

**The British Transplantation Society**

**Guidelines for Antibody Incompatible  
Transplantation**

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## **1.0 Need for guidelines**

Renal transplantation has benefited enormously over the last 35 years from the better identification of antibodies relevant to transplantation. This has allowed some transplants to take place in the presence of non-damaging antibodies, but there remain many circumstances where the presence of an antibody is currently prohibitive to transplantation.

Attempts have been made over many years actively to overcome antibody barriers that would otherwise preclude transplantation. In the last 5 years, there is a growing consensus that such measures are emerging from an experimental to a clinical context.

The applications of these newer techniques is however not straightforward, and the British Transplantation Society has produced these guidelines to inform the clinical teams, commissioners of transplant services, and patients of the special requirements of antibody incompatible transplantation.

## 2.0 Process of Writing

The British Transplantation Society formed a working party to produce these guidelines. The group met in April 2004 and reviewed its membership and invited more participants. The first draft of these guidelines was written mostly by Rob Higgins and Bob Vaughan. Extensive revision was made following reviews from the guideline production team and Dr Chas Newstead, Renal Unit, St James's University Hospital, Beckett St, Leeds LS9 7TF. The guideline was then open for public consultation on the British Transplantation Society website before final publication, and comments were received from Dr Stuart Roger, Glasgow Royal Infirmary, Glasgow G32 2ER, and Dr Phil Dyer, Manchester Royal Infirmary, Manchester M13 9WL.

### 2.1 Guideline development group

Dr Rob Higgins  
Department of Nephrology and Transplantation,  
University Hospitals Coventry and Warwickshire,  
Coventry CV2 2 DX  
Tel: 02476 535109  
email: Robert.Higgins@uhcw.nhs.uk

Dr David Briggs PhD,  
Consultant Clinical Scientist,  
National Blood Service,  
Longley Lane,  
Birmingham B15 2TT  
email: David.briggs@nbs.nhs.uk

Dr Brendan Clarke  
St James's University Hospital,  
Beckett St,  
Leeds LS9 7TF  
Email: brendan.Clark@leedsth.nhs.uk

Dr Mike Picton,  
Manchester Royal Infirmary,  
Oxford Road,  
Manchester M13 9 WL.  
email: Michael.Picton@cmmc.nhs.uk

Dr Paul Sinnott MRCSHC, PhD, FRCPATH  
Clinical Transplantation Laboratory  
Barts and The London NHS Trust  
London E1 1BB  
email: Paul.Sinnott@bartsandthelondon.nhs.uk

Dr Bob Vaughan PhD FRCPATH  
Consultant Clinical Scientist,  
Tissue Typing Laboratory,

New Guy's House,  
Guy's Hospital,  
London SE1 9RT  
robert.vaughan@kcl.ac.uk

Dr Anthony Warrens  
Consultant in Nephrology and Immunology  
Departments of Immunology and Renal Medicine,  
Imperial College London,  
Hammersmith Hospital,  
Du Cane Rd,  
London W12 0NN  
email a.warrens@ic.ac.uk

## 2.2 Methodology

These guidelines are based on published evidence, but the evidence and recommendations have not been graded for strength as almost all the published studies are descriptive. With only a handful of exceptions, conference presentations have not been included, as interpretation of data requires the level of detail about methodology, especially in the histocompatibility laboratory, that is only found in full publications. The publication cut off date for evidence included was July 2005. Review date – July 2008.

## 2.3 Abbreviations and terms

ABOi	Blood group incompatibility
AHG	Anti-human globulin
AiT	Antibody incompatible transplantation
CDC	Complement dependent cytotoxic (crossmatch)
DFPP	Double filtration plasmapheresis
DSA	Donor specific antibody
FC	Flow cytometry (crossmatch)
HLAi	Donor specific HLA antibody incompatibility
IA	Immunoabsorption
IVIg	Intravenous immunoglobulins
LDT	Living donor transplantation
PP	Plasmapheresis
PRA	Panel-reactive antibodies
standard transplantation	Transplantation without antibody incompatibility
XM	Crossmatch

## **3.0 Recommendations**

### **3.1 For Transplant Units**

1. AiT should be considered as part of an ongoing structured programme, and should not be performed on an occasional basis.
2. To initiate a programme, a unit should be able to demonstrate a demand of at least 5 cases a year, appropriate support from clinical transplant, PP and histocompatibility teams. PP should be available 7 days a week, in a location suitable for access by potentially sick patients post-transplant. An AiT programme requires funding for additional staff and consumables, and all programmes should receive Commissioner support.
3. The exact treatment protocols should be based on those successfully used in other centres, for example, the Johns Hopkins University, USA or Mayo Clinic, USA (PP-based protocols for HLAi and ABOi) (1); or Stockholm, Sweden (antigen-specific IA for ABOi)(2).
4. Post-transplant immunosuppression should consist of a regime similar or equivalent to anti-IL2 receptor antibody, tacrolimus, mycophenolate mofetil, prednisolone and Rituximab/splenectomy in selected cases.
5. All patients who start treatment with IVIg or PP should be reported to the national Registry, whether or not they receive a transplant.
6. Protocols that follow recommendation 3.1.3 do not require Ethics Committee approval. However, the standard of consent should include detailed written information which describes the risks of the procedure. The transplant donor should receive equivalent information to the recipient, so they are aware of the risks of the procedure to the recipient, whether it results in a transplant or not.

### **3.2 For Histocompatibility and Haematology Laboratories**

1. Laboratories should be able to define antibodies to the standard defined in BTS/BSHI document 'Guidelines for the detection and characterisation of clinically relevant antibodies in solid organ transplantation'(3). Sensitive and rapid techniques for the measurement of donor-specific HLA antibody levels must be available.
2. If ABOi transplantation is to be performed, blood group antibody titres need to be measured, with differentiation between A1 and A2 subgroups of recipient blood group A (when appropriate) and discrimination between IgG and IgM specific for ABO antibodies.
3. Data should be collected and reported to the standard set by the Registry as advised in the literature (1).
4. In LDT, it should not be necessary to provide a 24 hour service for antibody measurement, but a 7 day per week service with same day turn-around time is required.

5. A programme will require additional staffing in the laboratory, as well as additional consumable costs. Such costs must be included in the funding arrangements with Commissioners.

### **3.3 For Commissioners**

1. AiT (that is, transplantation across both HLAi and ABOi) is currently able to provide successful transplantation for significant numbers of patients. The numbers of cases may be up to 20% of the total LDT programme nationally.
2. AiT should be supported because of the improvements in quality of life after transplantation compared to dialysis. Additionally, many patients receiving antibody incompatible transplants may have no other chance of a transplant. The use of extra living donors is a real addition to the transplant pool. Lastly, transplantation is cost effective over time at about £15,000 per annum compared to dialysis averaged over a 10 year period.
3. It is likely that not every transplant unit should provide this specialised service, but arrangements should be in place so that patients can be transplanted appropriately regardless of their place of residence in the UK.
4. Commissioners should note the guidance given above for transplant units who want to set up an AiT service, and should support business plans that fulfil these criteria.
5. Commissioner support for AiT programmes should include funding for PP machines consumables and staff; transplant coordinator staff; drugs not routinely used in uncomplicated transplantation, eg mycophenolate and rituximab; clinical staff to coordinate the programme; equipment, consumables and staff in the histocompatibility laboratory.
6. Commissioners should require transplant units to participate in the national AiT Registry, and to support initiatives such as clinical trials organised on a Registry basis.

### **3.4 Recommendations for Audit**

1. Every patient undergoing antibody incompatible transplantation should be audited on a local and national basis, with the national audit through the AiT Registry.
2. The AiT Registry will define the optimal dataset to be collected, and will be able to report AiT activity against benchmark outcome data from international reports and the national dataset of renal transplantation.
3. All cases who start treatment with a regime based on IVIg or plasmapheresis should be included in audit, not just those who receive transplants.

### **3.5 Recommendations for Research**

1. The concentration of HLA antibody that is optimal on the day of transplantation should be better defined, especially to determine whether there is any advantage to the removal of HLA antibodies prior to transplantation in those who have a negative CDC/ positive FC XM.
2. The relative value of low dose and high dose IVIg in AiT should be better defined.
3. The mechanisms of accommodation to both HLA and ABO antibodies should be better defined.
4. Previous studies of AiT in deceased donor transplantation have produced overall graft survival rates inferior to those in transplantation performed in the absence of DSA. Efforts should be made either to refine the current treatments available, or to introduce novel treatments that allow deceased donor transplantation to be performed with a success rate similar to that of otherwise uncomplicated transplantation. This may include randomised studies on the use of IVIg and Rituximab.

