BTS
British Transplantation Society

The Voice of Transplantation in the UK

Guidelines for Antibody Incompatible Transplantation

Third Edition

Compiled by a Working Party of
The British Transplantation Society
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British Transplantation Society Guidelines

Contents

1 INTRODUCTION

1.1 The Need for Guidelines

Transplantation has benefited enormously over the last 40 years from the improved identification of antibodies relevant to transplantation. This has allowed a better understanding of risk so that some transplants can take place in the presence of potentially damaging antibodies.

The applications of these newer techniques is 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.

Because of the limited experience and evidence base in other areas, these guidelines deal almost exclusively with antibody incompatible transplantation (AIT) in adults and, except for the final section, concentrate on issues related to kidney transplantation.

1.2 Process of Writing and Methodology

The British Transplantation Society formed a working party to produce the first edition of these guidelines in April 2004. The first guideline was mostly written by Dr Rob Higgins and Dr Robert Vaughan and was published in 2006. Following extensive revision, the second edition was published in January 2011.

This third edition has been written under the auspices of the BTS Standards Committee and has been produced by a series of writing teams coordinated by Professor Rob Higgins. An important change has been to add guidance regarding the strength of the evidence base underlying the statements of recommendation, and to ensure that the guidance has been produced in line with the BTS Clinical Practice Guideline and the recommendations of NHS Evidence (1).

These guidelines are based on published evidence and up-to-date analysis of clinical outcomes by NHSBT, using the National AIT Registry. Initially, a literature search was performed by Professor Rob Higgins using PubMed® and search terms including combinations of ABO, HLA, antibody, incompatible, transplant, transplantation, mediated, rejection, acute,

chronic, kidney, heart, lung liver, pancreas. Publications were included if published (fully or epub ahead of publication) before 01 July 2015. An exception was made for the randomised controlled trial "A randomized, open-label, multi-center trial to determine safety and efficacy of eculizumab in the prevention of antibody mediated rejection (AMR) in living donor kidney transplant recipients requiring desensitization therapy" (NCT01399593). This has not been published in full but the trial was closed on 06 November 2014 and the results were released in preliminary form on 07 January 2015.

Transplant centres in the UK were informed of the development process and asked for nominations of interested authors. The first draft of the guidelines was written by Dr Michelle Willicombe, Dr David Lowe, Professor David Talbot, Dr Vaughan Carter, Dr Sian Griffin, and Professor Rob Higgins, with contributions from Dr Rommel Ravanan, Dr Brendan Clark, Professor Anthony Dorling, Dr Bob Vaughan, Professor David Briggs, Dr John Smith, and Dr Phil Mason. The preliminary draft guideline was reviewed by members of the Guideline Development Group and revised by Professor Rob Higgins. Further contributions were received from Dr Michelle Willicombe, Dr David Lowe, Dr Sian Griffin, Professor Susan Fuggle, Professor Anthony Dorling, Dr Sunil Daga, Dr Jack Galliford, Dr Andrew Bentall, Dr Phil Mason, Dr Vaughan Carter, Mr Ajay Sharma, Dr Bob Vaughan, Professor David Briggs, Professor Nizam Mamode, Dr Peter Andrews and Dr William McKane. Other contributors through the guideline development process were Dr Simon Ball and Miss Lorna Marson.

The guidelines were edited by Dr Peter Andrews, Chair of the BTS Standards Committee, and opened for public consultation through the website of the British Transplantation Society in December 2015. The final guidelines were published in February 2016.

It is anticipated that these guidelines will next be revised in 2020.

1.3 Guideline Development Group

Professor Rob Higgins MD FRCP
Consultant Nephrologist, Department of Nephrology and Transplantation
University Hospitals Coventry and Warwickshire, Coventry CV2 2 DX
Email: robert.higgins@uhcw.nhs.uk

Dr Peter Andrews MD FRCP

Consultant Nephrologist, SW Thames Renal & Transplantation Unit
St Helier Hospital, Surrey SM5 1AA
Email: peter.andrews@esth.nhs.uk

Dr Siân Griffin PhD FRCP
Consultant Nephrologist, Department of Nephrology and Transplantation
University Hospital of Wales, Cardiff CF14 4XW
Email: sian.griffin2@wales.nhs.uk

Dr David Lowe PhD
Clinical Scientist Histocompatibility and Immunogenetics
Royal Liverpool and Broadgreen University Hospital NHS Trust, Liverpool L7 8XP
Email: david.lowe@rlbuht.nhs.uk

Dr Michelle Willicombe
Clinical Lecturer, Imperial College Renal and Transplant Centre
Hammersmith Hospital, London W12 0HS
Email: michelle.willicombe@imperial.nhs.uk

Professor David Talbot
Consultant Transplant Surgeon, Freeman Hospital
Newcastle upon Tyne NE7 7DN
Email: david.talbot@nuth.nhs.uk

Dr Vaughan Carter PhD DMS FRCPath
Consultant Clinical Scientist & Associate Clinical Lecturer, Institute of Cellular Medicine, University of Newcastle upon Tyne
Head of Centre/Deputy Head of Department NHSBT Newcastle, Newcastle upon Tyne NE2 4NQ
Email: vaughan.carter@nhsbt.nhs.uk

Mr Ajay Kumar Sharma MBBS MS DNB FRCS (Glas) FRCS (Edin) FRCS (General Surgery)
Consultant Surgeon in Transplantation and General Surgery
Royal Liverpool and Broadgreen University Hospital NHS Trust, Liverpool L7 8XP
Email: ajay.sharma@rlbuht.nhs.uk

Professor Anthony Dorling PhD FRCP

Professor of Transplant Inflammation and Repair, Honorary Consultant Nephrologist
King's College London MRC Centre for Transplantation, Guy's Hospital, London SE1 9RT
Email: anthony.dorling@kcl.ac.uk

Professor Susan Fuggle DPhil FRCPath
Consultant Clinical Scientist, Transplant Immunology and Immunogenetics Laboratory
Oxford University Hospitals Foundation Trust, Churchill Hospital, Oxford OX3 7LE
Email: susan.fuggle@nds.ox.ac.uk

Professor Anthony N Warrens DM PhD FRCP FRCPath FEBS FHEA
Dean for Education, Barts and The London School of Medicine and Dentistry, Queen Mary University of London
Professor of Renal and Transplantation Medicine, Honorary Consultant Physician,
Barts Health Education Academy, London E1 2AD
Email: a.warrens@qmul.ac.uk

Professor David Briggs,
Director, NHSBT H&I Laboratory, Vincent Drive, Birmingham
Email: david.briggs@nhsbt.nhs.uk

Dr John Smith PhD FRCPath
HCS Consultant Head of Tissue Typing Service, Harefield Hospital
Royal Brompton & Harefield NHS Foundation Trust, Uxbridge UB9 6JH
Email: j.smith@rbht.nhs.uk

Dr Rommel Ravanan
Renal Unit, Southmead Hospital, Bristol BS10 5NB
Email: rommel.ravanan@nbt.nhs.uk

Dr Phil Mason
Consultant Nephrologist, The Churchill Hospital, Oxford OX3 7LJ
Email: phil.mason@ouh.nhs.uk

Robert Vaughan PhD FRCPath
Director, Clinical Transplantation Laboratory, Guy's Hospital, London SE1 9RT
Email: robert.vaughan@viapath.co.uk

Olivia Shaw PhD FRCPath
Consultant Clinical Scientist, Deputy Clinical Director Clinical Transplantation Laboratory

Guy's Hospital, London SE1 9RT
Email: olivia.shaw@viapath.co.uk

Dr William McKane PhD FRCP
Consultant Nephrologist, Sheffield Kidney Institute
Northern General Hospital, Sheffield S5 7AU
Email: william.mckane@sth.nhs.uk

Professor Nizam Mamode BSc MB ChB MD FRCS
Professor of Transplant Surgery, Guy's Hospital, London SE1 9RT
Email: nizam.mamode@gstt.nhs.uk

1.4 Declarations of Interest

Editors, authors and contributors have undertaken to work to the standards detailed in the BTS Clinical Practice Guideline accessible at:

> http://www.bts.org.uk/MBR/Clinical/Guidelines/Current/Member/Clinical/Current_Guidelines.aspx (5).

The following declarations have been notified:

Professor Anthony Dorling: within the last 5 years, unrestricted educational grant from Novartis Pharmaceuticals, consultancy fees from Chiesi Ltd, and accommodation and travel expenses to attend educational meetings from Thermofisher and Astellas.

Professor Susan Fuggle: Oxford University Hospitals Foundation Trust has received a fee, accommodation and travel expenses for participation in an Educational Symposium sponsored by Astellas Pharma Inc.

Dr Siân Griffin: attendance at educational meetings sponsored by Astellas, Novartis and Alexion.

Professor Rob Higgins: unrestricted educational grants to department from Roche, Alexion, LINC Medical and Miltenyi Bio. Accommodation expenses at educational meeting supported by Alexion and OneLamda. Honoraria and travelling expenses for lectures from Genzyme.
Dr William McKane: financial support for travel/education from Roche, Chiesi, Sandoz and Novartis and has undertaken paid consultancy for Novartis and Sandoz.

Professor David Talbot: funding for attending meetings from Roche, Astellas, Wyeth and Novartis.

Dr Peter Andrews, Dr David Lowe, Dr Phil Mason, Dr Rommel Ravanan, Dr John Smith: none

1.5 Grading of Recommendations

These guidelines represent consensus opinion from experts in the field of transplantation in the United Kingdom. They represent a snapshot of the evidence available at the time of writing. It is recognised that recommendations are made even when the evidence is weak. It is felt that this is helpful to clinicians in daily practice and is similar to the approach adopted by KDIGO (3).

In these guidelines, the GRADE system has been used to rate the strength of evidence and the strength of recommendations. This approach is consistent with that adopted by KDIGO, and also with guidelines from the European Best Practice Committee, and from the Renal Association (2,3). Explicit recommendations are made on the basis of the trade-offs between the benefits on the one hand, and risks, burden, and costs on the other.

For each recommendation the quality of evidence has been graded as:

A (high)
B (moderate)
C (low)
D (very low)

Grade A evidence means high quality evidence that comes from consistent results from well performed randomised controlled trials, or overwhelming evidence of another sort (such as well-executed observational studies with very strong effects).

Grade B evidence means moderate quality evidence from randomised trials that suffer from serious flaws in conduct, inconsistency, indirectness, imprecise estimates, reporting bias, or some combination of these limitations, or from other study designs with special strength.

Grade C evidence means low quality evidence from observational evidence, or from controlled trials with several very serious limitations.

Grade D evidence is based only on case studies or expert opinion.

For each recommendation, the strength of recommendation has been indicated as one of:

Level 1 (we recommend)
Level 2 (we suggest)
Not graded (where there is not enough evidence to allow formal grading)

A **Level 1** recommendation is a strong recommendation to do (or not do) something where the benefits clearly outweigh the risks (or vice versa) for most, if not all patients.

A **Level 2** recommendation is a weaker recommendation, where the risks and benefits are more closely balanced or are more uncertain.

1.6 Abbreviations

AAR	Accelerated acute rejection (second set response)
ABOi	Blood group incompatibility
AHG	Anti-human globulin
AIT	Antibody incompatible transplantation
AMR	Antibody mediated rejection
ATG	Antithymocyte globulin
CDC	Complement dependent cytotoxic (crossmatch)
cRF	Calculated reaction frequency
DDT	Deceased donor transplant
DFPP	Double filtration plasmapheresis
DSA	Donor specific antibody
DTT	Dithriothritol
EDTA	Ethylenediaminetetraacetic acid
FC	Flow cytometry (crossmatch)
HAR	Hyperacute rejection
HLA	Human leucocyte antigen
HLAi	Donor specific HLA antibody incompatibility
HSP	Highly sensitised patient
IA	Immunoadsorption
IVIg	Intravenous immunoglobulins
LD	Living donor

LDT	Living donor transplantation
MFI	Mean fluorescence intensity
NLDKSS	UK National living donor kidney sharing schemes
PP	Plasmapheresis
PRA	Panel-reactive antibodies
Standard transplantation	Transplantation without antibody incompatibility
TG	Transplant glomerulopathy
XM	Crossmatch

1.7 Disclaimer

This document provides a guide to best practice, which inevitably evolves over time. All clinicians involved in this aspect of transplantation need to undertake clinical care on an individualised basis and keep up to date with changes in the practice of clinical medicine.

These guidelines represent the collective opinions of a number of experts in the field and do not have the force of law. They contain information/guidance for use by practitioners as a best practice tool. It follows that the guidelines should be interpreted in the spirit rather than to the letter of their contents. The opinions presented are subject to change and should not be used in isolation to define the management for any individual patient. The guidelines are not designed to be prescriptive, nor to define a standard of care.

The British Transplantation Society cannot attest to the accuracy, completeness or currency of the opinions contained herein and do not accept any responsibility or liability for any loss or damage caused to any practitioner or any third party as a result of any reliance being placed on the guidelines or as a result of any inaccurate or misleading opinion contained in the guidelines.

References

1. Andrews PA. BTS Clinical Practice Guideline 2015. Accessed at http://www.bts.org.uk/MBR/Clinical/Guidelines/Current/Member/Clinical/Current_Guidelines.aspx

2. Uhlig K, Macleod A, Craig J, et al. Grading evidence and recommendations for clinical practice guidelines in nephrology. A position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney Int 2006; 70: 2058-65.

3. Kidney Disease Improving Global Outcomes (KDIGO) Transplant Work Group: KDIGO clinical practice guideline for the care of kidney transplant recipients. Am J Transplant 2009; 9(S3): S1-157.

