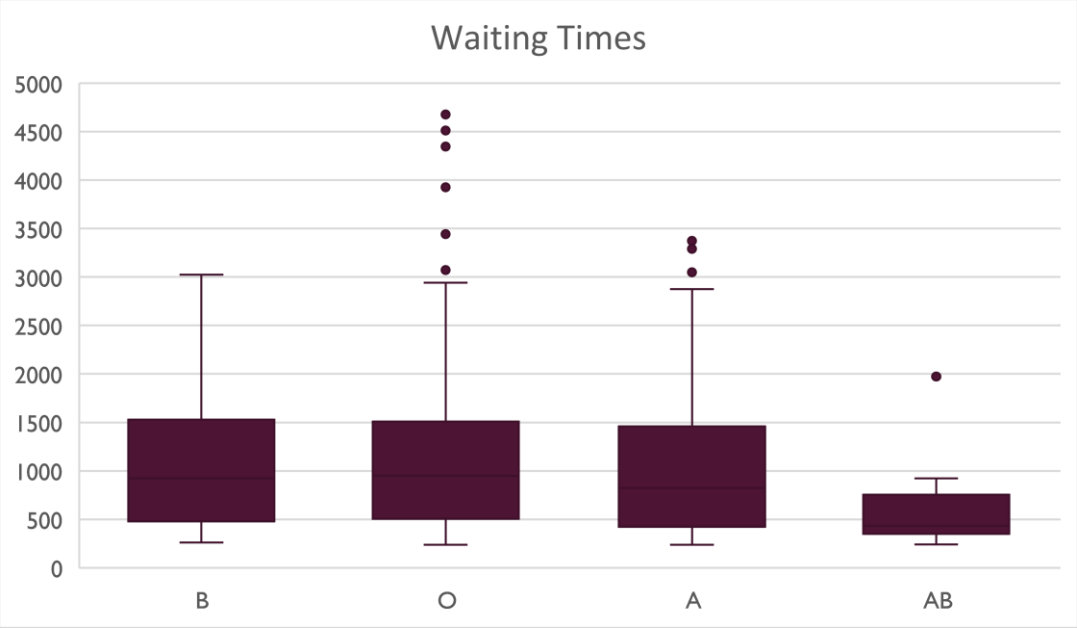


Figure 15 - Box and Whisker Plot

One main advantage of a box and whisker plot is that it can show the median, means and spread of the data in a single chart.

A disadvantage is that the chart can be difficult to explain to someone not familiar with the data.

LINE CHART

A line chart plots data over time, typically as a series of dots and draws a line joining the dots together. A line chart should be used to show changes in data over time e.g. changes in DSA MFI levels following several rounds of plasma exchange