## 4.0 Introduction

The development of techniques to achieve successful transplantation across antibody barriers has been continuous and evolutionary over the last 30 years (1, 4-7). Treatment protocols are emerging from an experimental setting to a routine, albeit very specialised, field of clinical care. However, there remain patients in whom the currently available techniques are not successful, and further development is required.

Renal transplantation is limited by a shortage of organs, but does offer the best quality of life for those with end stage renal failure. Living donor transplantation (LDT) is more than an expedient response to a donor shortage from deceased donors. It offers better long term graft survival than deceased donor transplantation, and also offers a choice as to when transplantation is performed, in particular allowing transplantation before dialysis is required. In addition, for many people with HLA antibodies, LDT across an antibody barrier may be the only realistic chance of a transplant.

Likewise there is also a large cohort of patients who do not have the option of live donor transplantation because none of their family or other potential living donor is ABO compatible. Overcoming this barrier allows transplantation for these individuals, and increases the overall number of transplants that may take place.

Many people have benefited from successful transplantation in the last 30 years because harmful antibodies have been avoided. This is due to better identification of antibody specificities in the laboratory and more sophisticated organ allocation systems. Indeed, the numbers of patients who have been transplanted by avoiding harmful antibodies greatly exceeds the numbers who have had protocols based upon plasmapheresis (PP) or intravenous immunoglobulins (IVIg). However, many sensitised subjects seem unlikely to be transplanted even with a well developed antibody avoidance policy, even if systems such as paired kidney exchange are developed.

Table 1 shows some milestones in AiT, and is divided into three sections. First, those therapies which have advanced understanding, but have remained specialist and largely experimental; ABOi transplantation in Japan is perhaps unfairly included in this group, and many such transplants have been performed, but the regimens have not been adopted extensively across the world. Second (in green) those therapies that are regarded, in these guidelines, as being of clinical value in the NHS. Lastly, there are ambitions that have not yet been achieved and still require a more experimental approach.

The justification for AiT in the NHS depends upon both clinical and cost effectiveness. Graft and patient survival are shown in Table 2, comparing recent results in AiT with recent standard LDT from the UK. Three to five year follow up of HLAi transplantation in the UK and USA (5, 7, 22) and up to 12 years follow up of ABOi transplantation in Japan (10) indicate that good graft function is maintained, with quality of life that appears equivalent to those who undergo standard transplantation. Taken together, these results indicate that AiT is clinically effective.

Economic analysis indicates that standard transplantation is cost effective compared to dialysis, by a margin of about £15,000 per annum over a 10 year period (23, 24). A meta-analysis of papers published between 1968 and 1998 indicated a cost of US\$55,000 – 80,000 per life year saved by in centre haemodialysis, compared to a cost of US\$10,000 per life year saved by transplantation, giving a cost saving for transplantation of US\$45,000 -70,000 per patient per

year (25). The Johns Hopkins University has estimated that US\$45,000 is added to the cost of a transplant with their PP and IVIg regimen (7). Although outcomes in the short term after transplantation across HLAi and ABOi are less good than with standard transplantation, the excellent long term survival (Table 2) (5, 7, 10, 22) means that cost-effectiveness over dialysis is still achieved by a considerable margin.

In addition, the predominant use of LDT in AiT means that one cannot argue that deceased donor kidneys are being reallocated from uncomplicated to complicated recipients, reducing both transplant survival rates and cost effectiveness. Indeed, the use of living donors who might not otherwise donate a kidney increases the total number of transplants that might be performed.

The demand for AiT in the UK is not fully known. A recent conference presentation indicated that as many patients were turned away from LDT because of antibody barriers as the number of living donor transplants actually performed in those units (26). This would amount to over 400 cases per annum in the UK. If these potential donors had proceeded through work-up towards transplantation, it seems likely that the drop out during work up in uncomplicated LDT would apply, namely about 50%. There would be further drop out during work up for AiT, because of more stringent recipient fitness criteria, and the unsuitability of some cases with very high antibody levels. A reasonable estimate of demand in the UK might be 50-100 cases per annum, but further work is required to define this better. This estimate is based on the use of PP-based protocols for living donor transplants. If it were possible to reduce HLA antibody levels in all of those awaiting transplantation, there would be in excess of 1000 sensitised patients on the UK transplant list who would potentially benefit.

## 5.0 Modulation and Accommodation

Successful AiT depends upon cessation of or reduction in antibody production (modulation), and/or adaptation of the graft to the presence of potentially damaging antibodies (accommodation). However, the mechanisms whereby modulation and accommodation occur are poorly understood, and there is even less understanding of how these could be manipulated therapeutically. Antibody removal with PP buys time while these processes take place, but by itself does not achieve either of these goals.

### 5.1 Waiting for Modulation

Although some patients with HLA antibodies will stop producing these spontaneously, for others the antibodies will remain at high titre and of broad specificity over many years. The factors which govern the natural down-regulation of antibody levels are not adequately understood (27). It has been postulated that anti-idiotypic antibodies (i.e. antibodies to the epitope-binding region of an antibody) play a role in the natural decline of an antibody response, although in certain circumstances they may be stimulatory and act to sustain a response (28).

A decision to wait rather than to proceed with transplantation from a suitable living donor requires assessment of the risks and benefits to the patient and donor. Waiting on dialysis may reduce the benefits of transplantation, probably because of the resulting cumulative cardiovascular damage.

### 5.2 Modulation with Immunosuppression

It has not generally been possible to down-regulate HLA specific antibody by the simple administration of immunosuppressive drugs. Mycophenolate has been shown to inhibit antibody production by B cells *in vitro* (29) and has antibody-lowering effects *in vivo* (30), but it has not been reported as being directly effective in the context of HLA-specific antibody reduction in patients awaiting transplantation. However, in 18 patients treated with mycophenolate mofetil for 4 weeks before ABOi transplantation, the clinical outcomes were superior to historical controls in the same centre who did not receive pre-treatment (31).

A chimeric humanised monoclonal antibody specific for the B cell surface antigen CD20 (Rituximab) has shown potential in modulating antibody in some auto-immune diseases (32) and may have a role in modulating alloantibody. Recently it has been reported to reduce HLA alloantibody levels in five of the nine renal patients treated (33). Rituximab has also been used as part of a protocol including plasmapheresis, IVIg and splenectomy for successful pre-transplant HLA antibody removal (16).

### 5.3 Intravenous Immunoglobulin (IVIg)

The background to the use of IVIg in transplantation has recently been reviewed in the BSHI/BTS guidelines on antibodies in solid organ transplantation, and will not be repeated in detail (3). Glotz et al have also recently reviewed the use of IVIg in renal transplantation (34).

IVIg has been reported to play a role in modulation of antibody production, either when used on its own prior to kidney (35-40) and cardiac transplantation (41, 42), or in conjunction with PP prior to kidney transplantation (15 43-5).

Being a complex mixture, the mechanism of IVIg action is obscure, but it is likely to be multifactorial and involve complement down-regulation, Fc receptors, and anti-idiotypic interactions. IVIg is well tolerated and a major advantage of IVIg over other methods of antibody modulation pre-transplant is that it may not require the simultaneous administration of immunosuppressive drugs.

The dosage of IVIg varies quite widely. It has been administered at 2 g/kg in adults, 500 mg/kg in a paediatric patient (37) and 500 mg/kg spread over 7 days to cover PP (43). A report on patients awaiting cardiac transplantation (42) compared PP with IVIg at a dose of 2 g/kg, the two methods reduced alloantibody (principally to HLA Class I) to a similar degree, but PP required more time. The consensus is that IVIg used alone to modulate antibody production is administered at a dose of 2 g/kg, at monthly intervals if more than one dose is required (38-40). When used either to augment antibody removal by PP or to restore resistance to infection, doses of 250-500 mg/kg may be used, or 100 mg/kg after each session of PP (10).

## 5.4 Accommodation

The response of the kidney to antibody that may potentially bind and cause damage is critical. Clinical observation suggests that sometimes donor-specific antibody may not obviously affect the graft function after transplant, especially in ABOi transplants (46). In other cases, donor-specific HLA antibodies may be associated with a reduction in graft function when first synthesised after a transplant, and then graft function improves 4-5 days later in the presence of the same serum level of antibody.