2 EXECUTIVE SUMMARY OF RECOMMENDATIONS

Transplant Units and Laboratories

We recommend that:

- Data on AIT must be collected and reported to the NHSBT AIT Registry to the standard set by the BSHI/BTS guidelines and as requested by NHSBT. In view of these revised guidelines, it is recommended that the content of this data set be reviewed. (1C)
- Laboratories must be able to define antibodies to the standard defined in the BSHI/BTS document 'Guidelines for the Detection and Characterisation of Clinically Relevant Antibodies in Allotransplantation'. Sensitive and rapid techniques for the assessment of donor-specific HLA antibody levels must be available. (1B)
- If ABOi transplantation is to be performed, blood group antibody titres must 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. (1C)
- An AIT 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. (1C)
- All transplant units performing AIT must follow appropriate clinical guidelines. (1C)
- If a transplant unit does not perform AIT, there must be a mechanism for informing patients of this option where appropriate, and the option of referral to another unit with an established AIT programme. (1C)
- MDT meetings must review all potential AIT, with representation by clinicians and laboratory colleagues. Laboratories must define the level of safe/unsafe antibody thresholds for HLAi that can be reproduced locally. (1C)

We suggest that:

- In living donor transplantation, it is not 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. (2C)

Selection, Risk Assessment and Making Choices

We recommend that:

- Any patient considering AIT must be fully counselled regarding the procedures, risks, and potential outcomes, and must also be informed of the alternative routes to

standard transplantation including exchange transplantation and the option of deceased donor transplantation. (1D)

- Patient counselling must include a risk assessment of the likelihood of accelerated acute, acute and chronic antibody mediated rejection, graft loss, and death using appropriate local and national/international data. (1D)
- In HLAi transplantation, patients must be risk assessed according to the principal risk factors for adverse outcome. These include a positive CDC crossmatch or a high FC crossmatch and may include high levels of cumulative DSA beyond MFI 10000, multiple donor specific antibodies, transplantation of a kidney from a deceased donor, and repeat mismatches including those related to pregnancy. (1C)
- In ABOi transplantation, patients must be risk assessed for acute AMR. Risk factors include ABO antibody titres above 1/256 and additional HLA antibody incompatibility. (1C)

Conditioning Treatment before Transplantation

We recommend that:

- Extracorporeal therapies must be used to remove HLA or ABO antibodies so that they are at levels at the time of implantation where the risks of AMR and graft loss are reduced. A reduced risk transplant may be considered where HLA antibody levels give a negative cytotoxic crossmatch or microbead measurement of MFI <5000, but this level may be flexible depending on an overall risk assessment. In ABOi, a haemagglutination titre of <1/8 is considered to be acceptable. (1C)
- In HLAi, the usual drug therapy before the transplant and at induction should be indicated in the unit's guidelines. Tacrolimus and mycophenolate may be started before the transplant. Combinations of IVIg and rituximab may also be used. (1C)
- In ABOi, the usual drug therapy during pre-transplant conditioning should be specified in the unit's guidelines. Combinations of IVIg, rituximab, and mycophenolate may be used. (1C)

We suggest that:

- There are several methods available for extracorporeal antibody removal (plasma exchange, cascade plasmapheresis, immunoglobulin immunoadsorption, specific antigen adsorption (ABOi only)). At present there is no evidence that one particular method produces superior clinical outcomes. (2C)

Initial Therapy and Monitoring in the Early Post-Transplant phase

We recommend that:

- The highest risk period for acute AMR is the first 2 weeks after transplantation. Patients must be monitored carefully in hospital or in clinic during this period. (1C)
- In HLAi, drug therapy during the first two weeks post-transplant should include tacrolimus and mycophenolate. Prednisolone, basiliximab, alemtuzumab, IVIg, ATG and bortezomib may be used according to local guidelines and risk assessment. (1C)
- In ABOi, drug therapy during the first two weeks post-transplant should include tacrolimus and mycophenolate. Prednisolone, basiliximab, alemtuzumab, IVIg, and ATG may be used according to local guidelines. (1C)

We suggest that:

- Daily measurement of HLA or ABO antibody levels is not mandatory, but daily samples should be taken when in hospital and at each clinical visit and be available for urgent analysis if required. (2D)

Diagnosis and Treatment of Acute Antibody Mediated Rejection

We recommend that:

- The diagnosis of acute antibody mediated rejection (AMR) is made on allograft biopsy. (1C)
- Patients with histologically proven acute AMR are screened for the presence of donor specific antibodies (DSA) at the time of diagnosis. (1C)
- Patients with acute AMR receive (or are switched to) baseline immunosuppression including tacrolimus, mycophenolate mofetil and corticosteroids, and are treated with high dose steroids. (1C)
- Patients with acute AMR in the presence of a detectable DSA receive extracorporeal antibody removal with five cycles of treatment or until the DSA is no longer detectable. (1C)
- In ABOi renal transplantation, AMR may occur rapidly so multiple therapies may need to be used. (1C)

We suggest that:

- IVIg, ATG, rituximab or bortezomib may be used in combination with other agents until evidence emerges to the contrary. (2D)

- Eculizumab may be considered for rescue therapy in resistant acute AMR in cases which are C4d positive or the DSA have complement fixing properties. (2D)
- Splenectomy may be considered (with or without additional eculizumab) to rescue acute AMR presenting with acute onset oligo/anuria in the early period after AIT. Where possible, the diagnosis should be confirmed pre-splenectomy by biopsy. (2D)

Diagnosis and Treatment of Chronic Antibody Mediated Rejection

We recommend that:

- The diagnosis of chronic antibody mediated rejection (cAMR) is made on renal allograft biopsy. (1C)
- Patients with histological changes consistent with cAMR are screened for the presence of DSA. (1C)
- In ABOi transplantation, the risks of late AMR related to blood group antibodies are very low. If there is a suspicion of AMR, the patient's current HLA antibody status should be checked. (1C)
- Other causes of 'glomerular double contours' are excluded. (1C)

We suggest that:

- In order to prevent cAMR, there is no evidence that maintenance immunosuppression needs be more intense than for 'standard' transplants, but there should be careful attention to advising and supervising adherence to care. (2C)
- Immunosuppressive agents used for the treatment of acute AMR may be considered for the treatment of cAMR in the presence of coexisting acute features of AMR. (Not graded)

Heart, Lungs, Liver and other Solid Organs apart from Kidney

We recommend that:

- Heart and liver transplantation may be carried out across ABO incompatibility in infants who have no detectable ABO antibodies. (1C)
- HLAi heart, lung and liver transplantation may be performed when there is no suitable compatible organ available and there has been a risk assessment in conjunction with the patient and the H&I laboratory. (1C)
- Transplantation of a liver at the same time as other organs (e.g. kidney, pancreas or small bowel) may confer protection against AMR and may be performed following risk assessment and informed patient consent. (1C)

We suggest that

- ABOi heart, lung and liver transplantation may be performed when there is no suitable compatible organ available and there has been a risk assessment in conjunction with the patient and the H&I laboratory; and if approved by NHSBT in light of other factors such as organ shortage. (2C)
- Antibody incompatible transplantation of pancreas, islets and small bowel is high risk (unless performed together with a liver transplant) and should only be performed following laboratory assessment and informed patient consent. (2C)

3 TRANSPLANT UNITS AND LABORATORIES

Statements of Recommendation

We recommend that:

- Data on AIT must be collected and reported to the NHSBT AIT Registry to the standard set by the BSHI/BTS guidelines and as requested by NHSBT. In view of these revised guidelines, it is recommended that the content of this data set be reviewed. (1C)
- Laboratories must be able to define antibodies to the standard defined in the BSHI/BTS document 'Guidelines for the Detection and Characterisation of Clinically Relevant Antibodies in Allotransplantation'. Sensitive and rapid techniques for the assessment of donor-specific HLA antibody levels must be available. (1B)
- If ABOi transplantation is to be performed, blood group antibody titres must 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. (1C)
- An AIT 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. (1C)
- All transplant units performing AIT must follow appropriate clinical guidelines. (1C)
- If a transplant unit does not perform AIT, there must be a mechanism for informing patients of this option where appropriate, and the option of referral to another unit with an established programme. (1C)
- MDT meetings must review all potential AIT, with representation by clinicians and laboratory colleagues. Laboratories must define the level of safe/unsafe antibody thresholds for HLAi that can be reproduced locally. (1C)

We suggest that:

- In living donor kidney transplantation, it is not 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. (2C)

3.1 Introduction

Antibodies directed against transplants are increasingly recognised as a critical barrier to further improvement in allograft survival and the access of patients to transplantation.

The modern era of renal transplantation began in the 1960s with the introduction of azathioprine. Within a few years, hyperacute rejection caused by blood group incompatibility and HLA-specific antibodies was recognised, and transplanting at risk was vetoed (1). Once hyperacute rejection was avoided, it was observed that a third of grafts were lost in the first year from T lymphocyte-mediated cellular rejection, which became the focus of intense research. This has resulted in a therapeutic toolkit that has eliminated the vast majority of graft losses from this cause in adherent patients. The therapies required to prevent and to treat T lymphocyte-mediated rejection comprise effective multipoint targeting of the interleukin-2 pathway, together with inhibition of lymphocyte proliferation and lymphocyte depletion therapy. These therapies are, however, relatively ineffective at dealing with T cell memory responses.

With effective treatments of T lymphocyte-mediated rejection largely established, evidence has accumulated that anti-donor antibodies also play an important role in acute and chronic allograft rejection, and that this phenomenon occurs in all types of solid organ transplantation (2). With this recognition, an international focus from clinicians, scientists and industry on developing new treatments for antibody mediated rejection (AMR) began about 15 years ago. We are currently in an exciting era characterised by new discoveries about anti-graft antibodies, their mechanisms of production, their action, and of the treatment of AMR.

This document should be used alongside the BSHI/BTS document ‘Guideline for the detection and characterisation of clinically relevant antibodies in allotransplantation’ (3rd edition), published in 2014 (3).

3.2 Definition of Antibody Incompatible Transplantation

Antibody incompatible transplantation (AIT) could be defined simply as the transplantation of an organ into a recipient who is ABO incompatible or who has current or pre-conditioning donor specific HLA antibodies. However, that definition would not be useful for clinicians, patients or commissioners because it would include some patients who lose their grafts from hyperacute rejection; some patients with early accelerated or acute AMR which can be successfully treated with good long term outcomes; and also many patients who will not develop acute or chronic AMR. Improvements in the sensitivity and specificity of risk

assessments for the key outcomes in AIT are required in order to produce more effective clinical guidance.

Hyperacute rejection (HAR) is a key risk in AIT. This can be avoided by not transplanting in the presence of a strongly positive flow cytometry (FC) crossmatch or a positive complement dependent cytotoxic (CDC) crossmatch in the immediate pre-operative period. However, there are patients who do not experience HAR despite being transplanted in such circumstances. Research that more accurately identified those patients who do not go on to develop HAR or untreatable acute or chronic AMR would be of great benefit.

A second key risk in AIT is the development of accelerated or acute AMR in the early period after transplantation, usually the first 2 weeks. This may be rapidly progressive with an associated risk of graft loss. Tools such as plasmapheresis and immunosuppressive drugs can successfully prevent and treat this type of rejection. Guidance is given in chapters 6, 7 and 8. These tools carry significant clinical risks and costs, however, so it is important to direct such therapies towards those patients most likely to benefit.

The currently available gold standard to identify those at increased risk of early AMR is a positive pre-treatment FC crossmatch. However, many patients with a positive FC crossmatch do not experience early AMR, even if they receive the same immunosuppression as in standard transplantation. Conversely, a smaller percentage of patients with detectable donor specific antibodies (DSA) but a negative FC crossmatch do experience early acute AMR (4-6). There is some evidence that points a route towards the improvement of risk assessment (section 4.2). This improved risk assessment may use additional parameters such as the source of sensitisation (7), detailed characteristics of DSA, and biomarkers measured either pre-conditioning or in the very early post-transplant period. An enhanced risk assessment will lead to safer and more efficient use of resources. However, even though much of the published research is promising, a clinically validated tool is not currently available. It is hoped that validated guidance can be given in the next edition of these guidelines.

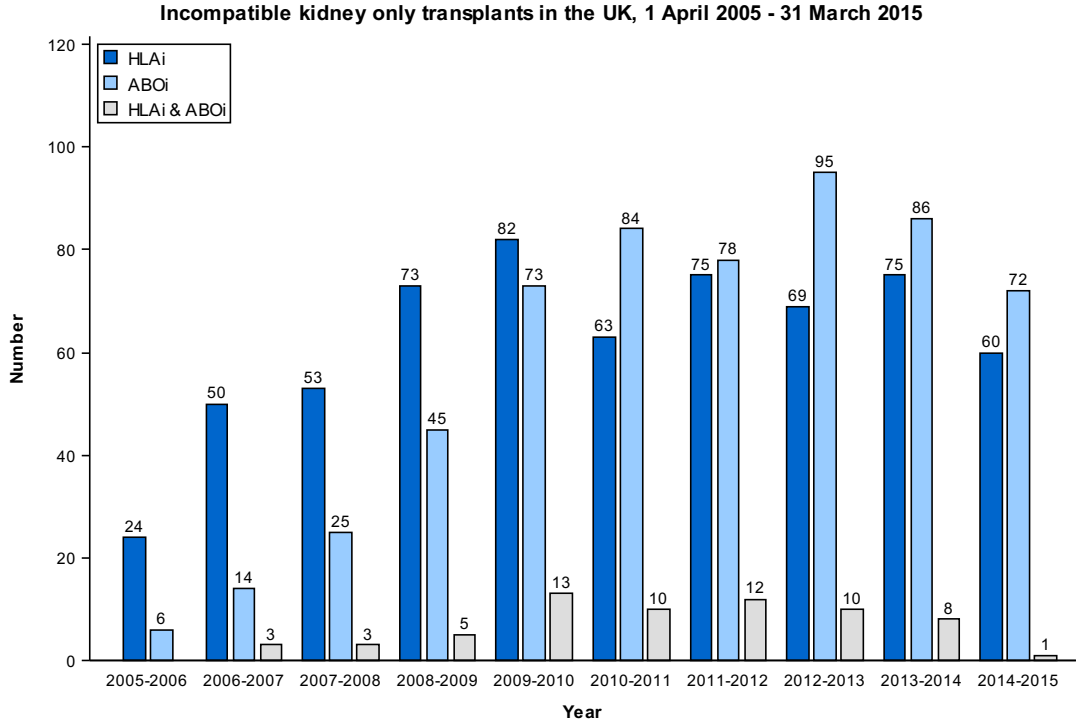
Chronic AMR is an important cause of graft failure after AIT, and also occurs in many patients who develop de novo HLA antibodies after standard transplantation. There are no are graded recommendations for the effective prevention or treatment of chronic AMR (chapter 8). Therefore being able to predict the risk of chronic AMR is less pressing in terms of clinical guideline development than for acute AMR. While chronic AMR is more frequent in those who have experienced acute AMR and the same risk factors may apply, there may be additional information that may refine the risk assessment for chronic AMR, such as the levels of DSA

after the early post-transplant period. It is hoped that evidence will accumulate so that recommendations can be made about the assessment of risk and the effective prevention and treatment of chronic AMR in subsequent editions of these guidelines.

In ABO incompatible transplantation, the donor to recipient blood group combinations of A to O, B to O, AB to O, B to A, AB to A, A to B and AB to B are incompatible, irrespective of whether the donor is blood group A1 or A2.

Other antibodies may play a role in allograft rejection; these include antibodies against the angiotensin receptor, non-ABO blood groups (e.g. Lewis), and endothelial antigens. There is insufficient evidence to incorporate recommendations about such antibodies in these guidelines.

The number of AIT performed in the UK is shown in Figure 3.1. It is seen that the numbers of HLAi and ABOi transplants increased sharply from 2000–09, but have since levelled off. A large driver behind this recent plateau has been the development of the UK National Living Donor Kidney Sharing Schemes (see chapter 4).



Source: Transplant activity in the UK, 2014-2015, NHS Blood and Transplant

Figure 3.1 Number of AIT performed annually in the UK, 2005-2014

3.3 Registry of Antibody Incompatible Transplantation

The Registry of Antibody Incompatible Transplantation, established by NHSBT, is a unique resource. Units must report details relating to all AIT as required by NHSBT. The data will allow better governance of activity (for example, definition of some of the high risk transplants performed by a unit), and is an important resource for further understanding the outcomes of AIT, and ultimately the development of new therapies. Transplants across an HLA antibody barrier which are microbead positive but FC crossmatch negative should continue to be reported to the Registry, in order to better define the risks of such transplants in the future.

3.4 Histocompatibility and Immunogenetics Laboratories

The ability to understand the degree of immunological incompatibility between donor and recipient is crucial in defining the level of risk prior to AIT. This can be determined in the laboratory by assessing the level of anti-donor reactive antibodies using both highly sophisticated solid phase assays and traditional serological assays. It is equally important to assess the sensitisation history of the recipient as this should relate to the HLA antibody status, and will influence risk assessment. Similarly, monitoring the post-transplant behaviour of such antibodies is important to patient management.

The three commonly used methods for assessing HLA-specific antibody levels are the complement dependent cytotoxic crossmatch (CDC), the flow cytometry crossmatch (FC), and HLA microbead analysis, although it should be noted that the CDC and FC techniques detect antibodies other than HLA (8). For a conventional antibody compatible transplant, donor/recipient compatibility is characterised by negative CDC and FC crossmatches and low levels of detectable donor-specific antibodies (DSA) by microbead analysis. However, in the case of AIT, these techniques can be used in combination to estimate the risk to graft survival when the transplant is performed in the presence of circulating DSA (9).

We recommend that pre-transplant assessment includes the use of microbead analysis plus a cellular crossmatch method. A positive cellular crossmatch in the absence of detectable donor specific HLA antibodies by microbead may be a false positive and should be investigated further. A positive microbead test without a positive cellular crossmatch should also be investigated. In general, a transplant will not fulfil the requirements to be defined as antibody incompatible for HLA specific antibodies unless an appropriate cellular crossmatch is used.