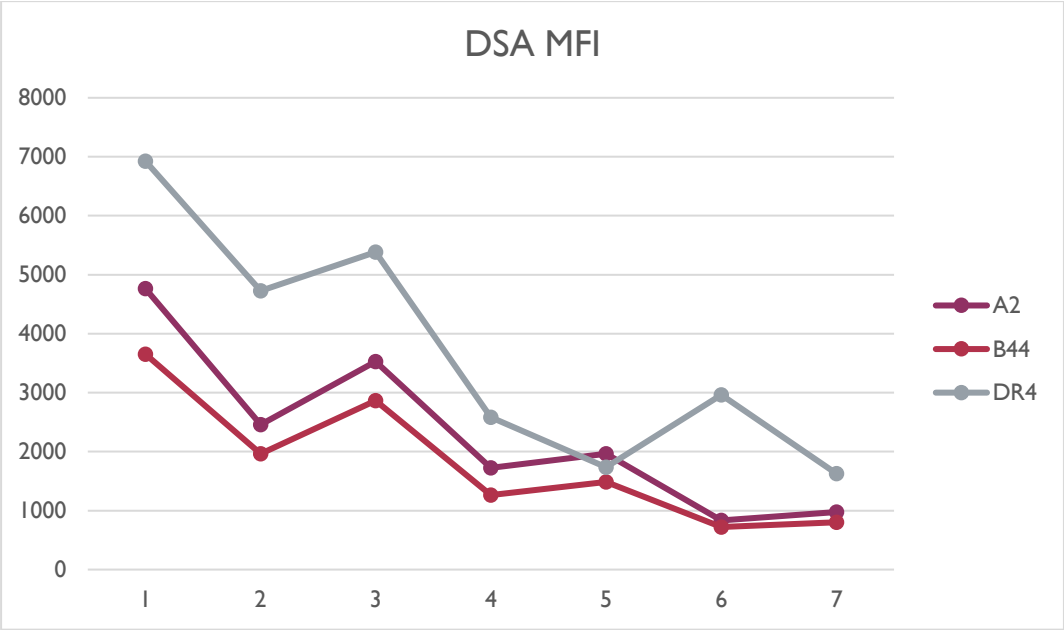


Figure 16 - Line Chart

The advantages of a line charts are that they are simple, they typically show all the data points and they can be used to show multiple sets of data.

The main disadvantage is that they only make sense in the context of ordered data.

RUN CHART

A run chart plots data over time, typically as a series of dots and draws a line joining the dots together and plots the median of the data on the same chart. A line chart is typically used to show changes in data over time for a single category being tracked

and are very useful for monitoring trends e.g. changes if a laboratory runs a control sample in every Luminex antibody run, changes in the MFI level of the control sample can be tracked to spot trends which may affect interpretation of the data.

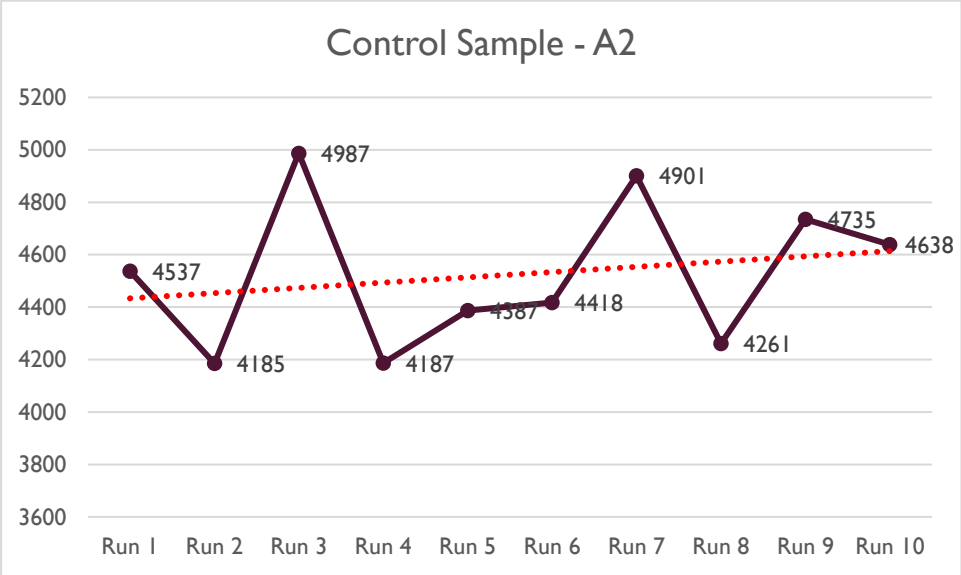


Figure 17 - Run Chart

The advantages of a line charts are that they are simple and easy to interpret.

The main disadvantage is that they only make sense in the context of ordered data.

STACKED BAR

A stacked bar chart is a used to break down and compare data items which form parts of a whole. Each bar in the chart represents a whole and segments in the bar represent different categories of that whole. Different colours are used to illustrate the different categories in the bar. A stacked bar chart can be used to changes in both the whole as well as the categories that form the whole. e.g. comparison of DBD, DCD and living kidney transplants over several years.