Multiple factors affect the sensitivity of an organ to circulating DSA, and these have recently been reviewed (47). The factors leading to accommodation may be grouped in several areas. First, antigen density on a transplant may vary. Examples that may be relevant to AiT include the lower expression of HLA DR on living donor than deceased donor kidneys at the time of transplantation (48). In ABOi transplantation, down regulation of blood group antigen expression has been described in a kidney that had functioned for some years. In other cases blood group antigen may be secreted at an enhanced rate from the transplanted organ, and could bind to circulating antibodies (47, 49). Antibody binding to a transplant is not necessarily by itself damaging, even with early complement binding, and rejection may depend, at least in part, on full activation of the complement cascade, balanced by defensive mechanisms in the graft, such as decay accelerating factor (DAF), and membrane cofactor protein (MCP). These have been given most attention in the context of xenotransplantation (50). Other factors that may be involved in protective pathways for the kidney are Bcl-xL, nitric oxide and haem oxygenase-1 (47, 51).

Accommodation may develop as a response to antibody exposure. Particularly interesting in this respect are *in vitro* experiments, in which human umbilical vein endothelial cells (HUVEC) incubated with subsaturating concentrations of HLA antibody showed increased expression of the cell surface molecule Bcl-xL, the expression of which is known to increase in kidneys which have accommodated to antibody exposure (51). Additionally these HUVEC were rendered refractory to endothelial cell activation and became resistant to complement-mediated lysis. In

contrast, HUVEC incubated with saturating concentrations underwent activation and expressed low levels of Bcl-xL. This study suggested that endothelial Bcl-xL expression defines the accommodation process in human allografts and this phenotype may be initiated by exposure of endothelium to low concentrations of anti-donor HLA antibodies.

A study of renal biopsies from 5 patients 1 year after ABOi transplantation was undertaken using gene probing, and did not show increased transcription of Bcl-x1, even though donor-specific ABO antibodies were present at the time in all patients (52). There were however differences between these biopsies and those of control patients in the gene expression of several potential pathways, including the disruption of normal signal transduction, attenuation of cellular adhesion and the prevention of apoptosis. These data are at potential conflict with the *in vitro* studies of Salama et al (51), but it is possible that different mechanisms may operate during the induction and maintenance of accommodation.

It is interesting to note that 25/49 (59%) of patients transplanted for HLAi at the Johns Hopkins University had donor specific HLA antibodies detectable at the time of transplantation, by FC XM in all cases, and by CDC XM in some cases (53). Likewise, 8/12 patients transplanted at the Mayo Clinic had a positive FC XM, and 10/11 had antibodies detectable by single antigen flowbeads (54). However, it is not clear whether the presence of low levels of DSA conferred any benefit to the outcomes, which in any case were excellent. Those cases transplanted in the presence of antibody were not deliberately selected, but were the cases where the PP protocol in use had failed to remove all DSA during the standard pre-transplant treatment schedule. These data, together with those from the Mayo Clinic detailed in the HLAi section (Selected Clinical Studies, IVIg, section 7.1.3) (55-6), offer an encouraging basis for further work on defining the optimal level of DSA present at the time of surgery in HLAi transplantation.

## 6.0 Techniques for antibody removal

When techniques of antibody removal are being considered, the dynamics of antibody production, distribution and action must be considered. These may differ in the immediate pre- and post-transplant periods.

Removal of IgG is constrained by its volume of distribution, which is approximately twice that of plasma. Moreover, the rate of redistribution of IgG from extravascular distribution into the plasma is slow. Any technique that removes antibody from the circulation, however efficiently and rapidly, will only have a temporary effect as redistribution occurs over the next 24 hours or so (Figure 1). Although it is difficult to separate re-synthesis of antibody from redistribution, the rebound in IgG levels between antibody removal sessions seems likely to be mostly due to redistribution, at least during a period of 7-10 days pre-transplant, when immunosuppression is being given and stimulation of production by antigen exposure is yet to occur.

In the post-transplant period, DSA production may be of rapid onset, and occur at a high rate, and this may be associated with impairment of graft function (1, 5). If DSA are causing significant vascular rejection, consideration will need to be given to the technique and schedule of antibody removal therapy required to keep pace with the rate of production. It should also be noted that, ultimately, modulation and accommodation may be necessary for successful engraftment and that antibody removal may only 'buy time' while these take place.

During the post-transplant period, the serum levels of DSA may not accurately reflect the effects of antibody on the graft. It is possible that antibodies may be produced and adsorbed by the transplant so avidly that the serum level remains low. Renal biopsy with C4d staining may be required to establish a diagnosis of rejection: the histological features of this type of rejection have been described (5, 57).

### 6.1 Plasmapheresis (PP)

PP may take the form of plasma exchange, where a patient's plasma is removed and replaced with human albumin and fresh frozen plasma or another substitute. This was one of the first methods to show success in alloantibody reduction. It has been used to reduce pre-transplant antibody levels (11, 43, 58-9). It is unlikely that whether centrifugation or filtration is used to separate plasma makes a major difference to the clinical outcome, so long as equivalent volumes of plasma are treated.

A disadvantage of PP is that plasma proteins need replacement, it has been estimated that the removal of one gram of IgG antibody by PP results in the loss of 150 gm of albumin together with other proteins and clotting factors (60). Albumin is normally the best replacement fluid, although the use of fresh frozen plasma has been recommended within 48 hours of a transplant to avoid haemorrhagic complications (43). These constraints make it difficult to treat more than 60ml/kg of plasma in a single treatment session, about 4 litres for an average sized person.

## **6.2 Double Filtration, or Cascade, Plasmapheresis (DFPP)**

Rather than discarding plasma removed by simple PP, it can be filtered a second time, retaining high molecular weight fractions of plasma, allowing molecules of lower molecular weight to be re-infused to the patient. As well as retaining antibodies, the secondary filter will also retain any molecule of high molecular weight, including, for example complement components and some clotting factors. Whether this is of clinical significance is not known.

DFPP has been used for transplantation in Japan, and more recently in Europe (19, 61). In the context of AiT, a major advantage is that up to 250 ml/kg of plasma can be treated per day, compared to 60 ml/kg with plasma exchange, although few units have so far tried to achieve the upper end of plasma volume treatment.

## **6.3 Immunoabsorption (IA) – Protein A or antibody affinity**

Immunoglobulins may be removed by passage of plasma over an affinity matrix. Protein A is the most widely used affinity column in clinical practice and has been used in the reduction of alloantibody pre-transplant (12-3, 62-6). Protein A is effective at binding all sub-classes of IgG except IgG3 which it binds poorly (67). Two columns are usually used alternately, by switching the extracorporeal blood plasma flow between the columns so that one column can be regenerated by acid elution of bound antibody. The columns are perfused with plasma, so are used in a circuit distal to a plasma separator device. IA has advantages over PP, it does not require the replacement of plasma proteins and allows the treatment of higher plasma volumes when regeneration of the protein A columns is used. It has been estimated that a Protein A column is capable of adsorbing 50% of the IgG from a volume of plasma (68), and multiple passages can result in 90% depletion of plasma IgG levels (13, 60, 69). Up to 500 ml/kg of plasma has been treated with Protein A immunoabsorption in a single session prior to transplantation (13).

## **6.4 Immunoabsorption – antigen-specific column**

The use of purified antigen is the most logical method to remove DSA. This is hard to achieve in HLAi because of the wide range of DSA specificities, but is easier in ABOi, where there are only 2 antigen types. Blood group antigen attached to Sepharose is available in a column that does not saturate with antibody after perfusion with 4 litres of plasma, and causes little bio-incompatibility. It does need to be perfused with plasma rather than whole blood, so is used in a circuit distal to a plasma separator device. Unlike cascade filtration, all the components of the plasma are returned to the patient apart from the ABO antibody, and routine treatment sessions of 150 ml/kg seem possible with few adverse effects (2, 19, 70).