Unlike CDC and FC crossmatching, microbead analysis does not rely upon a constant supply of fresh donor lymphocytes to allow for daily monitoring. Instead, target HLA proteins are purified and attached to polystyrene beads (10,11), while the beads themselves are individually labelled with specific ratios of fluorescent marker dyes. The beads are incubated with patient sera and any HLA specific antibody present will bind to the HLA protein coupled to the microbead. A fluorescently labelled anti-human IgG antibody is then added and then both the bead sets and the bound antibody are classified by two colour laser analysis on a suitable platform.

Microbeads also allow the laboratory to monitor other HLA antibody specificities that may not be present on the graft. This helps to distinguish between a general upregulation of the immune response, such as that which may be associated with an inflammatory response, and a specific anti-graft response (12).

Although the development of Luminex-based technology has led to substantial progress in HLA antibody detection compared with cell-based techniques, technical issues can confound assay interpretation.

Samples with high levels of antibodies exhibit a phenomenon known as *prozone*, whereby high-titre antibodies can agglutinate in suspension and can prevent themselves from binding to target antigen. This results in a falsely low mean fluorescence intensity (MFI) level, or in some cases high levels of antibodies not being detectable at all (13). A similar effect whereby false low readout can be obtained in the *high-dose hook effect* (14). This may cause inhibition of antibodies in the assay binding due to steric hindrance. The presence of prozone or the high dose hook effect can be overcome by serially diluting sera, although it is not always obvious when bead reactivities are being detected at false low levels. The prozone concept also applies to cellular assays.

One potential solution to overcome the prozone effect is to add a small amount of ethylenediaminetetraacetic acid (EDTA) to the serum prior to testing (13). EDTA has the ability to chelate metal ions, most notably calcium ions (Ca^{2+}). A suggested mechanism for the prozone effect is the binding of complement component C1 to the Fc portion of IgG1 and IgG3, and the addition of EDTA disrupts this Ca^{2+}-dependent process. The presence of C1 bound to the HLA specific antibody is then thought to prevent the binding of the anti-human IgG labelled secondary detection antibody, resulting in a lower detectable level of fluorescent marker. Further support for this hypothesis is gathered by addition of C1 inhibitor (C1INH), which also leads to a marked reduction in the prozone effect. Addition of DTT to the sera or

heat inactivation of the sera prior to testing are both equally effective alternatives to the addition of EDTA.

Similarly, the presence of HLA-specific antibodies of IgM isotype have been put forward as a possible cause of reduced IgG binding in the Luminex assay (15,16). It is hypothesised that the presence of HLA-specific antibodies of both IgM and IgG isotype within the same serum can lead to competitive inhibition and reduced binding of IgG antibodies. Therefore some laboratories recommend the routine treatment of all sera with dithiotretol (DTT) in order to reduce IgM binding capacity. We recommend that all laboratories involved in HLAi transplantation, in cases where a prozone or hook effect is considered, have a protocol for interrogating sera, for example with EDTA or serial serum dilution. DTT controls should always be applied and reported for the CDC assay.

If a laboratory is now routinely adding EDTA to the microbead assay, this may create difficulties interpreting any guidance based on MFI thresholds obtained using a 'non-EDTA' or heat inactivation method. Evidence elsewhere in this guideline that mentions MFI levels was all obtained without the use of EDTA in the assay, and this should be born in mind when translating guidance into local practice.

Another technical issue surrounding the use of HLA antigen coated beads has recently emerged. During the manufacturing process there appears to be a significant proportion of HLA protein coupled to the microbeads that has been to some extent denatured. The presence of denatured antigen on the bead surface can lead to the presentation of a number of non-native HLA epitopes due to altered conformation of the protein. Literature is available describing the presence of HLA-specific antibodies in previously untransfused and untransplanted male volunteers as detected by microbead analysis (8). These were previously explained as the presence of 'naturally occurring' anti-HLA antibodies, but it now seems that a more likely explanation is that these reaction patterns are due to binding to non-native epitopes presented by a proportion of denatured antigen found on the microbead (8). Strategies to identify reactions caused by denatured antigen binding have been described, with the most common being to acid treat the microbead set to fully denature the beads' protein repertoire. Often, it is observed that the initial result is due to a combination of binding to both intact and denatured antigen, and that - crucially - antibody that recognises non-native HLA epitopes are not to be considered clinically significant (17,18). A simple and effective way of avoiding the detection of 'naturally' occurring antibody artefacts is to use two manufacturer's kits. The proportion of denatured antigen differs due to differences in the recombinant HLA manufacturing and folding process. A number of laboratories in the UK are doing this, and

their estimate of microbead specificity is thereby enhanced. Antibody to denatured antigen should always be considered in cases of negative crossmatch with positive microbead assay results. Alternative screening methods should be considered in these cases to confirm, or deny, the presence of true donor HLA specific antibody before classification as potential HLAi.

Modifications of the Luminex assay have also been developed so that antibodies can be divided into those that fix complement and those that do not, with many of the early studies appearing to indicate that the presence of donor-specific complement fixing anti-HLA antibodies is associated with increased risk of rejection and graft loss (19-25). However, the clinical utility of these assays in renal transplantation is not clear cut and further research is therefore needed before a full recommendation can be made re the use of C1q binding assays in risk stratification.

In ABOi transplantation, measurement of blood group antibody levels is required. At present this is performed by haemagglutination testing. It is known that this method is not perfect, with considerable inter- and intra-laboratory variation, although there is a degree of standardisation with gel-card based assays (26,27). Other methods for the measurement of ABO antibodies have been suggested, particularly flow cytometry (28,29). There are insufficient data to recommend the routine use of any other technique at present, although we encourage the evaluation of potentially more reliable and meaningful methods.

Laboratories measuring ABO titres must participate in national quality assurance schemes, and should participate in future initiatives that may make the results of antibody testing more clinically relevant.

We recommend that the local clinical guidelines for an AIT service are written in conjunction with the laboratory service so that the requirements for testing are defined. It should not be necessary, in living donor transplantation, to perform antibody testing at night, and it is not necessary to perform daily measurement of antibody levels. However, a seven day a week service for antibody testing is desirable.

The laboratory workload required to support a programme of AIT will depend on the size of the programme, but is likely to be significant in terms of human and financial resources. In establishing the resource requirement, account should be taken of the extra work during the early work up period, and in repeating antibody levels before the immediate pre-transplant period.

HLA antibody risk assessment is complex and cannot be performed simply by using a printed laboratory report. We recommend that patient assessment is performed in a multidisciplinary manner.

3.5 Transplant Units

It is not recommended that AIT is performed on an 'ad hoc' basis, but that units performing this type of transplant have appropriate clinical guidelines, resource allocation, and established working arrangements with the appropriate laboratories.

Some transplant units may choose not to perform AIT, or may perform just ABO or just HLA AIT. If this is the case, patients should be informed of their choices in respect of AIT and be offered appropriate referral to another transplant unit if they wish. They should also be provided with appropriate information about the potential advantages of the Kidney Sharing Schemes.

References

1. Patel R, Terasaki PI. Significance of the positive crossmatch test in kidney transplantation. New Engl J Med 1969; 280: 735–9.
2. Terasaki PI. A personal perspective: 100-year history of the humoral theory of transplantation. Transplantation 2012; 93: 751-6.
3. BSH/BTS Guideline for the detection and characterisation of clinically relevant antibodies in allotransplantation (3rd edition). http://www.bts.org.uk/BTS/Guidelines_Standards/Current/BTS/Guidelines_Standards/Current_Guidelines.aspx?hkey=e285ca32-5920-4613-ac08-fa9fd90915b5
4. Lefaucheur C, Loupy A, Hill GS, et al. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. J Am Soc Nephrol 2010; 21: 1398-406.
5. Singh N, Djamali A, Lorentzen D, et al. Pretransplant donor-specific antibodies detected by single-antigen bead flow cytometry are associated with inferior kidney transplant outcomes. Transplantation 2010; 90: 1079-84.
6. Willicombe M, Brookes P, Santos-Nunez E, et al. Outcome of patients with preformed donor-specific antibodies following alemtuzumab induction and tacrolimus monotherapy. Am J Transplant 2011; 11: 470-7.
7. Higgins R, Lowe D Daga S, et al. Pregnancy-induced HLA antibodies respond more vigorously after renal transplantation than antibodies induced by prior transplantation. Hum Immunol 2015; 76: 546-52.

8. El-Awar NR, Terasaki PI, Nguyen A, Sasaki N. Epitopes of human leukocyte antigen class I antibodies found in sera of normal healthy males and cord blood. Hum Immunol 2009; 70: 844-53.
9. Higgins R, Hathaway M, Lowe D, et al. Blood levels of donor-specific human leukocyte antigen antibodies after renal transplantation: resolution of rejection in the presence of circulating donor-specific antibody. Transplantation 2007; 84: 876-84.
10. Pei R, Lee J, Chen T, Rojo S, Terasaki PI. Flow cytometric detection of HLA antibodies using a spectrum of microbeads. Hum Immunol 1999; 60: 1293-302.
11. Sumitran-Karuppan S. The clinical importance of choosing the right assay for detection of HLA-specific donor-reactive antibodies. Transplantation 1999; 68: 502-9.
12. Higgins R, Lowe D, Hathaway M, et al. Rises and falls in donor-specific and third-party HLA antibody levels after antibody incompatible transplantation. Transplantation 2009; 87: 882-8.
13. Schnaidt M, Weinstock C, Jurisic M, Schmid-Horch B, Ender A, Wernet D. HLA antibody specification using single-antigen beads - a technical solution for the prozone effect. Transplantation 2011; 92: 510-5.
14. Lowe D, Hathaway M, Briggs D. The high-dose hook effect in the detection and monitoring of HLA specific antibody by Luminex assay. Int J Immunogenetics 2007; 34: 288-94.
15. Kosmoliaptsis V, Bradley JA, Peacock S, et al. Detection of immunoglobulin G human leukocyte antigen-specific alloantibodies in renal transplant patients using single-antigenbeads is compromised by the presence of immunoglobulin M human leukocyte antigen-specific alloantibodies. Transplantation 2009; 87: 813-20.
16. Kosmoliaptsis V, O'Rourke C, Bradley JA, Taylor CJ. Improved Luminex-based human leukocyte antigen specific antibody screening using dithiothreitol-treated sera. Hum Immunol 2010; 71: 45-9.
17. Poli F, Banezzi E, Innocente A, et al. Heart transplantation with donor-specific antibodies directed toward denatured HLA-A*02:01: a case report. Hum Immunol 2011; 72: 1045-8.
18. Pereira S, Perkins S, Lee JH, et al. Donor-specific antibody against denatured HLA-A1: clinically nonsignificant? Hum Immunol 2011; 72: 492-8.
19. Smith JD, Hamour IM, Banner NR, Rose ML. C4d fixing, Luminex binding antibodies - a new tool for prediction of graft failure after heart transplantation. Am J Transplant 2007; 7: 2809-15.
20. Chen G, Sequeira F, Tyan DB. Novel C1q assay reveals a clinically relevant subset of human leukocyte antigen antibodies independent of immunoglobulin G strength on single antigen beads. Hum Immunol 2011; 72: 849-58.

21. Chin C, Chen GE, Sequeria F, et al. Clinical usefulness of a novel C1q assay to detect immunoglobulin G antibodies capable of fixing complement in sensitized pediatric heart transplant patients. J Heart Lung Transplant 2011; 30: 158-63.
22. Fontaine MJ, Kuo J, Chen G, et al. Complement (C1q) fixing solid-phase screening for HLA antibodies increases the availability of compatible platelet components for refractory patients. Transfusion 2011; 51: 2611-8.
23. Yabu JM, Higgins JP, Chen G, Sequeira F, Busque S, Tyan DB. C1q-fixing human leukocyte antigen antibodies are specific for predicting transplant glomerulopathy and late graft failure after kidney transplantation. Transplantation 2011; 91: 342-7.
24. Loupy A, Lefaucheur C, Vernerey D, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. N Engl J Med 2013; 369: 1215-26.
25. Yell M, Muth BL, Kaufman DB, Djamali A, Ellis TM. C1q binding activity of de novo donor-specific HLA antibodies in renal transplant recipients with and without antibody-mediated rejection. Transplantation 2015; 99: 1151-5.
26. Kumlien G, Wilpert J, Säfwenberg J, Tydén G. Comparing the tube and gel techniques for ABO antibody titration, as performed in three European centers. Transplantation. 2007; 84(S12): S17-9.
27. Tanabe K. Interinstitutional variation in the measurement of anti-A/B antibodies: the Japanese ABO-incompatible transplantation committee survey. Transplantation. 2007; 84(S12): S13-6.
28. Bentall A, Regan F, White J, et al. No progress in ABO titer measurement: time to aim for a reference? Transplantation. 2014; 97: e19-21.
29. Krishnan NS, Fleetwood P, Higgins RM, et al. Application of flow cytometry to monitor antibody levels in ABO incompatible kidney transplantation. Transplantation 2008; 86: 474-7.

4 SELECTION, RISK ASSESSMENT AND MAKING CHOICES

Statements of Recommendation

We recommend that:

- Any patient considering AIT must be fully counselled regarding the procedures, risks, and potential outcomes, and must also be informed of the alternative routes to standard transplantation including exchange transplantation and the option of deceased donor transplantation. (1D)
- Patient counselling must include a risk assessment of the likelihood of accelerated acute, acute and chronic antibody mediated rejection, graft loss, and death using appropriate local and national/international data. (1D)
- In HLAi transplantation, patients must be risk assessed according to the principal risk factors for adverse outcome. These include a positive CDC crossmatch or a high FC crossmatch and may include high levels of cumulative DSA beyond MFI 10000, multiple donor specific antibodies, transplantation of a kidney from a deceased donor, and repeat mismatches including those related to pregnancy. (1C)
- In ABOi transplantation, patients must be risk assessed for acute AMR. Risk factors include ABO antibody titres above 1/256 and additional HLA antibody incompatibility. (1C)

4.1 Informed Choices and Kidney Sharing

Current data indicate that the results of AIT are not as good as those of standard transplantation. The level of risk should be discussed with the patient and any potential living donor so an informed choice can be made, as alternatives include finding an alternative living donor, entering into a kidney sharing scheme, waiting for a deceased donor organ, or dialysis.

We recommend that patients with living donors should be encouraged to enter a kidney sharing scheme in order to have the chance of being offered a standard transplant. A few patients are not suitable for the sharing scheme due to medical urgency or other reasons and every patient and their potential donor should be able to make individualised choices, but there are significant advantages to participation in the sharing scheme wherever possible. Two or three ‘rounds’ of allocation in the sharing scheme offer the best chance of being offered a standard transplant.

Highly sensitised patients (HSPs), particularly those with a calculated reaction frequency (cRF) >95% are difficult to transplant with an immunologically low risk organ. The proportion of HSPs with a cRF >95% on the deceased donor transplant list was 21% in 2014/15. This group receive less than 8% of deceased donor transplants, and for those who are transplanted their waiting time is considerably longer than the national average.

For sensitised patients with a living donor, paired or pooled donation is a potential opportunity to receive an immunologically low risk transplant. The UK National Living Donor Kidney Sharing Schemes (NLDKSS) were introduced in April 2007 (1) following the success of schemes in other nations, including the USA and the Netherlands (2,3). Since 2012 it has been possible for an altruistic donor to donate to the scheme, and since April 2015 it has been possible for an altruistic donor to start a short chain to facilitate three transplants.

Since the programme was introduced, around 400 patients have received a transplant through the schemes. However, those with a cRF >95% remain difficult to match, and around 50% of patients in any one run will have this level of sensitisation. The transplant rate for this group in 2012 – 15 was 17%, compared to 40% for less sensitised recipients.