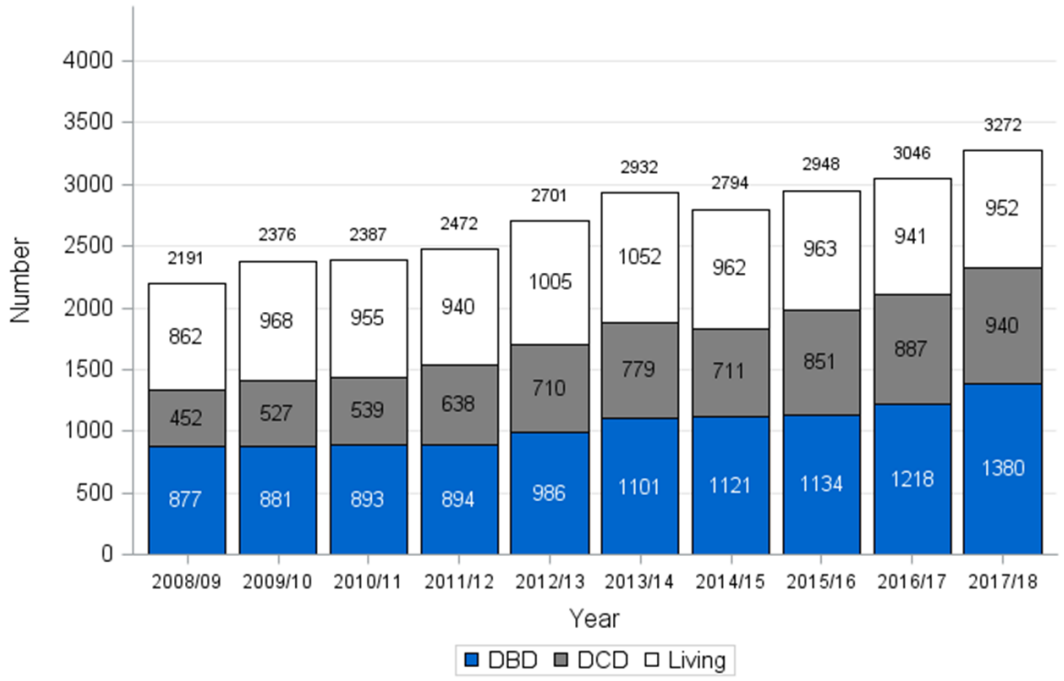


Figure 18 - Stacked Bar Chart (graph from NHSBT)

One main advantage of a stacked bar chart is that it shows both changes in the whole as well as changes in the segments that form part of the whole.

The major disadvantage of stacked bar charts is that they are harder to read as the number of segments in each bar increases. In addition, it can become harder to compare similar segments to each other as they are not aligned.

PIE CHART

A pie chart shows data as percentages of a whole. The size of each slice of the pie represents the proportion of the whole for that category. A pie chart should be used when the number of categories is less than 8 e.g. comparison of the number of different disease association tests completed each year.