## **6.5 Immunoabsorption - Liver transplantation**

The liver is capable of withstanding moderately high titres of HLA Class I antibody without apparent harm, and reports of hyperacute rejection of the liver are rare (71). It has proved possible to perform HLAi transplantation if a liver transplant is vascularised prior to the kidney (4, 72-3). This method is not guaranteed however (74) but it is not clear whether this is a

consequence of antibody titre, specificity or other factors. There are preliminary reports from Gothenberg, Sweden, of deceased donor renal transplantation in HLAi where the recipients have no liver disease, by the use of combined orthotopic transplantation of a lobe of a liver, the liver being used solely to remove DSA (75).

## 7.0 HLAi Renal Transplantation

The basic characterisation and clinical significance of HLA antibodies are not discussed here in detail, having been covered extensively in the recent BSHI/BTS guidelines (3), and in a review paper by Fuggle and Martin (76).

A laboratory supporting a programme of AiT must have robust methods to distinguish between total HLA antibodies, often reported as 'panel reactive antibodies' (PRA), and HLA antibodies directed against the donor. DSA levels may change independently of PRA, especially in the post-operative period. The introduction of more sensitive methods using purified HLA antigens either in ELISA formats or attached to beads are a major advance in monitoring DSA before and after transplantation. While the newer techniques allow for rapid and sensitive monitoring of DSA levels, CDC and FC XM should still be performed in all individuals. If the CDC XM indicates reactivity, serum should be titred in order to measure the highest dilution at which the CDC XM is positive. The titre of the CDC XM is regarded as an effective measure of the amount of antibody present, and is correlated with the amount of PP needed pre-transplant. It is also associated with the risk of antibody-mediated rejection post-transplant. However, there are circumstances where a transplant may be performed with a current positive CDC XM (see below).

The methods for performing antibody testing are not internationally standardised. In particular, it is common in the USA to perform the CDC with enhancement mediated by an intermediate incubation with anti-human globulin (AHG), and some laboratories use a final incubation with complement of greater than 1 hour (39). It is therefore possible that some patients we might regard as lower risk in the UK, where they are negative CDC/positive FC XM, would be regarded as higher risk in the USA, where they could be positive CDC/positive FC XM.

The level of HLAi that causes hyperacute rejection depends on factors including the antigen type, antigen density, and the antibody level. The CDC XM was once thought to represent an adequate *in vitro* evaluation of the risk of hyperacute rejection, as antigen and antibody-dependent factors both influence the test result. However, it has recently become clear that for some types of HLAi, a positive CDC XM does not necessarily indicate that hyperacute rejection will occur. This is most apparent for Class II HLA antibodies, especially the products of non DRB1 genes, ie HLA-DR51/52/53. Transplants with positive CDC XM titres of up to 1/16 have been performed in such cases with good immediate graft function (53). The relatively low level of expression of HLA-DR 51/52/53 is thought to be the main factor that reduces the risk of hyperacute rejection (77-8). The use of LDT may also facilitate transplantation in the presence of Class II DSA, as the relevant antigens are expressed to a rather lower extent than on deceased donor transplants (48).

Although low titre positive CDC class II DSA may be tolerated by the graft *at the time of transplantation* this has only been shown to be safe in the context of live donor transplants (53). Hyperacute rejection of deceased donor grafts due to Class II DSA is well described. The risk of hyperacute rejection due to HLA Class I specific antibodies is reduced to almost zero with a negative CDC XM, and antibodies detectable by FC only do not appear to be a risk factor for hyperacute rejection (53). A hierarchy of risk is shown in Table 3. It should be emphasised that often patients have more than one DSA specificity and other risk factors such as those listed in

Table 4 will influence a particular donor-recipient pair. These tables are therefore only a guide to the intensity of preconditioning treatment required and the likelihood of subsequent success.

The rationale for performing pre-transplant PP in subjects with low levels of HLAi, for example negative CDC/positive FC XM, is empirical. There is not expected to be a risk of hyperacute rejection, and any treatment is designed to reduce the chances of antibody-mediated rejection in the early post operative period. PP-based protocols have been used to treat patients with a negative CDC/positive FC XM, with good clinical outcomes (15). However, it has also been suggested that such subjects may be treated preoperatively with a single high dose of IVIg but without PP (55-6). Although it may appear to make sense to remove all DSA prior to a transplant, this is not necessarily the case. *In vitro* data indicate that low levels of DSA may upregulate defensive mechanisms in vascular endothelium, perhaps enhancing the ability of a kidney to resist antibody-mediated rejection at a later stage (51). The optimal treatment protocol for subjects with HLAi that is negative CDC/positive FC XM remains uncertain.

The likelihood of re-synthesis of DSA after the transplant cannot be predicted accurately in advance. It is associated with the amount of antibody present before treatment was started, but this is not always the case, and other risk factors have been defined on the basis of clinical experience (7, 79) (Table 4). When re-synthesis does occur, it may be rapid, leading to rejection with oliguria over a period of less than 24 hours. In other cases little or no change in graft function may occur. Therefore careful clinical and laboratory monitoring of all patients seems sensible, regardless of the pre-treatment levels of antibody.

The clinical significance of very low levels of antibody (for example negative CDC/negative FC XM but positive using purified antigen methods, eg antigen coated beads) remains to be elucidated. It is probably low risk, certainly at the time of surgery, though there may be an increased risk of antibody-mediated rejection subsequently. It would be reasonable to treat such patients without pre-transplant PP, but to monitor them very carefully post transplantation (5, 76).

## **7.1 Selected Clinical Studies**

### **7.1.1 Deceased donor transplantation**

- 1 The removal of anti-HLA antibodies prior to deceased donor transplantation by PP or IA has generally been followed by resynthesis of antibody, but repeated treatment may allow a period of time in which transplantation may be undertaken. Five patients received PP and IVIg, under cover of cyclophosphamide treatment at King's College Hospital, London (11). Four of five patients were successfully transplanted, but neutropenia occurred in all patients and one died of sepsis. In another study, 10 patients received protein A immunoadsorption, and 6 of 7 transplants grafts were functioning at the time of the report (12).
- 2 In a subsequent protocol also at King's College Hospital, London, antibodies were removed immediately before transplantation using protein A IA (13). Neither IVIg nor IA was administered after transplantation. Hyperacute rejection did not occur, though there was a high rate of antibody-mediated rejection. Of the 13 transplants, 8 had pre-treatment positive CDC XM against HLA Class I (titre 1:2 to >1:512), and 1 had positive CDC XM against HLA Class II (titre 1:256). One subject died and another 5 grafts failed. Modulation of DSA, but with concomitant maintenance of third party anti-HLA antibody production, was

observed in those subjects who were successfully transplanted (80). None of the subjects in whom the transplant was successful and in whom anti-HLA modulation occurred received IVIg before or after the transplant.

- 3 At the National Hospital, Oslo, Norway, 100 patients with over 50% panel reactive antibodies awaiting renal transplantation were treated with PP or IA (59). The PRA fell in 54 (60%) of the 90 patients treated with PP and a similar proportion of 10 patients treated with IA. IA did appear to be more effective in that it reduced the PRA of 4 patients where PP had no demonstrable effect. The average waiting time was just 10 weeks in these highly sensitised patients but the incidence of rejection episodes in the first three months was also high (89%). Overall, 1 year graft survival was 70% in first transplant deceased donor grafts, 61% for repeat transplants with deceased donors, and 77% with living donors.
- 4 At the University Hospital, Uppsala, Sweden, 23 patients with circulating PRA >50% were treated with PP and immunosuppressive drugs (cyclophosphamide 50-100mg/day and prednisolone) (81). Three sessions of PP were performed weekly for 4 weeks. 22 patients were transplanted, after a median time waiting for a transplant of 6 months. Pre-treatment XM data were not given. Cumulative five year graft survival was 59%, 8 of the grafts being lost from irreversible vascular rejection.
- 5 The University of Vienna, Austria, has recently reported on 40 patients who received deceased donor transplants, each after a single session of protein A IA, with a median treatment volume of about 9 litres (66). Nine patients had a positive CDC XM before IA, and 31 had negative CDC/positive FC XM, although the titres and antibody specificities are not given. IA was continued post-transplant, using immunosuppression with cyclosporin, mycophenolate mofetil and polyclonal antibody induction. At a median of 32 months follow up, 3 patients had died, 5 had lost their grafts from acute rejection, and 3 had lost their grafts from other causes.