To advise individual donor-recipient pairs of their chance of a match in the KSS, NHS Blood and Transplant (NHSBT) has developed a simulation to estimate the chance of a transplant (http://www.odt.nhs.uk/transplantation/guidance-policies/tools/). This simulation takes into account recipient and donor blood group, recipient cRF and age, and whether the recipient has high or low level ABOi or HLAi incompatibility with their donor (low/high). The availability of a blood group O donor greatly increases the chance of transplant though the NLDKSS.

Since January 2012 it has been possible for patients to have a modified profile of unacceptable antigens listed for the NLDKSS compared to the deceased donor waiting list, with removal of those antigens against which the recipient has only low level antibodies. The aim of this approach is to identify a transplant that, although it may be immunologically incompatible, the incompatibility would be due to the presence of antibodies amenable to removal by desensitisation. Half of the UK transplant units have taken advantage of this facility and to date 70 patients have been registered with different profiles, of which 23 have been transplanted.

Following the April 2014 matching run, an additional exercise was undertaken. All transplant units were contacted and invited to modify the unacceptable antigen profile of their patients and remove antibodies that were felt to be amenable to desensitisation according to local policy. A hypothetical matching run was then performed using the modified profiles.

The run included 178 patients and 6 altruistic donors. Sixty six patients (37%) had been in 5 or more previous matching runs, including 29 (16%) who had been in 10 or more runs. In the actual run, 16 patients (9%) were listed with different profiles to the deceased donor waiting list. This increased to 113 patients (63%) in the hypothetical run. In the actual run, 14 potential transplants were identified, this number increasing to 49 in the hypothetical run. For those patients with a cRF 0 – 84%, an additional 5 transplants were identified. This approach has obvious potential for future organ transplantation.

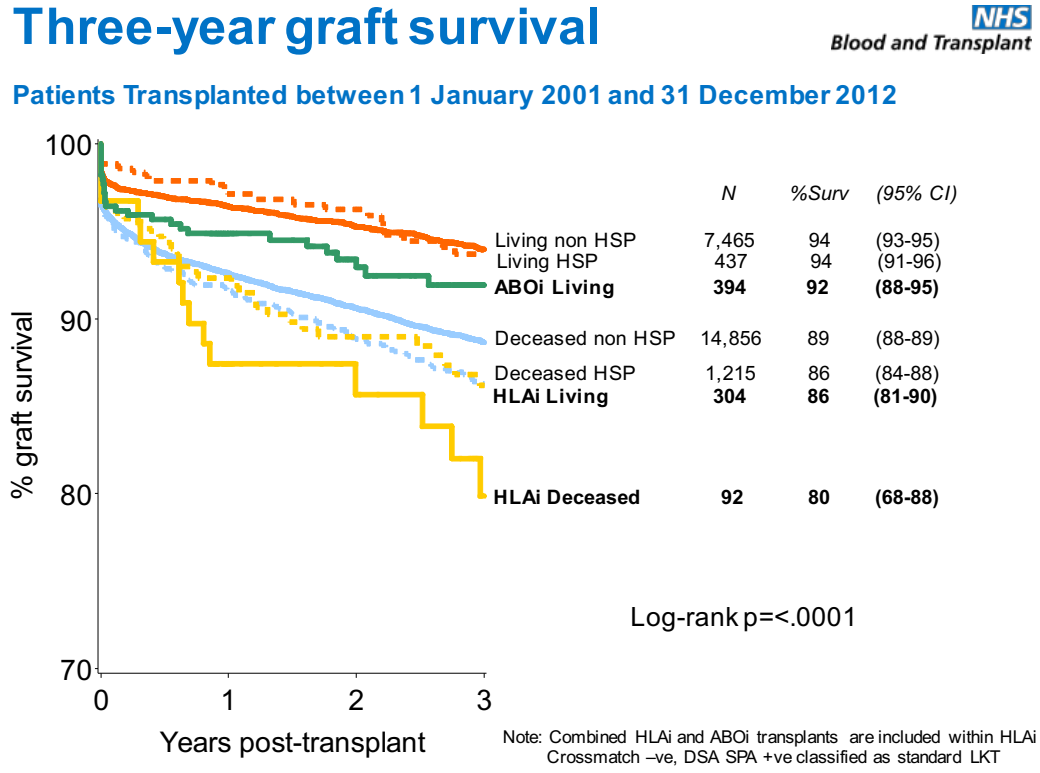
If a standard transplant does not become available through the sharing scheme after two runs, the risks and benefits of an antibody incompatible transplant should be discussed with the patient.

4.2 Outcomes and Risk factors for HLAi

The outcomes of AIT are not well defined, partly because of short term follow up and partly because of likely publication bias, those single centres with better results being more likely to present their outcomes for publication.

The UK has a comprehensive Registry of AIT which is unique as it fully separates the HLAi transplants from antibody compatible transplants. ABOi transplants are easier to identify in Registries, but if HLAi transplants cannot be separated from other transplants, the true outcome of ABOi transplants compared to antibody compatible transplants may be obscured. Some outcomes of AIT in the UK are shown in Figure 4.1.

The outcomes of HLAi transplants have also been reported in large collaborative studies from the USA (4,5). Five year unadjusted graft loss was 16.6% in transplants with no known antibody incompatibility, 20.1% in those with DSA detectable by microbead but crossmatch negative, 28.8% in those who were FC crossmatch positive and CDC crossmatch negative, and 39.9% in those who were CDC crossmatch positive. Five year mortality in these groups was 9.3%, 9.6%, 12.9%, and 19.1% respectively. Mortality is an important additional risk factor in HLAi transplantation, partly due to the more intense immunosuppression needed, and partly because many of the recipients have experienced long periods of previous dialysis and transplantation and may have acquired significant comorbidities. Over time, however, the risk of death is statistically higher in those patients that remain on dialysis.



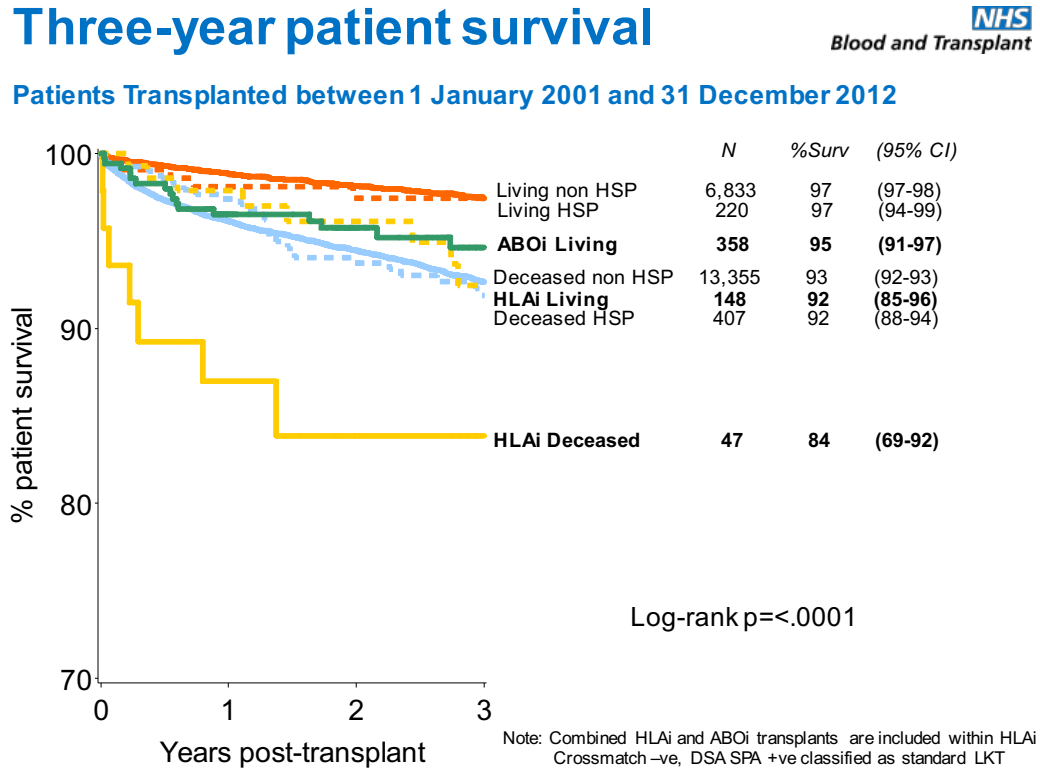


Figure 4.1 Outcomes of antibody incompatible kidney transplantation in the UK

Single centre reports of HLAi outcomes have also been reported (6-10). These show broadly similar outcomes to the national studies, although one study shows graft survival close to antibody compatible transplantation (6).

Table 4.1 shows the major reported risk factors for early antibody mediated rejection in HLAi transplantation.

Factor	**Higher risk**	**Lower risk**
Antibody level	CDC +ve	CDC –ve
Antibody level	Microbead MFI >10000	Microbead MFI <500
Antibody level	Flow cytometric XM +ve	Flow cytometric XM -ve
DSA specificities	Class I plus Class II	Single DSA
DSA specificities	Class I and DR	DP, DQ and DRB3, 4, 5
Sensitisation history	Pregnancy induced sensitisation, donor is patient's child or father of a child (6,11)	Transplant induced sensitisation with low DSA levels
Antibody characteristic*	Complement binding microbead positive	Complement binding microbead negative
ABO donor-recipient status	ABO incompatible	ABO compatible
Donor source	Deceased donor	Living donor

*not recommended as part of routine assessment

Table 4.1. Risks associated with HLAi kidney transplantation.

The most important of these risk factors is a positive CDC crossmatch, ABOi, or a single or cumulative DSA measured by microbead with MFI >10000. These are associated with 5 year graft survival rates as low as 50% (4-6,12,13) and transplantation in the face of such factors should only be performed with careful pre-transplant assessment and informed consent.

The definition of an antibody incompatible transplant is currently based on a limited risk assessment, this essentially being based on the level of antibody at the time of a transplant or pre-transplant conditioning (see section 3.2). As Table 4.1 indicates, other factors have been reported to impact on the risks of rejection and graft failure. There are also other emergent

studies into biomarkers and more subtle characteristics of DSA (14,15). It is not yet possible to determine which combination of risk factors gives the best prospective measure of the likelihood of AMR and graft loss post-transplant, and there is a need for work to integrate the known prognostic factors into a validated, clinically useful tool.

HLAi transplantation from a deceased donor has less good results in the current NHS BT Registry outcomes. Two approaches are being considered to address this. First, to remove unacceptable antigens to which the patient has generated only low level antibodies. This means the patient will receive an AIT, although this should have relatively low risks and probably a lower risk than continuing on dialysis. Second, pharmacological intervention to modulate the antibody profile and increase the chance of a transplant, which is discussed further in section 5.1. If these approaches are used, transplant units should act within their clinical guidelines for managing sensitisation, and all AIT transplants should be reported to the NHSBT Registry.

4.3 Outcomes and Risk Factors for ABOi

The outcomes of ABOi transplants have been described in large studies from the USA (16), Japan (17,18), Europe (19) and the UK (20). The USA study, and to a lesser extent the European study, show an early graft loss with a relative risk of around 2 compared to other transplants. This represents an increase in early graft losses from around 1 in 80 to 1 in 40. UK results showed results comparable with standard ABO compatible transplantation from one centre, but slightly reduced graft survival in early Registry analyses (20, 21) There is also an increased risk of complications, consequent on the more intense treatment given (22).

Acute AMR in ABOi is far less frequent than in HLAi, but may be of rapid onset and be hard to treat. Such rejection may occur even if the pre-treatment level of ABO antibody is low. The antibody level in ABOi is measured using a haemagglutination technique which has been shown to have poor inter- and intra-laboratory reproducibility. This makes it hard to define an upper limit of antibody level at which transplantation is high risk. Although it can occur with any pre-transplant level, AMR is more likely when the ABO titre is greater than 1/256 (23-26). Most reports have shown no significant association between maximum anti-A/B IgM titres and graft survival. However, AMR has been reported in association with high IgM titres despite low IgG titres in blood group O recipients of A2 kidneys, although there are insufficient supporting data to make a recommendation about the clinical interpretation of IgM blood group titres (27).

Although higher risk might be expected in blood group O recipients, as they tend to have higher antibody titres than blood group A or B patients, there do not seem to be large differences in outcomes according to recipient blood group. Grafts from blood group A2 donors appear to be at lower risk than those from A1 donors, as the antigen is expressed at lower levels on tissue, but despite this rejection and graft loss has been reported in this donor group.

The major increased risk of graft loss occurs very early post-transplantation and ABOi transplants that last greater than 2 weeks have the same survival as standard transplants.

These risks are summarised in Table 4.2:

Factor	**High risk**	**Low risk**
Haemagglutination titre	>1/256	Any level
If donor is group A	Group A1	Group A2
Donor type	Deceased donor	Living donor
HLA antibody incompatibility	Yes	No

References

1. Johnson RJ, Allen JE, Fuggle SV, Bradley JA, Rudge C; Kidney Advisory Group, UK Transplant NHSBT. Early experience of paired living kidney donation in the United Kingdom. Transplantation 2008; 86: 1672-7.
2. Segev DL, Kucirka LM, Gentry SE, Montgomery RA. Utilization and outcomes of kidney paired donation in the United States. Transplantation 2008; 86: 502-10.
3. Roodnat JI, Zuidema W, van de Wetering J, et al. Altruistic donor triggered domino-paired kidney donation for unsuccessful couples from the kidney-exchange program. Am J Transplant 2010; 10: 821-7.
4. Orandi BJ, Garonzik-Wang JM, Massie AB, et al. Quantifying the risk of incompatible kidney transplantation: a multicenter study. Am J Transplant 2014; 14: 1573-80.
5. Montgomery RA, Lonze BE, King KE, et al. Desensitization in HLA-incompatible kidney recipients and survival. N Engl J Med 2011; 365: 318-26.
6. Higgins R, Lowe D, Hathaway M, et al. HLA antibody incompatible renal transplantation: excellent medium term outcomes with negative cytotoxic crossmatch. Transplantation 2011; 92; 900-6.

7. Willicombe M, Brookes P, Santos-Nunez E, et al. Outcome of patients with preformed donor-specific antibodies following alemtuzumab induction and tacrolimus monotherapy. Am J Transplant 2011; 11: 470-7.
8. Couzi L, Manook M, Perera R, et al. Difference in outcomes after antibody-mediated rejection between ABO-incompatible and positive cross-match transplantations. Transpl Int 2015; 28: 1205-15.
9. Vo AA, Sinha A, Haas M, et al. Factors predicting risk for antibody-mediated rejection and graft loss in highly human leukocyte antigen sensitized patients transplanted after desensitization. Transplantation 2015; 99: 1423-30.
10. Singh N, Djamali A, Lorentzen D, et al. Pretransplant donor-specific antibodies detected by single-antigen bead flow cytometry are associated with inferior kidney transplant outcomes. Transplantation 2010; 90: 1079-84.
11. Higgins R, Lowe D Daga S, et al. Pregnancy-induced HLA antibodies respond more vigorously after renal transplantation than antibodies induced by prior transplantation . Human Immunology 2015; 76: 546-52.
12. Lefaucheur C, Loupy A, Hill GS, et al. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. J Am Soc Nephrol 2010; 21: 1398-406.
13. Bentall A, Cornell LD, Gloor JM, et al. Five-year outcomes in living donor kidney transplants with a positive crossmatch. Am J Transplant 2013; 13: 76-85.
14. Banham G, Prezzi D, Harford S, et al. Elevated pre-transplant soluble BAFF is associated with an increased risk of acute antibody-mediated rejection. Transplantation 2013; 96: 413-20.
15. Khovanova N, Daga S, Shaikhina T, et al. Subclass analysis of donor HLA specific IgG in antibody incompatible renal transplantation reveals a significant association of IgG_4 with rejection and graft failure. Transplant International, in press. doi: 10.1111/tri.12648
16. Montgomery JR, Berger JC, Warren DS, James NT, Montgomery RA, Segev DL. Outcomes of ABO-incompatible kidney transplantation in the United States. Transplantation 2012; 93: 603-9.
17. Tanabe K, Ishida H, Inui M, et al. ABO-incompatible kidney transplantation: long-term outcomes. Clin Transpl 2013: 307-12.
18. Aikawa A, Saito K, Takahashi K. Trends in ABO-incompatible kidney transplantation. Exp Clin Transplant 2015 Apr; 13 Suppl 1: 18-22.
19. Opelz G, Morath C, Süsal C, Tran TH, Zeier M, Döhler B. Three-year outcomes following 1420 ABO-incompatible living-donor kidney transplants performed after ABO antibody reduction: results from 101 centers. Transplantation 2015; 99: 400-4.
20. Barnett AN, Manook M, Nagendran M, et al. Tailored desensitization strategies in ABO blood group antibody incompatible renal transplantation. Transpl Int 2014; 27: 187-96.