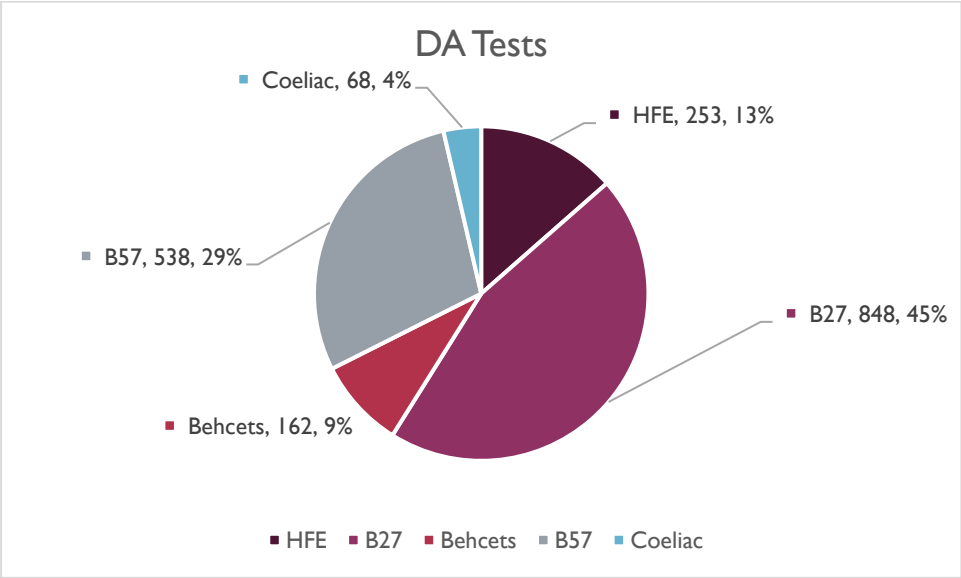


Figure 19 - Bar Chart

A pie chart has the advantage of being simple and quick and visually shows proportions of one category against another. Disadvantages include the fact that it is not possible to visually show the size of the whole as each pie is a simple circle though the whole can be represented in text. Another disadvantage is that the relative proportions of slices with only small differences does not stand out, e.g. 38% vs. 42% though again, this can be represented in text.

SCATTER PLOT

A scatter plot shows a plot of data points where each point is affected by two continuous variables which are plotted on the x and y axis. Scatter plots are typically used to show data where one of the continuous variables is under the control of the experimenter and the other depends on it or when both continuous variables are independent. e.g. DSA MFI levels vs. FCXM MCS.

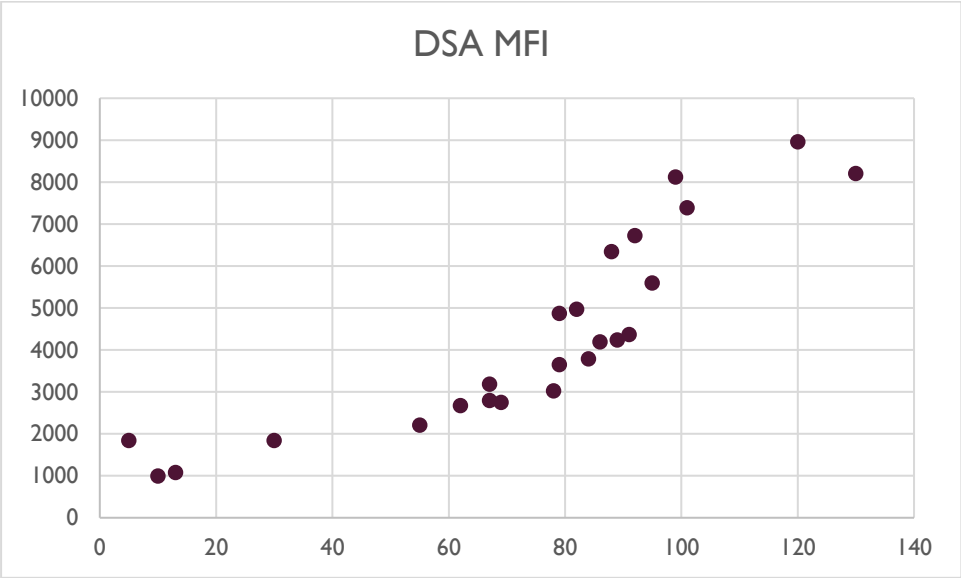


Figure 20 - Scatter Plot

One main advantage of a bar chart is that it shows all the data points and can highlight potential correlations between the two variables.

One disadvantage is that interpretation of the data can be subjective and of course correlation does not imply causation.