### **7.1.2 Living donor transplantation**

1. Removal of HLA antibodies before LDT was reported in 2000 by the Johns Hopkins University, USA (5, 7, 15). Alternate day PP (1-1.5 plasma volumes per session using a centrifugal separator) and IVIg (Cytogam, MedImmune, Gaithersburg, MD, USA, 100mg/kg after each session) were administered. In the initial report there were 18 subjects, 8 of whom had positive CDC XM, and 10 of whom had negative CDC/positive FC XM. Immunosuppression was given with tacrolimus, mycophenolate mofetil, prednisolone and daclizumab. Post transplant, PP was administered on days 2, 4, and 6, and augmented according to antibody screening data. Five patients developed acute vascular rejection and were treated with steroids and further PP. Recent reports indicate 62 patients transplanted, with 3 yr graft survival of 86.7% and 3 yr patient survival of 94.4%. The protocol has become individualised according to perceived immunological risk (see Table 4), and includes Rituximab (375 mg/m<sup>2</sup> body surface area) in selected patients.
2. Successful transplantation of patients with both ABOi and HLAi has also been reported from the Johns Hopkins University (82). Two cases had positive CDC XM with their donor due to HLA antibodies and the third was negative CDC/positive FC XM. Two cases were ABOi due to blood group A2, with anti-A2 titres of 1/256 and 1/2 respectively, the third was blood group A1 incompatible with a recipient anti-A1 titre of 1:16. All patients received the

treatment regimen detailed in the paragraph above together with pre-operative splenectomy. All three grafts were functioning at over 9 months follow-up, one of them developing antibody-mediated rejection at day 14.

3. The Mayo Clinic, USA, (17), has reported the transplantation of 14 patients with HLAi, all of whom had positive CDC XM against their donors before PP, at titres ranging from 1:1 to 1:16. PP, 1 plasma volume, was performed on transplant days -4, -3, -1 and on the morning of transplantation. Following each PP, 100mg/kg intravenous immunoglobulins, Gamimune (Bayer Biological, Elkhart, IL, USA) was given. PP was performed on days +1 and +3 after surgery. All patients had splenectomy. Immunosuppression was with tacrolimus, mycophenolate, prednisolone and rabbit anti-human T-cell polyclonal antibody for 10 days. Six patients had antibody-mediated rejection after transplantation, treated with further PP (1-10 sessions) and steroids. All patients with pre-treatment cytotoxic crossmatch titre of >1:4 experienced rejection. Two transplants failed during the first year (both associated with persistent anti-donor antibody production). One of these patients later died on dialysis, and a further patient died with a functioning transplant in the first year. DSA disappeared and did not return in patients with good graft function, though more recently the use of more sensitive methods for the detection of DSA have indicated that they may continue to be produced at very low levels in many patients (54).

### 7.1.3 IVIg

1. At the Hopital European Georges Pompidou, Paris, France, a series of 15 patients were treated with IVIg (38). Three doses of 2mg/kg (Baxter Gammagard, Baxter, Belgium) were given at 4 weekly intervals. Thirteen of the 15 were effectively desensitised and underwent transplantation. Sensitisation was defined on the basis of PRA levels, and most patients had a pre-treatment PRA of 50-70% (range 10-86%). Results of DSA were reported only by cytotoxic testing, and 3 transplants had a negative crossmatch on pre-treatment sera. At follow-up of up to 2 years, 8 of the 13 grafts were still functioning, with 2 deaths and 3 graft losses.
2. Forty two patients at the Cedars-Sinai Medical Center, UCLA School of Medicine, USA, with positive CDC XM against deceased donor kidney, living donor kidney or heart transplant donors were treated with IVIg (39). Patients were selected on the basis of in vitro measurement of inhibition of XM by IVIg. Forty three percent of the subjects had donor specific HLA antibodies, the nature of the antibodies that were not DSA is not given. IVIg was given at a single dose of 2 g/kg (maximum 140 g), repeated one month later in 2 living donor transplant recipients, in order to achieve a negative XM. Rejection occurred in 13 (31%) of the transplants after transplantation. Patient and graft survival was 97.6% and 89.1% respectively at 24 months.
3. A randomized, double blind, placebo controlled study was performed in 12 centres in the USA, coordinated by the Cedars-Sinai Medical Center, UCLA School of Medicine, USA (NIH IG02 study) (40). It should be noted that this is the only randomised study in AiT that contributes to these guidelines, and highlights the need for more randomised trials. Ninety-eight patients were analysed, all of whom had PRA >50% monthly for 3 months before study entry. Forty eight received IVIg (Gamimune, Bayer, Elkhart IL) at a dose of 2 g/kg monthly for 4 months, then again at 12 and 24 months, and 50 patients received placebo. IVIg significantly reduced PRA compared to placebo, and more IVIg patients (35%) than

placebo patients (17%) received transplants. Rejection episodes occurred in 9 of 17 IVIg patients and 1 of 10 placebo patient, and 2 year graft survival as similar in each group. Mean PRA levels were about 80% before treatment, but mean IgG PRA did not fall below 60% in the IVIg group, returning to near placebo group levels by 6 months. The excess of transplants in the IVIg group took place after PRA levels rose back towards placebo levels, and data on XM status using pre-treatment sera are not given. Of the 27 evaluable transplants, 7 had failed and 2 patients died with functioning grafts by 2 years.

4. In a preliminary report, the Mayo Clinic, USA, treated 18 patients with negative CDC/positive FC XM with IVIg, three doses of 0.5 g/kg in 13 LDT, and one dose of 0.5 g/kg in 5 deceased donor transplants (55-6). In the early post operative period, two patients experienced antibody-mediated rejection, which was treated with PP and 100 mg/kg IVIg per session of PP. Patient and graft survival at early follow up was 100%. In a more recent report, 26 subjects were treated as above, and compared with 51 who had positive CDC XM before treatment. No patient in the negative CDC/positive FC XM group developed hyperacute rejection, and 15% developed antibody-mediated acute rejection.

## 8.0 ABOi Renal Transplantation

The ABO blood group system forms a major barrier to solid organ transplantation. ABO antigens are variably expressed on almost all body tissues, and T-cell independent IgM and IgG antibodies to A and/or B antigens not present in the host are produced. The ramifications for this in solid organ transplantation can most easily be shown in the Tables 5 and 6. ABO antibodies are most easily measured by standard haemagglutination testing, which is adequate in clinical practice. ELISA testing is also available.

Blood group A occurs in several forms, of which A1 and A2 are the most frequent. The A2 blood group has qualitative and quantitative differences in expression that result in it being relatively less antigenic and expressed at lower levels than A1. Ceppellini's group showed that although A1 and B blood group skin was immediately rejected when grafted onto O recipients, blood group A2 skin grafts were rejected at a slower rate, comparable to O skin grafted onto O recipients (83). This provided a rationale for attempting A2 incompatible transplantation.

Antibody titres must be measured against reagent cells of donor ABO type and not against donor red cells, and IgG levels, and not IgM levels, are important in terms of defining risk of failure. There are differences in the methods for measuring the titres of anti-A and anti-B antibodies that may make comparison of studies from different centres and countries difficult. First, titres are not absolutely precise, so even with a similar method, titres may vary by one, or even two, dilutions. Secondly, there are various methods. Nelson et al (84) used saline agglutination and DTT to distinguish between IgG and IgM. Shimmura (85) used antiglobulin to test for IgG, and this method is also used in some laboratories in the USA. As with AHG enhancement of the CDC XM used to test for HLA antibodies, this may be more sensitive. It has been suggested that this could even increase sensitivity by as many as five dilutions, in other words a titre of 1:4 could be equivalent to 1:128 in another laboratory (86). Although in some papers reference is only made to a 'standard' isohaemagglutination test, it would appear that Japanese and Swedish groups did not use AHG enhancement (2, 10), while it was used at the Johns Hopkins University (18), but not in the Midwest Transplant Network in Kansas, USA (86).

Although there are many reports of transplantation across A2 incompatibility without hyperacute rejection, it is suggested that such transplants are performed with an anti-A2 titre at the time of transplantation of 1:8 or less, using plasmapheresis to lower the titre if necessary (86-8). Likewise an anti-blood group titre of 1:8 or less at the time of surgery seems safe in A1 or B incompatible transplantation (2, 88).

The risk of rejection in the post operative period is associated with the maximum pre-operative titre, and results from the Tokyo Women's Hospital indicated very poor survival in subjects with a titre of 1:128 or greater (85). Some recent reports do however indicate that these high antibody levels can be successfully crossed, though it should be noted that the antibody titres in this series were measured using an AHG-enhanced method (18).