21. Higgins R, Hudson A, Johnson R, et al. National registry of ABO and HLA antibody incompatible renal transplantation. American Transplant Congress, Philadelphia, 2011 (abstract).
22. Lentine KL, Axelrod D, Klein C, et al. Early clinical complications after ABO-incompatible live-donor kidney transplantation: a national study of Medicare-insured recipients. Transplantation 2014; 98: 54-65.
23. Masterson R, Hughes P, Walker RG, et al. ABO incompatible renal transplantation without antibody removal using conventional immunosuppression alone. Am J Transplant 2014; 14: 2807-13.
24. Krishnan N, Coates R, Daga S, et al. ABO incompatible renal transplantation without antibody removal using conventional immunosuppression alone. Am J Transplant 2015; 15: 1728-9.
25. Won D, Choe W, Kim HJ, Kwon SW, Han DJ, Park SK. Significance of isoagglutinin titer in ABO-incompatible kidney transplantation. J Clin Apher 2014; 29: 243-50.
26. Chung BH, Lim JU, Kim Y, et al. Impact of the baseline anti-A/B antibody titer on the clinical outcome in ABO-incompatible kidney transplantation. Nephron Clin Pract 2013; 124: 79-88.
27. Tierney J, Shaffer D. Transplantation of ABO A2 kidneys into O recipients: do IgM anti-A1 titers matter? Clin Transplant 2015; 29: 379-82.

5 CONDITIONING TREATMENT BEFORE TRANSPLANTATION

Statements of Recommendation

We recommend that:

- Extracorporeal therapies must be used to remove HLA or ABO antibodies so that they are at levels at the time of implantation where the risks of AMR and graft loss are reduced. A reduced risk transplant may be considered where HLA antibody levels give a negative cytotoxic crossmatch or microbead measurement of MFI <5000, but this level may be flexible depending on an overall risk assessment. In ABOi, a haemagglutination titre of <1/8 is considered to be acceptable. (1C)
- In HLAi, the usual drug therapy before the transplant and at induction should be specified in the unit's guidelines. Tacrolimus and mycophenolate may be started before the transplant. Combinations of IVIg and rituximab may also be used. (1C)
- In ABOi, the usual drug therapy during pre-transplant conditioning should be specified in the unit's guidelines. Combinations of IVIg, rituximab, and mycophenolate may be used. (1C)

We suggest that:

- There are several methods available for extracorporeal antibody removal (plasma exchange, cascade plasmapheresis, immunoglobulin immunoabsorption, specific antigen adsorption (ABOi only)). At present there is no evidence that one particular method produces superior clinical outcomes. (2C)

5.1 Strategies for Conditioning Therapy

There are three conditioning strategies that may be used before AIT is performed.

First, treatment may be given to reduce HLA antibody sensitisation over a period of weeks or months, which may enable a more compatible organ to be sourced if there is no living donor and may reduce the risks of transplantation in that HLA antibody production is reduced. This was the approach adopted as AIT was first developed in the 1980s (1). It was not easy to obtain a durable reduction in HLA antibody levels and the emphasis therefore moved to living donor transplantation with shorter conditioning periods. However, the longer term

'desensitisation' strategy continues to be used in some centres, especially the Cedars-Sinai Hospital in the USA (2-4).

Second, conditioning may be performed over a period of days or hours before a transplant. This strategy creates a safer window of opportunity for transplantation in the belief that the risks of early acute AMR may be reduced as the graft is presumed to be most vulnerable at the time of implantation. Again, this approach was first performed in deceased donor transplantation, but the short timescales available did not allow for effective antibody removal and downregulation of antibody production and the emphasis has now shifted to living donor transplantation (5,6). The majority of AIT is currently performed in this setting of short term conditioning.

Third, there may be no conditioning, although the immunosuppression given at the time of transplantation may be more intense than for a standard transplant. It may be possible to achieve good outcomes with this approach even with high DSA at the time of transplantation, as reported in France (7).

5.2 Extracorporeal Antibody Removal Therapy

There is considerable variation in the management of AIT within the UK and around the world, but most units perform extracorporeal antibody removal in living donor transplantation in the belief that this reduces the risk of early AMR, with the transplanted organ being most at risk immediately after implantation (8-11)

There is some evidence in HLAi that early rejection is associated with the levels of antibody at the time of transplantation (12), but this has not been systematically tested. In deceased donor transplantation, some groups have reported acceptable graft outcome rates without using pre-transplant antibody removal. In these series, patients are CDC crossmatch negative with their donor kidney, but may have microbead measured DSA of MFI >10000 (7). However, in living donor transplantation, which is a planned procedure, it seems more appropriate to electively remove antibody before transplantation.

Early AMR is more likely to be avoided if the antibody levels are reduced to CDC crossmatch negative, FC crossmatch negative and microbead MFI <5000. However, it is not an absolute requirement to achieve these levels. Indeed, a positive CDC crossmatch at transplant may be safe where there is only a single DSA of DP, DQ or DRB3-4 class. If the pre-treatment FC

crossmatch is negative, antibody removal should not be necessary, although this may be considered if the DSA microbead MFI level is >10000.

In ABOi, antibody removal is used to achieve a haemagglutination titre of <1/8 at the time of transplantation (11,13). There are published data where the ABO titre at the time of transplantation has been up to 1/32, but the safety of transplanting routinely at this level is not fully established (14).

In HLAi the available extracorporeal techniques include plasma exchange, double filtration plasmapheresis and immunoadsorption (Protein A or immunoglobulin specific adsorption). Any of these techniques may be used. We recommend careful monitoring of coagulation, including fibrinogen levels, and appropriate correction if necessary, especially around the time of transplantation.

In ABOi, the available extracorporeal techniques include plasma exchange, double filtration plasmapheresis and immunoadsorption (Protein A, immunoglobulin specific adsorption, or ABO specific adsorption). Any of these techniques may be used. We recommend careful monitoring of coagulation, including fibrinogen levels, and appropriate correction if necessary, especially around the time of transplantation. Even though the Sepharose-carbohydrate column used in specific immunoadsorption does not systematically remove coagulation factors, an increased bleeding risk has been reported and coagulation and fibrinogen levels should be monitored (15,16).

5.3 Pre-transplant Drug Therapy

There is no good evidence that any particular drug therapy given alongside extracorporeal antibody removal will effectively suppress donor specific antibody production.

There is randomised trial evidence that IVIg reduces sensitisation levels, though the trial showed only a transient fall in sensitisation and it is not clear whether the increased transplant rate in the treated group was purely due to IVIg (2).

Rituximab may have some benefit in HLAi, though there is no high quality trial evidence to support its use (17). Data on other agents, such as mycophenolate or proteasome inhibitors (18,19), do not indicate clearly whether there is significant benefit. Preliminary results from a randomised trial using an interleukin-6 receptor specific humanised monoclonal antibody have been reported, but further studies are required before recommendations can be made (4).

Many series of ABOi have reported the used of rituximab and IVIg. The schedules for administration of rituximab vary, from 30 days to 1 day pre-transplant. Likewise the use of IVIg is at various doses and treatment intervals (13). In the absence of good data, rituximab, IVIg or mycophenolate may be used prior to ABOi transplantation but it is not possible to make specific treatment recommendations.

Tyden published the results of the Stockholm protocol for 60 consecutive ABOi renal transplants and these form the basis of practice in many UK transplant units. The protocol uses antigen-specific immunoadsorption to remove ABO antibodies to a target titre of 1/8 (four sessions in the seven days before transplant and three sessions in the week after transplant), rituximab (375 mg/m^2) 30 days before the transplant, IVIg (0.5 g/kg) after the last session of immunoadsorption, IL2 receptor antibody, and tacrolimus, mycophenolate mofetil and prednisolone started from 13 days pre-transplantation. This group has reported allograft survival of 97% in ABOi group (n=60) versus 95% in ABO-compatible kidney recipients (n=276), and similar patient survival figures of 98% in both groups (20).

Splenectomy was used prior to ABOi transplantation after Alexandre reported its use was associated with successful outcomes in the 1980s (21). However, splenectomy is no longer recommended and many centres with excellent outcomes use rituximab (17,20). More recently, the transplant team from John Hopkins reported excellent graft outcomes of 98.3%, 92.9%, and 88.7% respectively at one, three, and five years without using rituximab or splenectomy. These results were comparable to UNOS data relating to standard living donor transplantation (22). Splenectomy is therefore not recommended as a treatment to prevent AMR in ABOi transplantation.

References

1. Palmer A, Taube D, Welsh K, Bewick M, Gjorstrup P, Thick M. Removal of anti-HLA antibodies by extracorporeal immunoadsorption to enable renal transplantation. Lancet 1989; 1(8628): 10-2.
2. Jordan SC, Tyan D, Stablein D, et al. Evaluation of intravenous immunoglobulin as an agent to lower allosensitization and improve transplantation in highly sensitized adult patients with end-stage renal disease: report of the NIH IG02 trial. J Am Soc Nephrol. 2004; 15: 3256-62.

3. Vo AA, Choi J, Cisneros K, et al. Benefits of rituximab combined with intravenous immunoglobulin for desensitization in kidney transplant recipients. Transplantation 2014; 98: 312-9.
4. Vo AA, Choi J, Kim I, et al. A phase I/II trial of the interleukin-6 receptor specific humanized monoclonal (Tocilizumab) + intravenous immunoglobulin in difficult to desensitize patients. Transplantation 2015; May 25 (epub ahead of print).
5. Higgins RM, Bevan DJ, Carey BS, et al. Prevention of hyperacute rejection by removal of antibodies to HLA immediately before renal transplantation. Lancet 1996; 348: 1208-11.
6. Montgomery RA, Zachary AA, Racusen LC, et al. Plasmapheresis and intravenous immune globulin provides effective rescue therapy for refractory humoral rejection and allows kidneys to be successfully transplanted into cross-match-positive recipients. Transplantation 2000; 70: 887-95.
7. Lefaucheur C, Loupy A, Hill GS, et al. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. J Am Soc Nephrol 2010; 21: 1398-406.
8. Higgins R, Lowe D, Hathaway M, et al. HLA antibody incompatible renal transplantation: excellent medium term outcomes with negative cytotoxic crossmatch. Transplantation 2011; 92; 900-6.
9. Higgins RM, Lowe D, Hathaway M, et al. Double filtration plasmapheresis in antibody incompatible kidney transplantation. Ther Apher Dial 2010; 14; 392-9.
10. Becker LE, Siebert D, Süsal C, et al. Outcomes following ABO-incompatible kidney transplantation performed after desensitization by nonantigen-specific immunoadsorption. Transplantation 2015; 99: 2364-71.
11. Lawrence C, Galliford JW, Willicombe MK, et al. Antibody removal before ABO-incompatible renal transplantation: how much plasma exchange is therapeutic? Transplantation 2011; 92: 1129-33.
12. Higgins R, Zehnder D, Chen K, et al. The histological development of acute antibody-mediated rejection in HLA antibody incompatible renal transplantation. Nephrol Dial Transplant 2010; 25: 1306-12.
13. Genberg H, Kumlien G, Wennberg L, Berg U, Tydén G. ABO-incompatible kidney transplantation using antigen-specific immunoadsorption and rituximab: a 3-year follow-up. Transplantation 2008; 85: 1745-54.
14. Couzi L, Manook M, Perera R, et al. Difference in outcomes after antibody-mediated rejection between ABO-incompatible and positive cross-match transplantations. Transpl Int 2015; 28: 1205-15.

15. de Weerd AE, van Agteren M, Leebeek FW, Ijzermans JN, Weimar W, Betjes MG. ABO-incompatible kidney transplant recipients have a higher bleeding risk after antigen-specific immunoadsorption. Transpl Int 2015; 28: 25-33.
16. Lentine KL, Axelrod D, Klein C, et al. Early clinical complications after ABO-incompatible live-donor kidney transplantation: a national study of Medicare-insured recipients. Transplantation 2014; 98: 54-65.
17. Macklin PS, Morris PJ, Knight SR. A systematic review of the use of rituximab for desensitization in renal transplantation. Transplantation 2014; 98: 794-805.
18. Aubert O, Suberbielle C, Gauthe R, Francois H, Obada EN, Durrbach A. Effect of a proteasome inhibitor plus steroids on HLA antibodies in sensitised patients awaiting a renal transplant. Transplantation 2014; 97: 946-52.
19. Woodle ES, Shields AR, Ejaz NS, et al. Prospective iterative trial of proteasome inhibitor-based desensitisation. Am J Transplant 2015; 15: 101-18.
20. Tydén G, Donauer J, Wadström J, et al. Implementation of a protocol for ABO-incompatible kidney transplantation - a three-center experience with 60 consecutive transplantations. Transplantation 2007; 83: 1153-5.
21. Alexandre GP, Squifflet JP, De Bruyère M et al. Present experiences in a series of 26 ABO-incompatible living donor renal allografts. Transplant Proc 1987; 19: 4538-42.
22. Montgomery RA, Locke JE, King KE et al. ABO incompatible renal transplantation: a paradigm ready for broad implementation. Transplantation. 2009; 87: 1246-55.

6 INITIAL THERAPY AND MONITORING IN THE EARLY POST-TRANSPLANT PHASE

Statements of Recommendation

We recommend that:

- The highest risk period for acute AMR is the first 2 weeks after transplantation. Patients must be monitored carefully in hospital or in clinic during this period. (1C)
- In HLAi, drug therapy during the first two weeks post-transplant should include tacrolimus and mycophenolate. Prednisolone, basiliximab, alemtuzumab, IVIg, ATG and bortezomib may be used according to local guidelines and risk assessment. (1C)
- In ABOi, drug therapy during the first two weeks post-transplant should include tacrolimus and mycophenolate. Prednisolone, basiliximab, alemtuzumab, IVIg, and ATG may be used according to local guidelines. (1C)

We suggest that:

- Daily measurement of HLA or ABO antibody levels is not mandatory, but daily samples should be taken when in hospital and at each clinical visit and be available for urgent analysis if required. (2D)

6.1 Occurrence of Acute AMR and Monitoring

It is clear from many reports that the highest risk period for acute AMR in AIT is in the first 2 weeks after transplantation. If graft loss from rejection occurs during the first 4 weeks, it will often be found that the onset of rejection was during the first 2 weeks (1,2). Close follow up is mandatory throughout this period.

It is not possible to recommend exact follow up regimens for all patients as this will depend on the intensity of anti-rejection therapy and any other complications, as well as the risk of AMR. However hospitalisation is likely to be longer and outpatient monitoring more frequent than for ‘standard’ transplants, and account should be taken of this in commissioning and planning services.

There is not a direct correlation between HLA or ABO antibody levels measured post-transplant and the onset of AMR. Antibody levels cannot be interpreted in the absence of

clinical information and an increase in antibody levels need not necessarily dictate a change in therapy if graft function is stable, though some clinical practice is more proactive in ABOi (see chapter 8) (3,4). Transplant units and laboratories should be able to measure antibody levels if required as a 7 day service, but need not do so routinely.