FUNNEL PLOT

A funnel plot is a scatterplot with upper and lower percentiles and is a good way to understand variation within a system. It is used primarily as a visual aid for detecting bias. It is used in the practice of process improvement because of its ability to identify where data points fall within the norm and where they are potential outliers. E.g. in DBD kidney donor acceptance rates in transplant centres, a funnel plot can be used to identify centre who fall into line with most other centre, those that accept more than other and are therefore above the funnel and those that accept less than others and therefore fall below the funnel.

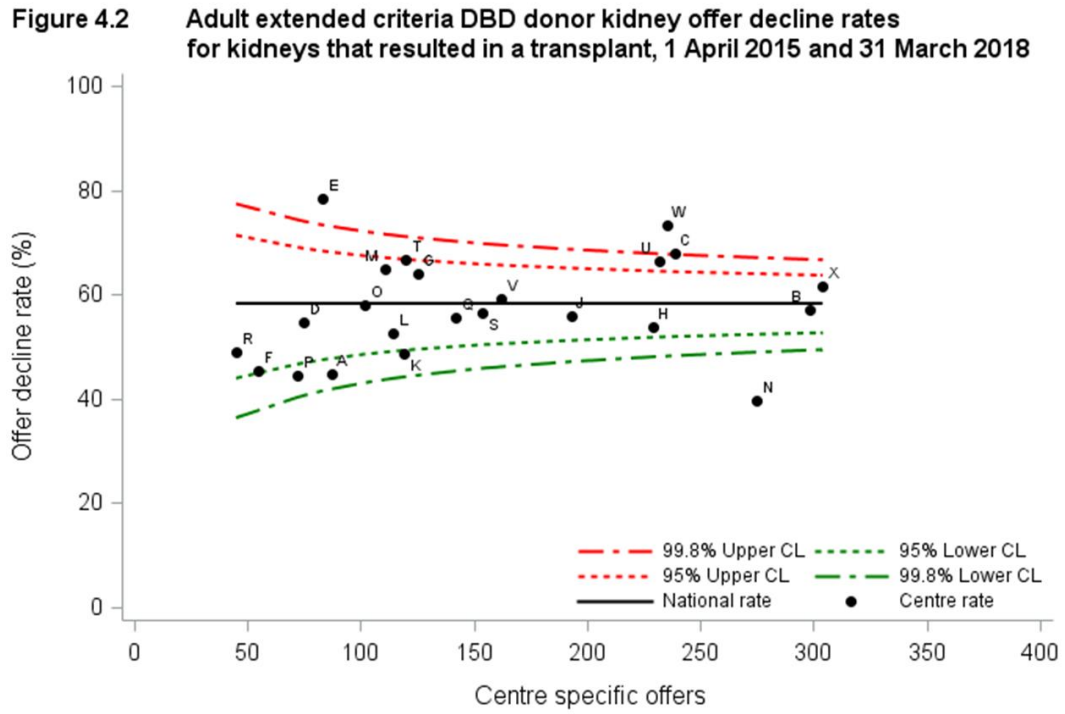


Figure 21 - Funnel Plot (graph from NHSBT)

One main advantage is that it is very easy to spot data items above the funnel and those that are below the funnel and potentially require attention.

One disadvantage is that Funnel plots show a snapshot of your system, not how it is changing over time.

HEAT MAP

A heat map is a graphical representation of data in which in which individual data value are colour coded in scale which reveal key information. A heat map is useful where data is dense and hard to follow as the colours can visually highlight trends. e.g. post-transplant DSA MFI levels in a patient undergoing plasma exchange.

Days	A2	B44	DR4
0	4765	3654	6927
1	2456	1965	4726
2	3527	2864	5382
3	1725	1265	2584
4	1965	1487	1736
5	834	722	2963
6	976	800	1627

Figure 22 - Heat Map

One main advantage of heat map is that it is visual and very easy to have your eyes drawn to important aspects of the data.

One potential disadvantage is that if not designed properly a heat map may potentially lead the investigator to discount a crucial aspect of the data.

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