After transplantation, Gloor et al found that all patients that developed antibody-mediated rejection had antibody titres of greater than or equal to 1:64 at baseline or of 1:8 at the time of transplantation (16). However, there is no clear association between antibody levels after transplantation and graft function. Excellent graft function with titres of up to 1/256 has been reported. The key to clinical outcome seems to be the transplanted graft rather than the presence

of antibody, and it presumed that successful transplants adapt to the presence of antibody (see section on accommodation). However, while the mechanisms of accommodation are not fully understood, the reasons for variation in accommodation are even less understood. In the context of ABO transplantation, it is interesting that those who are blood group secretor status may have higher antigen expression in their grafts (89-91). In addition, a study has suggested differences in antigen expression between different ethnic groups, independent of secretor status (91).

## 8.1 Details of Selected Clinical Studies

1. The first ABO incompatible renal transplantation was performed by Hume et al in 1955 (92). A blood group O recipient received a blood group B cadaver renal allograft and the recipient had early rejection on day 7.
2. Further early attempts at ABO incompatible renal transplantation met with poor results and these have been reviewed (93). ABO incompatible renal allografts were rejected with histological findings consistent with an anti-A and/or B blood group antibody binding to renal vascular endothelial cells and activating complement, leading to platelet aggregation and vascular thrombosis. Initial results with A2 kidney transplantation into O group recipients resulted in graft loss within a month for 40%, but long term graft survival for the remaining 12 (94). One reason for poor graft survival appeared to be the anti-A titre and with an anti-A titre of 1 in 4 or less the results were very good with a two year deceased donor graft survival rate of 94%.
3. Plasmapheresis was used to reduce the antibody titre to below 1 in 8 in a series from Portsmouth, UK with some patients also undergoing splenectomy. It was thought that the majority of plasma cells producing T-cell independent antibody reside in the spleen (95).
4. The first report of a large scale programme of ABO incompatible transplantation found that recipients who did not have splenectomy rapidly rejected their graft when compared to splenectomised recipients (9).
5. The Tokyo Women's Hospital Medical University, Japan, has reported on 141 ABOi transplants performed between 1989 and 2001 (85). In 68 cases there was A1 incompatibility, in 72 cases B incompatibility, and in 1 case both A1 and B incompatibility. Haemagglutination titre was reduced <1:32 prior to transplantation using DFPP or Biosynsorb A and/or B columns (Chemobiomed Ltd, Edmonton, Canada). Immunosuppression was with cyclosporin or tacrolimus, prednisolone, and mycophenolate mofetil, or prior to the use of mycophenolate, antilymphocyte globulin, deoxyspergualin and irradiation of the graft. One, 5 and 10 year graft survival was 82%, 76% and 56%, compared to 96%, 85% and 67% for ABO compatible grafts performed in the same institution. One year graft survival has been >90% since 1998, compared to about 95% for ABO compatible transplants. Before 1998 the outcome was strongly associated with the anti-A/B titre, with 10 year graft survival of <25% in those with a pre-treatment titre of 128 or above. Since 1998, the titre has not been associated with outcome, even for those with a titre of 128 or above, though the number of cases with very high titres since 1998 is not given.
6. The results of ABOi transplantation in 441 patients at 55 centres in Japan, including the Tokyo Women's Hospital, between 1989 to 2001 have been pooled and reported (10). Antibody removal was carried out with DFPP, on average, 2-3 times before transplantation in

the majority of patients. DFPP was not performed routinely after the transplant, unless antibody levels rose suddenly with biopsy-proven vascular rejection. Immunosuppression was with cyclosporin or tacrolimus, anti-metabolite and prednisolone, and 98% of patients had pre-transplant splenectomy. Patient and graft survival were 93% and 84% respectively at 1 year; 87% and 71% at 5 years, and 84% and 65% at 9 years. Graft survival was better in younger recipients; did not differ between A and B incompatibility; and was better in those given anticoagulation therapy after the transplant.

7. The Johns Hopkins University, USA, has reported on 18 patients transplanted with ABOi, providing most detail for the last 6 cases who were transplanted without prior splenectomy (18). Of these patients, the incompatibility was group A2 in 2 cases; B in 3 cases, and A1 in 1 case. The anti A1 titre was 1:128, and for the B incompatible subjects, 1:128, 1:32 and 1:32. PP was given on alternate days until an anti-A/B titre of <1:16 was achieved, using IVIg as in the HLA programme (15). Tacrolimus and mycophenolate were started with the first plasmapheresis, and Rituximab (375 mg/m<sup>2</sup>) was given the day before surgery. Daclizumab and prednisolone were added post-operatively, and PP was given on days 2, 4 and 6. No antibody mediated rejection occurred and antibody levels never rose above those observed pre-transplant. Graft and patient survival was 100% at 4-14 months follow up.
8. The Mayo Clinic, USA, has reported on 18 patients transplanted across ABOi (16). Ten patients were incompatible across blood group A2 (titres 1/16 to 1/126); five across group A1 (titres 1/16-1/512) and three across group B (titres 1/32 – 1/64). The first 8 incompatible at A2 had no preconditioning, but this was instituted after two patients experienced rejection. PP was given on days -4, -2, -1 and 0, at 1 plasma volume, with 10G IVIg (Gammimune, Bayer, Elkhart IN) with each treatment. Immunosuppression was with anti-thymocyte globulin, tacrolimus, mycophenolate and prednisolone, and one patient had splenectomy. One year graft survival was 89.1%, compared with 96% in uncomplicated transplants. Patients who developed rejection were those with higher baseline titres of blood group antibody.
9. The Karolinska Hospital in Stockholm, Sweden, has reported on 11 patients who were transplanted after antigen specific IA (2). Three subjects were transplanted across the A2 – O barrier, each with IgG anti-A2 titres of 1:64. Four subjects had group A1 incompatibility, with titres of 1:64, 1:128, 1:16, and 1:16, four had group B incompatibility with titres of 1:2, 1:8, 1:16 and 1:32. Rituximab (375 mg/m<sup>2</sup>) was given 2-4 weeks before treatment started, and tacrolimus, mycophenolate and prednisolone were started with IA. Glycorex columns were used, with 4 treatment sessions. If these did not achieve an antibody titre of <1:8, further treatment sessions were given. After the last pre-transplant session, 0.5 g/kg of IVIg (Gammagard, Baxter, Belgium) was given. Post operatively, no patient experienced rejection and patient and graft survival at reporting (3-34 months) was 100%.
10. The Midwest Transplant Network based in Kansas, USA, reported the outcomes of 56 patients transplanted with A2 incompatibility, over the period 1994-2003 (86). These transplants were performed into blood group B recipients, using deceased donor kidneys. Patients were selected who had anti-A titres of <1:8, and standard immunosuppression was given, without IVIg or plasmapheresis. Compared to group B recipients of blood group compatible kidneys, there was a trend to a higher early acute rejection rate (41% vs 28%, p=0.09), but 7 year graft survival was similar in both groups (72% vs 74%).

# 9.0 Information that may be used to support the consent process

## 9.1 HLA (Tissue type) antibodies

### Antibody Removal to allow Kidney Transplantation

Take time to decide whether or not you wish to proceed with an antibody incompatible transplant.

#### Why is antibody incompatible transplantation being considered?

A kidney transplant is prevented by an antibody (natural defence) in the blood that reacts against the donor kidney.

#### What are antibodies?

- Everyone has antibodies in their blood, these are part of the body's natural defence against infection.
- Antibodies against tissue types of other people are not normally present, but may develop after pregnancy, blood transfusion, or previous transplantation.
- For many years, it has not been possible to transplant across an antibody barrier, and people who have antibodies against a transplant do not go ahead with the operation. This means that many people with high antibody levels may never receive a transplant.

#### What is the procedure?

- Recently, techniques have become available to remove antibodies or to reduce their levels so that transplantation may be possible. The techniques are best developed for people who have living donors. Additionally, some people have antibody levels that are too high for the most modern treatment to overcome.
- The exact treatment schedule varies from case to case, but in the most common situation, antibodies are removed from the blood with a machine. The procedure is called plasmapheresis. This means being attached to a machine which pumps blood through a special filter – the procedure looks very much like haemodialysis. If someone is on peritoneal dialysis and does not have a fistula, it will be necessary to insert a dialysis catheter (plastic tube) into a vein in your neck, which will remain in place for the two weeks. The treatment removes all antibodies, some pooled antibodies will be given as replacement.
- Most people require 5 sessions of plasmapheresis in the ten days before the transplant, but an individual schedule will be designed for each person.
- The kidney transplant will take place in the normal way, except that more powerful anti-rejection drugs than usual will be used after the transplant. Plasmapheresis may be given in

the first week after the transplant; and there may be a routine kidney biopsy at 7 days after the transplant.