6.2 Choice of Immunosuppression in HLAi

Optimal post-transplant therapy for the prevention of acute AMR has not been defined in HLAi transplantation and there are few randomised trials to provide guidance. Tacrolimus and mycophenolate are recommended as part of maintenance immunosuppression. Azathioprine may be used if mycophenolate is not tolerated. Most units report also using prednisolone.

Good outcomes have been reported by one unit using tacrolimus, mycophenolate, prednisolone and basiliximab with escalation of therapy only if acute AMR is suspected or proven (1). However, most units report using more intense routine induction immunosuppression, especially if the DSA results in a positive FC crossmatch. ATG and alemtuzumab are the agents most commonly reported (5-7). We recommend either of these agents may be included in a unit's guidelines.

There is randomised trial evidence that IVIg reduces sensitisation levels, though the data do not extend to showing that early acute AMR rates are reduced by the use of IVIg (8). However, IVIg is effective in treating AMR and is used by many units performing AIT. We recommend that IVIg may be included in a unit's guidelines.

Agents that may reduce the rates of antibody production post-transplant include rituximab (9), and bortezomib (10). Evidence for their efficacy remains anecdotal and their use is not recommended in lower risk transplants.

Eculizumab is not recommended as routine preventative therapy for AMR in HLA AIT transplants. A phase 2 randomised controlled trial has recently been performed using this agent. This enrolled 102 patients receiving kidney transplants from living donors, all of whom were at risk of AMR based on elevated levels of donor-specific antibodies. After screening, patients were randomised into two groups of 51 patients each, with one group receiving eculizumab and a control group receiving the anti-rejection standard of care specified by the institution in which the patient's transplant took place. In the preliminary, unpublished, analysis of the 9-week data, there was no significant difference between treatment and control groups for the pre-determined composite endpoint (NCT01399593). Full publication of the data and

subgroup analyses are awaited. Preliminary results of a trial using a C-1 inhibitor have also been reported, but do not yet enable recommendations for practice to be made (11).

6.3 Choice of Immunosuppression in ABOi

The peak risk period for acute AMR is the first 14 days after ABOi transplantation, perhaps with a peak at day eight post-transplant (12). There are no randomised trials which indicate the optimal therapy after ABOi transplantation to prevent AMR. However, unlike HLAi, ABO antibodies do not seem to cause late AMR or chronic AMR, so the focus is on the prevention of early AMR.

Most reports use tacrolimus and mycophenolate for maintenance immunosuppression (13,14). Prednisolone-free immunosuppression has been reported with the use of alemtuzumab (15).

Early series of ABOi transplantation reported the used of rituximab, IVIg and splenectomy. Splenectomy is no longer undertaken in most units and is no longer necessary in ABOi (16,17). In patients with low pre-transplant ABO antibody levels, the successful use of standard immunosuppression alone has been reported, though there are also reports of graft loss due to a significant antibody rise in patients with low pre-transplant ABO antibody titres (18,19).

We recommend that IVIG, rituximab, ATG or alemtuzumab may be included in a unit's guidelines for ABOi. We do not recommend the routine use of bortezomib or eculizumab.

References

1. Higgins R, Lowe D, Hathaway M, et al. HLA antibody incompatible renal transplantation: excellent medium term outcomes with negative cytotoxic crossmatch. Transplantation 2011; 92; 900-6.
2. Garonzik Wang JM, Montgomery RA, Kucirka LM, Berger JC, Warren DS, Segev DL. Incompatible live-donor kidney transplantation in the United States: results of a national survey. Clin J Am Soc Nephrol 2011; 6: 2041-6.
3. Higgins R, Lowe D, Hathaway M, et al. Rises and falls in donor specific and third party HLA antibody levels after antibody incompatible transplantation. Transplantation 2009: 87: 882-8.

4. Ishida H, Kondo T, Shimizu T, Nozaki T, Tanabe K. Postoperative rebound of antiblood type antibodies and antibody-mediated rejection after ABO-incompatible living-related kidney transplantation. Transpl Int 2015; 28: 286-96.
5. Lefaucheur C, Loupy A, Hill GS, et al. Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. J Am Soc Nephrol 2010; 21: 1398-406.
6. Chouhan KK, Zhang R. Antibody induction therapy in adult kidney transplantation: a controversy continues. World J Transplant 2012 Apr 24; 2: 19-26.
7. Vo AA, Wechsler EA, Wang J, et al. Analysis of subcutaneous (SQ) alemtuzumab induction therapy in highly sensitized patients desensitized with IVIG and rituximab. Am J Transplant 2008; 8: 144-9.
8. Jordan SC, Tyan D, Stablein D, et al. Evaluation of intravenous immunoglobulin as an agent to lower allosensitization and improve transplantation in highly sensitized adult patients with end-stage renal disease: report of the NIH IG02 trial. J Am Soc Nephrol. 2004; 15: 3256-62.
9. Macklin PS, Morris PJ, Knight SR. A systematic review of the use of rituximab for desensitization in renal transplantation. Transplantation 2014; 98: 794-805.
10. Woodle ES, Shields AR, Ejaz NS, et al. Prospective iterative trial of proteasome inhibitor-based desensitization. Am J Transplant 2015; 15: 101-18.
11. Vo AA, Zeevi A, Choi J, et al. A phase I/II placebo-controlled trial of C1-inhibitor for prevention of antibody-mediated rejection in HLA sensitized patients. Transplantation 2015; 99: 299-308.
12. Fidler ME, Gloor JM, Lager DJ, et al. Histologic findings of antibody-mediated rejection in ABO blood-group-incompatible living-donor kidney transplantation. Am J Transplant 2004; 4: 101-7.
13. Opelz G, Morath C, Süsal C, Tran TH, Zeier M, Döhler B. Three-year outcomes following 1420 ABO-incompatible living-donor kidney transplants performed after ABO antibody reduction: results from 101 centers. Transplantation 2015; 99: 400-4.
14. Genberg H, Kumlien G, Wennberg L, Berg U, Tydén G. ABO-incompatible kidney transplantation using antigen-specific immunoadsorption and rituximab: a 3-year follow-up. Transplantation 2008; 85: 1745-54.
15. Galliford J, Charif R, Chan KK, et al. ABO incompatible living renal transplantation with a steroid sparing protocol. Transplantation 2008; 86: 901-6.
16. Lo P, Sharma A, Craig JC, et al. Preconditioning therapy in ABO-incompatible living kidney transplantation: a systematic review and meta-analysis. Transplantation 2015 Oct 1. [Epub ahead of print]

17. Song GW, Lee SG, Hwang S, et al. A desensitizing protocol without local graft infusion therapy and splenectomy is a safe and effective method in ABO-incompatible adult LDLT. Transplantation 2014; 97 Suppl 8: S59-66.
18. Masterson R, Hughes P, Walker RG, et al. ABO incompatible renal transplantation without antibody removal using conventional immunosuppression alone. Am J Transplant 2014; 14: 2807-13.
19. Krishnan N, Coates R, Daga S, et al. ABO incompatible renal transplantation without antibody removal using conventional immunosuppression alone. Am J Transplant 2015; 15: 1728-9.

7 DIAGNOSIS AND TREATMENT OF ACUTE ANTIBODY MEDIATED REJECTION

Statements of Recommendation

We recommend that:

- The diagnosis of acute antibody mediated rejection (AMR) is made following allograft biopsy. (1C)
- Patients with histologically proven acute AMR must be screened for the presence of donor specific antibodies (DSA) at the time of diagnosis. (1C)
- Patients with acute AMR receive (or are switched to) baseline immunosuppression including tacrolimus, mycophenolate mofetil and corticosteroids, and are treated with high dose steroids. (1C)
- Patients with acute AMR in the presence of a detectable DSA receive extracorporeal antibody removal with five cycles of treatment or until the DSA is no longer detectable. (1C)
- In ABOi renal transplantation, AMR may occur rapidly so multiple therapies may need to be used. (1C)

We suggest that:

- IVIg, ATG, rituximab or bortezomib may be used in combination with other agents until evidence emerges to the contrary. (2D)
- Eculizumab may be considered for rescue therapy in resistant acute AMR in cases which are C4d positive or the DSA have complement fixing properties. (2D)
- Splenectomy may be considered (with or without additional eculizumab) to rescue acute AMR presenting with acute onset oligo/anuria in the early period after AIT. Where possible, the diagnosis should be confirmed pre-splenectomy by biopsy. (2D)

7.1 Introduction and Diagnosis

These guidelines are directed primarily towards the management of AIT, but the principles of diagnosis and treatment may be used in patients without pre-formed donor specific antibodies at the time of transplantation who subsequently present with acute AMR.

Acute AMR can only be formally diagnosed on renal biopsy. Therefore a biopsy should be performed in all cases of suspected acute AMR, when safe to do so, in order to exclude other

pathology and to classify the rejection in line with the current Banff histological criteria (1). In some patients, it is recognised there may not be an opportunity to perform a renal allograft biopsy to make a formal diagnosis. This may be because of technical reasons or the need not to delay treatment.

Immunofluorescence staining to distinguish the predominant cell type involved in rejection (monocytes vs T cells) and analysis of gene transcripts, as pioneered by the Edmonton group, are two novel techniques that may significantly improve the accuracy of acute AMR diagnosis in the future.

Detection of circulating DSA at the time of histological evidence of AMR is required to meet the full criteria of acute AMR, so all patients should have serological testing for DSA (1). In the absence of a detectable DSA despite histological features, a diagnosis is categorised 'suspicious' for acute AMR (1). As well as assisting in diagnosis, knowledge of the quantity and characteristics of any DSA present at the time of onset of acute AMR may help predict prognosis, although this evidence is not graded (2-5).

Extrapolating from desensitisation protocols, it appears the relative strength of antibody present pre-transplant corresponds to outcome (6,7). Uncontrolled studies have shown that the risk of acute AMR and allograft failure is higher for positive pre-treatment CDC crossmatch, positive FC crossmatch, single bead positive DSA, and other factors such as the source of sensitisation (see section 4.2) (6-8).

There are observational data to suggest that measuring the DSA following treatment of acute AMR may help determine the response to therapy and overall prognosis, and it is possible that the change in DSA will become a surrogate end point for therapeutic studies in acute AMR (2,9).

In patients receiving high immunological risk transplants, the rate of acute AMR in the early post-transplant period has been reported to occur in up to 40% of patients (6,8,10-13). Severe acute AMR is associated with oliguria and requires immediate treatment. Measurement of DSA in these cases may be useful as the rejection episode is often associated with an acute rise in DSA levels (6,8,10-13).

7.2 Treatment of Acute AMR

Given the lack of randomised control trials, the optimal treatment for AMR remains uncertain. Current treatment protocols vary, making meaningful interpretation of reported series difficult. New treatment protocols are often extrapolated from desensitisation programmes rather than evidenced from randomised controlled trials (9,12,14-19). A recent systematic review on the treatment of AMR found only five randomised controlled trials, of which four were performed before formalisation of the histological definition of AMR by Banff (19). The lack of a definitive evidence base means that management protocols vary from unit to unit, as described in a recent study in the USA (15).

The current KDIGO guidelines suggest that acute AMR should be treated with one or more of the following, with or without steroids: plasma exchange, intravenous immunoglobulin, anti-CD20 antibody, and lymphocyte-depleting antibody; however, this advice was based upon level 2C evidence or less (14).

It is recommended that AIT recipients with acute AMR receive baseline maintenance treatment with tacrolimus, mycophenolate mofetil and steroids at the usual maintenance doses (20-24). This is based on evidence drawn from studies assessing maintenance immunosuppression protocols associated with lower rates of acute rejection (20-24). The more specific treatment options used in practice are described below and the most relevant references are shown in Table 7.1.

7.3 Extracorporeal Antibody Removal

Plasmapheresis, plasma exchange and immunoadsorption remove circulating DSA and are used in in the treatment of AMR (25-34). Whilst historic observational studies had mixed results regarding the benefit of plasmapheresis in the treatment of acute AMR, one of the few randomised controlled trials had to be terminated due to the significant benefit seen in the interventional arm receiving immunoadsorption (29). In this study, pre-sensitised patients who had pre-transplant immunoadsorption were excluded from study entry, although the majority of rejection episodes did occur soon after transplantation.

Whilst there are recognised differences between immunoadsorption and plasma exchange, the principles of antibody removal are similar (25-27). This is shown from the published data, predominantly from desensitisation protocols on the effectiveness of rapid HLA antibody removal following plasma exchange (7,8). Plasmapheresis is often administered as five

sessions on alternate days, or fewer if DSA are no longer detectable. However, there is no good evidence to make a general recommendation re the optimal regimen.

More recent data have indicated there may be limitations to the use of plasmapheresis in patients with rapidly rising DSA levels, and in some patients the DSA levels will fall after a few days regardless of whether plasmapheresis is given. The complication rate, especially infection, is higher when plasmapheresis is added to ATG therapy (34).

7.4 IVIg

Intravenous immunoglobulin (IVIg) has numerous potential effector mechanisms which could attenuate the treatment of AMR. These include the ability to neutralise circulating DSA, inhibition of complement activation, and blocking immune activation by competing for FcγRs (35). IVIg has been shown to reduce the degree of allosensitisation in highly sensitised renal patients on the transplant waiting list and is frequently used in protocols for the prevention or treatment of alloimmune injury (36). There are case reports of successful treatment of acute AMR when used as a single 'high' dose of 2 g/kg (37). The only randomised control trial of IVIg in allograft rejection is historic and pre-dates AMR as defined by Banff (38). In that study, the researchers found that IVIg alone was equivalent to OKT3 in the treatment of steroid resistant rejection (38). Another observational study showed that the use of IVIg could improve the outcome in patients with both steroid and anti-lymphocyte antibody resistant rejection (39).

7.5 ATG

Most of the evidence re the use of anti-thymocyte globulin (ATG) in the treatment of rejection predates the inclusion of AMR into the Banff criteria (40). ATG consists of polyclonal antibodies which predominantly act against T-cells; however it also contains antibodies against activated B-cell and plasma cell surface antigens (41). It is commonly incorporated into induction protocols for high immunological risk renal transplantation (8).

In a randomised control trial, ATG was shown to be superior to steroids for the treatment of first rejection episodes, although the complication rate in the former did not justify its routine use in this setting (42,43). Most of the trials assessing the use of ATG in the treatment of steroid-resistant rejection were performed to assess its efficacy over other polyclonal antibody (anti-lymphocyte globulin (ALG), horse ATG) or monoclonal antibody preparations (OKT3) (40,42,44). Observational studies indicate that ATG on its own or in conjunction with

plasmapheresis is effective at reversing histologically proven AMR (45,46). ATG may also be effective in treating concurrent acute T cell mediated rejection.

7.6 Rituximab

Biological agents targeting B-cells, plasma cells and their derivatives have been increasingly used as adjuvant therapy in both desensitisation and AMR treatment protocols over the past decade (8,19).

Rituximab is a B-cell depleting monoclonal antibody directed against the cell surface molecule CD20. Following administration, rituximab depletes both immature and mature B-cells, but not plasma cells or memory B cells. Preliminary case reports and series suggested a benefit of the addition of rituximab to the standard treatment protocols of acute AMR (32,47). Rituximab is now the commonest add-on agent used in the treatment of acute severe AMR (15,19). However, two notable studies have emerged which cast considerable doubt over the putative benefit of rituximab for the treatment of acute AMR. The first is a retrospective case-control observation comparing the use of bortezomib with rituximab as an add-on immunosuppressive agent in patients with acute AMR receiving plasmapheresis and IVIg. In this study, there was a trend toward superior allograft survival and improved allograft function in the patients receiving bortezomib (48). The second study is a randomised controlled trial, Ritux ERAH (NCT01066689), which has published one year outcomes in abstract form (49). This double blinded randomised controlled trial analysed the effectiveness of rituximab versus placebo in patients with acute AMR receiving plasmapheresis, IVIg and corticosteroids. At one year, there was no benefit of adjuvant rituximab in terms of allograft survival or improvement in function (21,49). The longer term outcomes have yet to be reported.

7.7 Bortezomib

Bortezomib is a proteasome inhibitor which induces apoptosis in metabolically active plasma cells. The first description of the use of bortezomib for acute AMR was published in 2008 by Everly et al (50). They described six cases of mixed AMR and ACR which were successfully treated with Bortezomib. However, five of these patients also received rituximab or ATG, and all received plasmapheresis. In the largest series reported, bortezomib was used in conjunction with plasmapheresis and IVIg to treat 16 renal transplant recipients with AMR. Allograft survival was 85% at a median of 9.8 months but only 25% of grafts regained their baseline function. The authors concluded that bortezomib demonstrated anti-humoral activity

but appeared to be most effective when used early (50). Subsequent observational studies have also suggested a positive role for bortezomib in the treatment of acute AMR (48,51).

The use of bortezomib in chronic AMR is being addressed in two clinical trials. The first, bortezomib for the treatment of late antibody mediated rejection, BORJECT Study (NCT01873157), aims to examine its effectiveness in preserving allograft function in patients found to have a *de novo* DSA on routine screening coupled with subclinical AMR. The investigators will be using bortezomib in isolation (22).

7.8 Eculizumab

Eculizumab is a humanised monoclonal antibody directed against the complement component C5. Its use to treat severe, refractory AMR is limited to case reports and small case series where there is likely to be a strong element of publication bias (11,52,53). A single centre study indicated that it reduced the incidence of acute AMR post-transplant, but longer term reported outcomes suggest it did not prevent the development of chronic AMR (13) and a preliminary report from a recent randomised controlled trial showed no difference between treatment and control groups in relation to the composite primary endpoint (see section 6.2). There have also been reports of failure of eculizumab to rescue allografts with acute AMR. Of note in a case series of 2 patients where treatment failed, the allograft showed C4d negative AMR and the DSA present were non-complement fixing (54). The authors noted that antibody-mediated injury may occur in a non-complement dependent manner and eculizumab may not be of benefit in this setting (54).

There are numerous active clinical trials involving the use of eculizumab for the prevention of AMR in a number of different settings (52). There is no active trial investigating its use in the treatment of acute AMR; however, there is an ongoing study assessing its use in the treatment of chronic complement-mediated injury of renal allografts (NCT01327573) (24).

7.9 Splenectomy

Splenectomy has been used for rescue therapy in patients with severe AMR in HLAi (10). One study from the Johns Hopkins University reported on five patients, all who were failing to respond to plasmapheresis and IVIg (± rituximab and ATG). All five patients had return of allograft function within two days of splenectomy (10). In a more recent study, the same group reported on outcomes of HLAi patients with severe acute AMR post-transplant who underwent rescue therapy either with splenectomy alone, eculizumab alone, or splenectomy plus

eculizumab (11). They described superior outcomes in the group who received treatment with splenectomy plus eculizumab. Of note, the eculizumab alone group had the worst outcome (11).

Table 7.1 Summary of studies into the use of agents to treat acute AMR

Mechanism of action	Agent (predominant mechanism)	References
Extracorporeal DSA removal	Plasma exchange/immunoadsorption (physical removal)	28*, 29*, 30, 31*, 32-34
Reduce DSA production	ATG (anti-T cell, B cell apoptosis)	43*, 44*, 45, 45
	Rituximab (anti-B cell)	32, 47, 48, 49*
	Bortezomib (Anti-plasma cell)	48, 50, 51
	Splenectomy	10, 11
Reduce DSA injury	Eculizumab (blocks complement activation)	11, 53, 54
Multiple level effects	IVIg	32, 38*
	ATG (anti-T cell, B cell apoptosis)	43*, 44*, 45, 46
	Steroids	46

*Randomised controlled trials

7.10 AMR in ABOi

In contract to HLAi transplantation, acute AMR is uncommon following ABOi transplantation but it can be catastrophic when it occurs, leading to allograft infarction in a matter of hours. Whilst most acute AMR after HLAi transplantation may respond, at least initially, to treatment, rejection after ABOi transplantation may be of rapid onset and antibody levels more resistant to removal. Reports of successful treatment often include the use of multiple therapies, sometimes initiated as soon as graft dysfunction is observed, and sometimes with a biopsy performed after initial therapy.

While it is not possible to make graded recommendations based on evidence, one anecdotal approach that has been used successfully in ABOi is described here. Daily antibody level measurement may indicate an early sign of immune activity. Minor changes (for example titres

changing by one dilution (e.g. 1/4 to 1/8)) may prompt a plan of plasmapheresis or immunoadsorption for the following day if the following day's antibody titre is worse. If the change is two dilutions, e.g. 1/4 to 1/16 from the previous day, then treatment may be initiated that day. Rising titres despite immunoabsorption combined with graft dysfunction mean that the graft may thrombose as the antibody is being synthesised at a faster rate than it can be removed. Vascular patency needs to be confirmed by ultrasound and then the recipient treated with complement blockade (eculizumab), which may need to be administered within 24-48 hours of graft dysfunction if antibody titres are rapidly increasing. Histological confirmation of antibody prior to treatment serves to delay this decision and may be postponed. Combining extracorporeal antibody removal with eculizumab is expensive and serves to reduce the effectiveness of eculizumab.

Graft dysfunction with low levels of AB antibody and without cell fragmentation should prompt the normal approach of ultrasound scan and biopsy and the more normal steroid based treatment, as T cell mediated rejection may be present.

Further studies should be undertaken to determine whether biomarker/s can reliably predict the onset of oliguria due to AMR in ABOi kidney transplantation. These could include a change in ABO antibody levels as suggested above, and possibly other markers of early allograft injury.

References

1. Haas M, Sis B, Racusen LC, et al. Banff 2013 meeting report: inclusion of C4d-negative antibody-mediated rejection and antibody-associated arterial lesions. Am J Transplant 2014; 14: 272-83.
2. Everly MJ, Everly JJ, Arend LJ, et al. Reducing de novo donor-specific antibody levels during acute rejection diminishes renal allograft loss. Am J Transplant 2009; 9: 1063-71.
3. Konvalinka A, Tinckam K. Utility of HLA antibody testing in kidney transplantation. J Am Soc Nephrol 2015; 26: 1489-502.
4. Lefaucheur C, Viglietti D, Bentlejewski C, et al. IgG donor-specific anti-human HLA antibody subclasses and kidney allograft antibody-mediated injury. J Am Soc Nephrol 2015 Aug 20 pii: ASN.2014111120. [Epub ahead of print]
5. Loupy A, Lefaucheur C, Vernerey D, et al. Complement-binding anti-HLA antibodies and kidney-allograft survival. New Engl J Med 2013; 369: 1215-26.

6. Bentall A, Cornell LD, Gloor JM, et al. Five-year outcomes in living donor kidney transplants with a positive crossmatch. Am J Transplant 2013; 13: 76-85.
7. Gloor JM, Winters JL, Cornell LD, et al. Baseline donor-specific antibody levels and outcomes in positive crossmatch kidney transplantation. Am J Transplant 2010; 10: 582-9.
8. Marfo K, Lu A, Ling M, Akalin E. Desensitization protocols and their outcome. CJASN 2011; 6: 922-36.
9. Archdeacon P, Chan M, Neuland C, et al. Summary of FDA antibody-mediated rejection workshop. Am J Transplant 2011; 11: 896-906.
10. Locke JE, Zachary AA, Haas M, et al. The utility of splenectomy as rescue treatment for severe acute antibody mediated rejection. Am J Transplant 2007; 7: 842-6.
11. Orandi BJ, Zachary AA, Dagher NN, et al. Eculizumab and splenectomy as salvage therapy for severe antibody-mediated rejection after HLA-incompatible kidney transplantation. Transplantation 2014; 98: 857-63.
12. Stegall MD, Dean PG, Gloor J. Mechanisms of alloantibody production in sensitized renal allograft recipients. Am J Transplant 2009; 9: 998-1005.
13. Stegall MD, Diwan T, Raghavaiah S, et al. Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. Am J Transplant 2011; 11: 2405-13.
14. KDIGO clinical practice guideline for the care of kidney transplant recipients. Am J Transplant 2009; 9 (Supplement 3).
15. Burton SA, Amir N, Asbury A, Lange A, Hardinger KL. Treatment of antibody-mediated rejection in renal transplant patients: a clinical practice survey. Clin Transplant 2015; 29: 118-23.
16. Djamali A, Kaufman DB, Ellis TM, Zhong W, Matas A, Samaniego M. Diagnosis and management of antibody-mediated rejection: current status and novel approaches. Am J Transplant 2014; 14: 255-71.
17. Levine MH, Abt PL. Treatment options and strategies for antibody mediated rejection after renal transplantation. Semin Immunol 2012; 24: 136-42.
18. Puttarajappa C, Shapiro R, Tan HP. Antibody-mediated rejection in kidney transplantation: a review. J Transplant 2012; 2012: 193724.
19. Roberts DM, Jiang SH, Chadban SJ. The treatment of acute antibody-mediated rejection in kidney transplant recipients - a systematic review. Transplantation 2012; 94: 775-83.
20. Kasiske BL, Chakkera HA, Louis TA, Ma JZ. A meta-analysis of immunosuppression withdrawal trials in renal transplantation. JASN 2000; 11: 1910-7.

21. Mycophenolate mofetil for the treatment of a first acute renal allograft rejection: three-year follow-up. The mycophenolate mofetil acute renal rejection study group. Transplantation 2001; 71: 1091-7.
22. Pascual J, Quereda C, Zamora J, Hernandez D. Spanish group for evidence-based medicine in renal transplantation. Steroid withdrawal in renal transplant patients on triple therapy with a calcineurin inhibitor and mycophenolate mofetil: a meta-analysis of randomized, controlled trials. Transplantation 2004; 78: 1548-56.
23. Rath T. Tacrolimus in transplant rejection. Expert Opin Pharmacother 2013; 14: 115-22.
24. Webster AC, Woodroffe RC, Taylor RS, Chapman JR, Craig JC. Tacrolimus versus ciclosporin as primary immunosuppression for kidney transplant recipients: meta-analysis and meta-regression of randomised trial data. Br Med J 2005; 331(7520): 810.
25. Ahmed T, Senzel L. The role of therapeutic apheresis in the treatment of acute antibody-mediated kidney rejection. J Clin Apher 2012; 27: 173-7.
26. Schwenger V, Morath C. Immunoadsorption in nephrology and kidney transplantation. Nephrol Dial Transplant 2010; 25: 2407-13.
27. Winters JL. Plasma exchange: concepts, mechanisms, and an overview of the American Society for Apheresis guidelines. Hematology Am Soc Hematol Educ Program 2012; 2012: 7-12.
28. Allen NH, Dyer P, Geoghegan T, Harris K, Lee HA, Slapak M. Plasma exchange in acute renal allograft rejection. A controlled trial. Transplantation 1983; 35: 425-8.
29. Bohmig GA, Wahrmann M, Regele H, et al. Immunoadsorption in severe C4d-positive acute kidney allograft rejection: a randomized controlled trial. Am J Transplant 2007; 7: 117-21.
30. Brown CM, Abraham KA, O'Kelly P, Conlon PJ, Walshe JJ. Long-term experience of plasmapheresis in antibody-mediated rejection in renal transplantation. Transplant Proc 2009; 41: 3690-2.
31. Kirubakaran MG, Disney AP, Norman J, Pugsley DJ, Mathew TH. A controlled trial of plasmapheresis in the treatment of renal allograft rejection. Transplantation 1981; 32: 164-5.
32. Lefaucheur C, Nochy D, Andrade J, et al. Comparison of combination plasmapheresis/IVIg/anti-CD20 versus high-dose IVIg in the treatment of antibody-mediated rejection. Am J Transplant 2009; 9: 1099-107.
33. Rocha PN, Butterly DW, Greenberg A, et al. Beneficial effect of plasmapheresis and intravenous immunoglobulin on renal allograft survival of patients with acute humoral rejection. Transplantation 2003; 75: 1490-5.
34. Higgins RM, Lowe D, Hathaway M, et al. Double filtration plasmapheresis in antibody incompatible kidney transplantation. Ther Apher Dialysis 2010; 14; 392-9

35. Jordan SC, Toyoda M, Kahwaji J, Vo AA. Clinical aspects of intravenous immunoglobulin use in solid organ transplant recipients. Am J Transplant 2011; 11: 196-202.
36. Jordan SC, Tyan D, Stablein D, et al. Evaluation of intravenous immunoglobulin as an agent to lower allosensitization and improve transplantation in highly sensitized adult patients with end-stage renal disease: report of the NIH IG02 trial. JASN 2004; 15: 3256-62.
37. Jordan SC, Quartel AW, Czer SCL, et al. Post-transplant therapy using high-dose human immunoglobulin (intravenous gammglobulin) to control acute humoral rejection in renal and cardiac allograft recipients and potential mechanism of action. Transplantation 1998; 66: 800-5.
38. Casadei DH, del CRM, Opelz G, et al. A randomized and prospective study comparing treatment with high-dose intravenous immunoglobulin with monoclonal antibodies for rescue of kidney grafts with steroid-resistant rejection. Transplantation 2001; 71: 53-8.
39. Luke PP, Scantlebury VP, Jordan ML, et al. Reversal of steroid- and anti-lymphocyte antibody-resistant rejection using intravenous immunoglobulin (IVIG) in renal transplant recipients. Transplantation 2001; 72: 419-22.
40. Webster AC, Pankhurst T, Rinaldi F, Chapman JR, Craig JC. Monoclonal and polyclonal antibody therapy for treating acute rejection in kidney transplant recipients: a systematic review of randomized trial data. Transplantation 2006; 81: 953-65.
41. Zand MS, Vo T, Huggins J, et al. Polyclonal rabbit antithymocyte globulin triggers B-cell and plasma cell apoptosis by multiple pathways. Transplantation 2005; 79: 1507-15.
42. Uslu A, Nart A. Treatment of first acute rejection episode: systematic review of level I evidence. Transplant Proc 2011; 43: 841-6.
43. Hoitsma AJ, van Lier HJ, Reekers P, Koene RA. Improved patient and graft survival after treatment of acute rejections of cadaveric renal allografts with rabbit antithymocyte globulin. Transplantation 1985; 39: 274-9.
44. Mariat C, Alamartine E, Diab N, de Filippis JP, Laurent B, Berthoux F. A randomized prospective study comparing low-dose OKT3 to low-dose ATG for the treatment of acute steroid-resistant rejection episodes in kidney transplant recipients. Transplant Int 1998; 11: 231-6.
45. Shah A, Nadasdy T, Arend L, et al. Treatment of C4d-positive acute humoral rejection with plasmapheresis and rabbit polyclonal antithymocyte globulin. Transplantation 2004; 77: 1399-405.
46. Higgins R, Lowe D, Hathaway M, et al. HLA antibody incompatible renal transplantation: excellent medium term outcomes with negative cytotoxic crossmatch. Transplantation 2011; 92; 900-6.