- It will be necessary to use more powerful anti-rejection drugs than usual to control rejection in the first few weeks after the transplant, and to have daily plasmapheresis during a rejection.
- The risks of death, transplant failure and serious infection are about twice as high as with an uncomplicated transplant. The weeks just before and after the transplant are very stressful, and support from family and friends is essential.
- A successful transplant behaves just like an ordinary transplant after the first few weeks; the body stops producing antibody against the transplant, and/or the transplant stops being affected by antibody that is present in the blood. In the longer term, then, doses of anti-rejection drugs can be reduced to the same levels as in other transplanted patients.

#### Is the treatment experimental?

Plasmapheresis treatment for transplantation is not in general use throughout the world, and is still under development. Results in the USA, Japan and Sweden over the last 10 years have been very encouraging, though the treatment is not successful in all cases. Guidelines for transplantation across incompatible blood groups have been drawn up by transplant professionals in the UK, and the treatment you will receive is in line with recommendations made by national experts.

#### What are the alternatives?

One alternative to this treatment is to continue to wait for a kidney transplant from a deceased donor. However, it is possible that a perfect match will never come up, and that all other kidneys will be impossible to transplant because of the antibodies. It is currently not feasible routinely to remove antibodies from people quickly enough to allow a transplant from a deceased donor, which is why living donor transplantation is currently preferred. Research is moving quickly, and kidney units will have up to date information about any new developments.

It may also be possible to be considered for a 'paired', or 'exchange' transplant. This may be possible if a donor is compatible with another person with kidney failure, and that other person has a donor that is compatible with the first recipient. Each donor could then give their kidney to the other recipient, allowing both transplants to take place in the standard manner, without antibody incompatibility. To explore the possibility of paired donation further, someone should discuss their particular circumstances with their own kidney unit.

#### What are the side effects of having the transplant?

There are a number of possible side effects, some of which are serious.

- There is a chance of dying from complications before or after the transplant. The usual death rate after a kidney transplant is about 1 in 100 – the exact risk depends on the recipient's physical fitness. The risk of death is likely to be increased to between 1 in 25 and 1 in 50 after antibody removal.

- The chance of the transplant failing soon after the transplant is increased. Normally 1 in 20 transplants will fail in the first year after the transplant. The risk of transplant failure is about 1 in 10 with this procedure.
- Most people get a rejection episode after the transplant with this procedure, compared to about 1 in 3 patients receiving a kidney transplants generally. This rejection is usually treatable, but more powerful drugs and extra plasmapheresis may be needed.
- Infections may occur before and after the transplant, because the treatment reduces resistance to infection.
- There is a risk of developing cancer after a transplant, because of the anti-rejection drugs. The risk of serious cancer (lymphoma) is about 1 in 50 in the first year; the risk of this may be increased because of the extra treatment required to overcome antibody barriers.
- The procedure may not result in removal of enough antibody to make the transplant possible, so it would be cancelled or postponed just before the operation.

Will my medical details be kept confidential?

All information which is collected will be kept strictly confidential. Some details of every transplant are kept by United Kingdom Transplant (UKT), to measure the success of different types of transplant, and to monitor the performance of individual transplant units. Additional information on all patients who receive treatment to reduce their antibody levels will also be retained by UKT. Pooled data will be used to monitor and improve the results of transplantation across antibody barriers, but individuals will not be identifiable from any published articles or presentations.

## 9.2 ABO (blood group) antibodies

### Antibody Removal to allow Kidney Transplantation

Take time to decide whether or not you wish to proceed with an antibody incompatible transplant.

Why is blood group incompatible transplantation being considered?

A kidney transplant in your case is prevented by an antibody (natural defence) that reacts against the donor kidney.

What are antibodies?

- Everyone has antibodies in their blood, these are part of the body's natural defence against infection, and are not usually likely to damage a kidney.

- Antibodies against incompatible blood groups are likely to damage a kidney transplant.
- A blood group incompatible transplant occurs when someone who is blood group O receives a kidney from someone who is group A, or group B, or Group AB; or when someone who is blood group A receives a kidney from someone who is group B or group AB; or when someone who is blood group B receives a kidney from someone who is group A or group AB.
- There are two types of blood group A, called A1 and A2. It is easier to do a transplant from a donor who is blood group A2 than someone who is group A1. Typing for A1 and A2 is not routinely performed, but will be performed if someone is being assessed as a transplant donor.
- For many years, it has not been possible to transplant across an antibody barrier, and people who have antibodies against a transplant do not go ahead with the operation.

### What is the procedure?

- Recently, techniques have become available to remove antibodies or to reduce their levels so that transplantation may be possible. The techniques are best developed for people who have living donors. Additionally, some people have antibody levels that are too high for the most modern treatment to overcome.
- The exact treatment schedule varies from case to case, but in the most common situation, antibodies are removed from the blood with a machine. The procedure is called plasmapheresis. This means being attached to a machine which pumps blood through a special filter – the procedure looks very much like haemodialysis. If someone is on peritoneal dialysis and do not have a fistula, it will be necessary to insert a dialysis catheter (plastic tube) into a vein in your neck, which will remain in place for the two weeks. The treatment removes all antibodies, some pooled antibodies will be given as replacement.
- Most people require 5 sessions of plasmapheresis in the ten days before the transplant, but an individual schedule will be designed for each person.
- The kidney transplant will take place in the normal way, except that more powerful anti-rejection drugs than usual will be used after the transplant, and plasmapheresis may be given in the first week after the transplant. There may be a routine kidney biopsy at 7 days after the transplant.
- It will be necessary to use more powerful anti-rejection drugs than usual to control rejection in the first few weeks after the transplant, and to have daily plasmapheresis during a rejection.
- The risks of death, transplant failure and serious infection are about twice as high as with an uncomplicated transplant. The weeks just before and after the transplant are very stressful, and support from family and friends is essential.
- A successful transplant behaves just like an ordinary transplant after the first few weeks; the body stops producing antibody against the transplant, and/or the transplant stops being

affected by antibody that is present in the blood. In the longer term, then, doses of anti-rejection drugs can be reduced to the same levels as in other transplanted patients.

### Is the treatment experimental?

Plasmapheresis treatment for transplantation is not in general use throughout the world, and is still under development. Results in the USA, Japan and Sweden over the last 10 years have been very encouraging, though the treatment is not successful in all cases. Guidelines for transplantation across incompatible blood groups have been drawn up by transplant professionals in the UK, and the treatment you will receive is in line with recommendations made by national experts.

### What are the alternatives?

One alternative to this treatment is to continue to wait for a kidney transplant from a deceased donor. The waiting time for a transplant from a deceased donor depends largely on the blood group and tissue type. The deceased donor will have to have a blood group compatible with the recipient. If someone is blood group O, A or AB waiting time is not affected, if someone is group B there may be a longer waiting time. If someone has an unusual tissue type, the wait for a deceased donor kidney that has a good match may be longer. A kidney unit will be able to give some idea of the average waiting time for a deceased donor transplant for someone with each person's blood group and tissue type.

It may also be possible to be considered for a 'paired' or 'exchange' transplant. This may be possible if someone's donor is compatible with another person with kidney failure, and that other person has a donor that is compatible with the first recipient. Each donor could then give their kidney to the other recipient, allowing both transplants to take place in the standard manner, without antibody incompatibility. To explore the possibility of paired donation further, discuss individual particular circumstances with the local kidney unit.

### What are the side effects of having the transplant?

There are a number of possible side effects, some of which are serious.

- There is a chance of dying from complications before or after the transplant. The usual death rate after a kidney transplant is about 1 in 100 – the exact risk depends on the recipient's fitness. The risk of death is likely to be increased to between 1 in 25 and 1 in 50 after antibody removal.
- The chance of the transplant failing soon after the transplant is increased. Normally 1 in 20 transplants will fail in the first year after the transplant. The risk of transplant failure is about 1 in 10 with this procedure.
- Most people get a rejection episode after the transplant with this procedure, compared to about 1 in 3 patients receiving a kidney transplants generally. This rejection is usually treatable, but more powerful drugs and extra plasmapheresis may be needed.
- Infections may occur before and after the transplant, because the treatment affects the resistance to infection.

- There is a risk of developing cancer after a transplant, because of the anti-rejection drugs. The risk of serious cancer (lymphoma) is about 1 in 50 in the first year; the risk of this may be increased because of the extra treatment required to overcome antibody barriers.
- The procedure may not result in removal of enough antibody to make the transplant possible, so it would be cancelled or postponed just before the operation.

Will my medical details be kept confidential?

All information that is collected about you will be kept strictly confidential. Some details of every transplant are kept by United Kingdom Transplant (UKT), to measure the success of different types of transplant, and to monitor the performance of individual transplant units. Additional information on all patients who receive treatment to reduce their antibody levels will also be retained by UKT. Pooled data will be used to monitor and improve the results of transplantation across antibody barriers, but individuals will not be identifiable from any published articles or presentations.

## 9.0 Authors' Potential Conflicts of Interest

Dr Higgins has received expenses for travel and accommodation to attend scientific meetings from Astellas, Novartis, Roche and Wyeth; honoria for teaching from Baxter, Roche and Wyeth. His research and that of colleagues has been sponsored in part by Novartis, Roche, Wyeth and Astellas (formerly Fujisawa).

Dr Briggs has received travel and conference support from VH Bio Ltd and Quest Biomedical Ltd and his laboratory has received research financial support from Astellas (formerly Fujisawa).

Dr Newstead has received multiple honoria for lectures and teaching as well as expenses for travel and accommodation to attend scientific meetings principally from Fujisawa, Novartis, Roche and Wyeth. Dr Newstead has received Honoria for contributions to advisory boards for Roche and Wyeth, but non since appointed chair British Transplantation Society Standards Committee. Research and that of collaborators has been in part sponsored by the above named companies as well as the Yorkshire Kidney Research Fund, Medical Research Council, Ipsen International, Jensen-Cilag, Alpha Blood Products, Baxter Healthcare and Biotrin International. A current statement is available via the BTS web site: [www.bts.org.uk](http://www.bts.org.uk).

Dr Vaughan has received expenses for travel and accommodation for visiting scientific meetings from Novartis and One Lambda and is part holder of a patent subject to a licencing agreement with Dynal UK/Invitrogen.

Dr Clark has accepted an invitation from One-Lambda to give a lecture at an EFI meeting in Oslo. One Lambda are the manufacturers of bead based assay systems for serum screening/specificity analysis.

Dr Picton, none declared

Dr Sinnott has received travel and conference support from Astellas (formerly Fujisawa), Novartis, Roche and One Lambda and his laboratory has received research financial support from Octopharma Ltd

Dr Warrens has received honoraria for teaching or providing professional advice, travel grants and research support variously from Astellas (formerly Fujisawa), Novartis, Roche and Wyeth. His group has also received research support from the Medical Research Council, the Juvenile Diabetes Research Foundation and Kidney Research (UK) (formerly the National Kidney Research Fund).

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## 11.0 Tables and Figures

**Table 1. Development of interventions in transplantation across antibody incompatibility**

Intervention	Centre	Reference
Plasmapheresis to treat rejection	Toronto 1977	8
Blood group incompatible transplants with splenectomy	Brussels, Belgium 1986 Various Japanese groups <sup>1</sup>	9, 10
HLA antibody removal and immunosuppression to prevent resynthesis	London 1980s	11, 12
HLA antibody removal immediately before transplant	London 1996	13
ABO incompatible heart transplants in neonates	Toronto, 2001	14
Living donor transplants after plasmapheresis	Johns Hopkins 2000	15, 16, 17
Blood group incompatible transplants with Rituximab	Gothenburg, Sweden 2005 Johns Hopkins University 2004	2, 18
Strategies to deal with:- <ul style="list-style-type: none"> <li>• High level antibodies</li> <li>• Deceased donor grafts</li> <li>• Modulation of antibody production without transplant in situ</li> </ul>	not yet established	

<sup>1</sup> These protocols were almost routine in Japan while elsewhere, there has been reluctance to take up their protocols despite good results, perhaps because of the use of routine splenectomy, and because in many other countries deceased donor programmes offer a better chance of an eventual transplant than in Japan.

**Table 2. Patient and graft survival in recently published series of antibody-incompatible renal transplantation.**

Centre	Antibodies	Early outcome	Longer term outcome	Number cases	Ref
Japan	ABO	84% 1 yr graft survival 93% 1 yr patient survival	71% 5 yr graft survival <sup>1</sup> 87% 5 yr patient survival	441 cases, 1989-2001 Japanese centres	10
Tokyo Women's University Hospital, Japan	ABO	82% 1 yr graft survival (>90% since 1998) 94% 1 yr patient survival		141 cases, 1989-2001	19
Stockholm, Sweden	ABO	100% graft survival 100% patient survival		11 cases 3-34 months up	2
Mayo Clinic, USA	ABO	85% graft survival 92% patient survival		26 cases 400 days mean follow up	20
Johns Hopkins University USA	ABO	94% graft survival 100% patient survival		18 cases >12 months up in 15/18 cases	5, 18
Johns Hopkins University USA	HLA		87% 3 yr graft survival 94% 3 yr patient survival	62 cases	5, 7
Mayo Clinic, USA	HLA	86% 1 yr graft survival 93% 1yr patient survival		14 cases	17
Conventional transplantation, for comparison	No antibody barrier	94% 1 yr transplant survival 98% 1 yr patient survival	84% 5 yr transplant survival 95% patient survival	1582 transplants, 1992	21

<sup>1</sup> This 5 year survival rate includes transplants performed in the late 1980's when survival rates were lower than currently; in the UK, 5 year transplant survival for living donor transplants performed in 1992 was 79% (95% CI 73-84) (data UK Transplant).

**Table 3. Hierarchy of risk in HLAi renal transplantation, according to pre-treatment DSA levels.**

This table is only a guide. Many patients have more than one DSA specificity, and risk factors in Table 4 also need to be taken into account.

	HLA Class II DR 51/2/3	HLA Class II DR 1-10	HLA Class I
Negative	Green	Green	Green
Historic positive Current negative	Green	Green	Green
Fluorescent bead positive FC negative CDC negative	Green	Green	Green
Cellular FC positive CDC negative	Amber	Amber	Amber
CDC positive, titre up to 1/32	Amber	Amber	Amber
CDC positive, titre 1/64 - 1/128	Amber	Amber	Red
CDC positive, titre 1/256 or greater	Amber	Red	Red

CDC, complement-dependent cytotoxic crossmatch

FC, flow cytometric crossmatch

Green – lowest risk, transplant may proceed with appropriate antibody monitoring

Amber – transplant may require antibody removal or modulation of production before proceeding

Red – highest risk, even with plasmapheresis

(adapted from Fuggle and Takahashi)

**Table 4. Risk factors in HLAi transplantation, additional to the level and type of DSA shown in Table 3.**

Wide breadth of HLA reactivity
Previous transplants
Previous early graft losses
Sustained antibody production to all or most previous mismatched antigens
Multiple repeat mismatches (spreading specificities during rejection)
Multiple donor reactive antibodies
Multiple sensitising events
High risk combinations (husband-to-wife or child-to-mother transplants)

(adapted from Montgomery 2004)

**Table 5. The basic organisation of the ABO blood group system**

Phenotype	Genotype	Antigens	Antibodies present	Frequency in UK (white European subjects)
O	OO	None	Anti-A & anti-B	44%
A	AA or AO	A	Anti-B	45%
B	BB or BO	B	Anti-A	8%
AB	AB	A & B	None	3%

**Table 6. Recipient and Donor compatibilities by ABO phenotype**

Recipient blood group	Compatible Donor Blood group	Donor blood group	Compatible recipient blood group
O	O	O	O, A, B, AB
A	A, O	A	A, AB
B	B, O	B	B, AB
AB	AB, A, B, O	AB	AB

**Figure 1. Typical rebound of antibody levels between sessions of plasmapheresis treatment, performed alternate days over an 11 day period before transplantation.**

Bead MFI: Antibody levels measured Luminex™ bead analysis. Courtesy David Briggs.