47. Kaposztas Z, Podder H, Mauiyyedi S, et al. Impact of rituximab therapy for treatment of acute humoral rejection. Clin Transplant 2009; 23: 63-73.
48. Waiser J, Budde K, Schutz M, et al. Comparison between bortezomib and rituximab in the treatment of antibody-mediated renal allograft rejection. Nephrol Dial Transplant 2012; 27: 1246-51.
49. Sautenet BG, Buchler M, Morelon E, et al. One year results of the effects of rituximab on acute humoral rejection in renal transplantation: RITUX ERAH, a multicenter randomized placebo controlled trial [abstract]. Am J Transplant 2013; 13 (suppl 5).
50. Everly MJ, Everly JJ, Susskind B, et al. Bortezomib provides effective therapy for antibody- and cell-mediated acute rejection. Transplantation 2008; 86: 1754-61.
51. Flechner SM, Fatica R, Askar M, et al. The role of proteasome inhibition with bortezomib in the treatment of antibody-mediated rejection after kidney-only or kidney-combined organ transplantation. Transplantation 2010; 90: 1486-92.
52. Legendre C, Sberro-Soussan R, Zuber J, et al. Eculizumab in renal transplantation. Transplant Rev (Orlando) 2013; 27: 90-2.
53. Locke JE, Magro CM, Singer AL, et al. The use of antibody to complement protein C5 for salvage treatment of severe antibody-mediated rejection. Am J Transplant 2009; 9: 231-5.
54. Burbach M, Suberbielle C, Brocheriou I, et al. Report of the inefficacy of eculizumab in two cases of severe antibody-mediated rejection of renal grafts. Transplantation 2014; 98: 1056-9.

8 DIAGNOSIS AND TREATMENT OF CHRONIC ANTIBODY MEDIATED REJECTION

Statements of Recommendation

We recommend that:

- The diagnosis of chronic antibody mediated rejection (cAMR) is made on renal allograft biopsy. (1C)
- Patients with histological changes consistent with cAMR are screened for the presence of DSA. (1C)
- In ABOi transplantation, the risks of late AMR related to blood group antibodies are very low. If there is a suspicion of AMR, the patient's current HLA antibody status should be checked. (1C)
- Other causes of 'glomerular double contours' are excluded. (1C)

We suggest that

- In order to prevent cAMR, there is no evidence that maintenance immunosuppression need be more intense than for standard transplants, but there should be careful attention to advising and supervising adherence to care. (2C)
- Immunosuppressive agents used for the treatment of acute AMR may be considered for the treatment of cAMR in the presence of coexisting acute features of AMR. (Not graded)

8.1 Diagnosis and Management

Chronic AMR is an important cause of graft loss in all types of solid organ transplantation. Given that it is associated with the appearance of HLA specific antibodies, patients transplanted across pre-existing DSA are at high risk. This is especially the case in those with very high pre-treatment DSA levels (e.g. positive CDC crossmatch), or those with early acute rejection episodes. There is, however, no evidence that the patients at risk require different management post-transplant than those receiving standard transplants.

Chronic AMR is a histological diagnosis, defined by the 2013 Banff meeting as the presence of one or more features of chronic injury: either double contours of the glomerular basement membrane on light microscopy or electron microscopy (transplant glomerulopathy); or severe peritubular capillary basement membrane multi-layering (by electron microscopy) or arterial

intimal fibrosis, where no other cause is identified (1). The histological changes are most likely the end result of previous episodes of active antibody mediated rejection. Chronic AMR is associated with a poor prognosis and there has been no report of effective sustained treatment (2). There is a higher incidence of chronic AMR in renal patients undergoing high immunological risk transplants, with a reported prevalence of 27% at one year in one series (3). The presence of the histological features consistent with transplant glomerulopathy may be caused by other pathogenic mechanisms other than chronic AMR including thrombotic microangiopathy and hepatitis C (4), and has also been associated with T-cell mediated rejection (5). The Banff 2013 criteria therefore require other causes of double contouring to be excluded before a diagnosis of chronic AMR is made.

Detection of circulating DSA at the time of histological evidence of AMR is required to meet the full criteria of acute AMR, therefore all patients should have serological testing for DSA (1).

There are few reports of late graft rejection attributable to ABO antibodies. Therefore, if chronic AMR is suspected in a recipient of an ABOi transplant, the HLA antibody status of the patient should be checked and the patient should be treated as for HLA antibody mediated chronic AMR if such antibodies are found.

There is a lack of evidence and no graded recommendations regarding the treatment of transplant glomerulopathy. A number of studies are being undertaken in this field as outlined previously. The RituxiCAN-C4 study aims to assess if rituximab can improve allograft function and reduce proteinuria in renal transplant recipients with chronic AMR (NCT00476164). The TRIBUTE study (NCT02201576) is an efficacy study which aims to assess the benefit of bortezomib in conjunction with plasmapheresis and IVIg in preventing progression of the histological features of chronic AMR. There is an ongoing study assessing the use of eculizumab in the treatment of chronic complement-mediated injury of renal allografts (NCT01327573).

A small number of observational studies suggest that the response to treatment directed at the humoral response may be greater in patients where there is significant microvascular inflammation; however, this has not been shown in an appropriately powered prospective trial (6). In the absence of strong evidence, we suggest that patients be maintained on triple immunosuppression including tacrolimus and mycophenolate. Additional measures to reduce proteinuria, including blockade of the rennin-angiotensin system, should also be taken.

References

1. Haas M, Sis B, Racusen LC, et al. Banff 2013 meeting report: inclusion of C4d-negative antibody-mediated rejection and antibody-associated arterial lesions. Am J Transplant 2014; 14: 272-83.
2. Issa N, Cosio FG, Gloor JM, et al. Transplant glomerulopathy: risk and prognosis related to anti-human leukocyte antigen class II antibody levels. Transplantation 2008; 86: 681-5.
3. Bentall A, Cornell LD, Gloor JM, et al. Five-year outcomes in living donor kidney transplants with a positive crossmatch. Am J Transplant 2013; 13: 76-85.
4. Baid-Agrawal S, Farris AB 3rd, Pascual M, et al. Overlapping pathways to transplant glomerulopathy: chronic humoral rejection, hepatitis C infection, and thrombotic microangiopathy. Kid Int 2011; 80: 879-85.
5. Hayde N, Bao Y, Pullman J, et al. The clinical and genomic significance of donor-specific antibody-positive/C4d-negative and donor-specific antibody-negative/C4d-negative transplant glomerulopathy. Clin J Am Soc Nephrol 2013; 8: 2141-8.
6. Kahwaji J, Najjar R, Kancherla D, et al. Histopathologic features of transplant glomerulopathy associated with response to therapy with intravenous immune globulin and rituximab. Clin Transplant 2014; 28: 546-53.

9 HEART, LUNG, LIVER AND OTHER SOLID ORGANS

Statements of Recommendation

We recommend that

- Heart and liver transplantation may be carried out across ABO incompatibility in infants who have no detectable ABO antibodies. (1C)
- HLAi heart, lung and liver transplantation may be performed when there is no suitable compatible organ available and there has been a risk assessment in conjunction with the patient and the H&I laboratory. (1C)
- Transplantation of a liver at the same time as other organs (e.g. kidney, pancreas or small bowel) may confer protection against AMR and may be performed following risk assessment and informed patient consent. (1C)

We suggest that

- ABOi heart, lung and liver transplantation may be performed when there is no suitable compatible organ available and there has been a risk assessment in conjunction with the patient and the H&I laboratory; and if approved by NHSBT in light of other factors such as organ shortage. (2C)
- Antibody incompatible transplantation of pancreas, islets and small bowel is high risk (unless performed together with a liver transplant) and should only be performed following laboratory assessment and informed patient consent. (2C)

9.1 Introduction

The relative success of AIT in kidney transplantation has not been replicated to the same degree in other organs. This is due to several factors, the main one being the lack of a living donor option and hence the inability to plan transplant dates and preparatory immunomodulation. However, there have been notable successes transplanting small groups of patients in certain categories.

There are different challenges involved in all forms of solid organ transplantation, especially where there is no readily available replacement therapy such as dialysis for those awaiting a kidney transplant. There have been huge advances in ventricular assist devices for those in end stage heart failure, but currently these are still seen as a bridge to transplantation in the

majority of cases, and there are no similar supportive measures for those requiring lung or liver transplantation.

Each organ has its own immunological challenge. In most cases, patients requiring a transplant will receive a limited number of offers and therefore it is incumbent on the transplant community to ensure that any immunological barriers that can be overcome are investigated thoroughly.

Organ specific antibody incompatibility is also considered in the BSHI/BTS guideline 'Guideline for the detection and characterisation of clinically relevant antibodies in allotransplantation' (1).

9.2 Transplantation in Infants

New-borns do not make their own antibodies, and in infancy the production of AB antibodies does not develop until weaning. Thus an ABO incompatible transplant may be performed safely if the ABO titre is 1 in 4 or less under the age of two years; and indeed the infant will subsequently not produce antibodies against the donor blood group (2,3). UNOS does not recommend ABO-incompatible transplantation beyond this age.

With the development of potential post-transplant therapies such as antibody removal columns and monoclonal antibodies and with increasing experience, these goalposts have changed with many centres now transplanting patients with higher antibody titres (4,5).

9.3 Liver Transplantation

It has been recognised for many years that the liver may absorb HLA antibodies without suffering severe acute AMR even if the pre-transplant CDC crossmatch is positive (6-9). However, if only a segment of a liver is transplanted, clinically significant rejection may occur, presumably as circulating antibody is deposited at a higher concentration. Evidence is accumulating that crossing pre-transplant Luminex detectable antibodies leads to increased complication rates in the early post-transplant phase. However, these complications are usually surmountable due to the ability of the liver to regenerate and repair, and therefore most centres will still transplant across such antibodies (9,10).

The liver may also 'protect' other organs against AMR, and it is well documented, for example, that a liver transplant may render a CDC crossmatch negative within a few minutes, and the

subsequent transplantation of a kidney may be successful (11-13). As well as the kidney, other visceral organs may be safely transplanted at the same time as the liver.

Likewise the liver may absorb ABO antibodies without suffering AMR, and ABOi liver transplantation may be performed if there is no alternative in clinically urgent cases. However the outcomes are reduced with occasional occurrence of AMR (14-17). There are fewer data on whether a high ABO antibody titre is a risk factor for AMR, though transplantation from donor group A2 into an O recipient may be lower risk (18).

9.4 Heart and Lung Transplantation

Following the introduction of more sensitive assays, the number of patients on transplant waiting lists who are considered to be sensitised has increased. In thoracic transplantation, >40% of patients are now considered to be sensitised (19). Successful transplantation across a pre-formed HLA antibody barrier may be successful, although a positive pre-transplant CDC crossmatch is associated with worse outcomes and emerging evidence indicates that lower levels of DSA also have a detrimental clinical effect (20-21).

BSHI/BTS clinical guidelines have recommended risk stratification as follows (1). No pre-transplant HLA antibody indicates a standard risk transplant. Cumulative MFI values of below 2,000 (i.e. the sum of MFI values of each defined antibody specificity corresponding to donor HLA mismatches) are considered to confer an additional, although manageable risk of early rejection with minimal risk of hyperacute rejection. Such enhanced immunological risk would be managed by increased immunosuppression and regular post-transplant monitoring. For patients with pre transplant DSA in the MFI range 2,000 – 5,000, the risk of hyperacute rejection is also reported to be low. Pre-transplant antibody reduction with enhanced immunosuppression and post-transplant antibody monitoring are suggested as techniques to manage the risk associated with antibody detected at this level. With antibody MFI above 5,000 the risk of hyperacute rejection is increased and therefore this is considered a contraindication to transplantation in all but exceptional cases. Other assays should be introduced in such cases including prospective crossmatching and/or complement fixing assays.

Clinical urgency is usually the main driver toward transplanting patients and often overrides the increased risk of AMR following an ABOi transplant. Given this, the associated risk from ABOi may be preferable to the risks of waiting for an ABO-compatible transplant. Irving et al recently published data documenting intentional ABO-incompatible transplantation of patients

with high pre-transplant ABO titres showing that the ABO barrier can be overcome in paediatric heart transplantation, and as time has passed more challenging ABOi heart transplants are now performed in older children (22).

Successful outcomes have been reported after ABOi heart or lung transplantation in adults, but there are not enough data to make recommendations in this field (23,24).

9.5 Other Organs

Transplants of the pancreas, islets and small bowel are known to be at increased risk of AMR if there are pre-formed HLA antibody barriers, but there are few data in these fields and it is not possible to make recommendations at present unless a simultaneous liver transplant is also being performed (see above) (11,25,26).

The risks of transplanting against known DSA need to be balanced against the risk to the patient of not transplanting and the future possibility of the patient receiving an alternative donor with a lower immunological risk. Bearing this in mind, the following approach is suggested in the BSHI/BTS guidance (1). No pre-transplant HLA antibody indicates a standard risk transplant. A cumulative MFI value of below 2,000 is considered to confer an additional risk of early rejection. For patients with pre-transplant DSA in the MFI range 2,000 – 8,000, the FC crossmatch is likely to be positive and the risk is considered to be intermediate. With antibody MFI above 8,000 there is a high risk the CDC crossmatch will be positive and a high risk of rejection.

9.6 Prevention, Diagnosis and Treatment of Rejection

The same therapies and drugs may be used to prevent and treat antibody mediated rejection as in chapters 6–8. The diagnosis of rejection is organ-specific and may be made either by biopsy, using organ specific diagnostic criteria, or in some cases by clinical and laboratory criteria without biopsy.

References

1. BSH/BTS Guideline for the detection and characterisation of clinically relevant antibodies in allotransplantation (3rd edition) http://www.bts.org.uk/BTS/Guidelines_Standards/Current/BTS/Guidelines_Standards/Current_Guidelines.aspx?hkey=e285ca32-5920-4613-ac08-fa9fd90915b5

2. Urschel S, Larsen IM, Kirk R, et al. ABO-incompatible heart transplantation in early childhood: an international multicenter study of clinical experiences and limits. J Heart Lung Transplant 2013; 32: 285-92.
3. Irving C, Gennery A, Kirk R. Pushing the boundaries: the current status of ABO-incompatible cardiac transplantation. J Heart Lung Transplant 2012; 31: 791-6.
4. Dipchand AI, Pollock BarZiv SM, Manlhiot C, West LJ, VanderVliet M, McCrindle BW. Equivalent outcomes for pediatric heart transplantation recipients: ABO-Blood group incompatible versus ABO-compatible. Am J Transplant 2009; 10: 389-397.
5. Roche SL, Burch M, O'Sullivan J, et al. Multicenter experience of ABO-incompatible pediatric cardiac transplantation. Am J Transplant 2008; 8: 208-15.
6. O'Leary JG, Demetris AJ, Friedman LS, et al. The role of donor-specific HLA alloantibodies in liver transplantation. Am J Transplant 2014; 14: 779-87.
7. Germani G, Theocharidou E, Adam R, et al. Liver transplantation for acute liver failure in Europe: outcomes over 20 years from the ELTR database. J Hepatol 2012; 57: 288-96.
8. Dyson JK, Carter V, Hudson M, Manas DM, Masson S. A positive complement dependent cytotoxic (CDC) crossmatch does not impact on patient survival or increase the risk of acute cellular rejection, or biliary strictures after liver transplantation. Gut 2014; 63: 186-7.
9. Alexandru I. Musat, Courtney M. et al. Pretransplant donor-specific anti-HLA antibodies as predictors of early allograft rejection in ABO-compatible liver transplantation. Transplantation 2013: 19: 1132-41.
10. Musat AI, Agni RM, Wai PY, et al. The significance of donor-specific HLA antibodies in rejection and ductopenia development in ABO compatible liver transplantation. Am J Transplant 2011; 11: 500-10.
11. Abu-Elmagd KM, Wu G, Costa G, et al. Preformed and de novo donor specific antibodies in visceral transplantation: long-term outcome with special reference to the liver. Am J Transplant 2012; 12: 3047-60.
12. Higgins RM, Bevan DJ. Antibody removal therapy in transplantation. Transplantation Reviews 1995; 9: 177-99.
13. Lowe D, Shabir S, Buckels J, et al. HLA incompatible combined liver-kidney transplantation: dynamics of antibody modulation revealed by a novel approach to HLA antibody characterisation. Transpl Immunol 2014; 30: 30-3.
14. Thorsen T, Dahlgren US, Aandahl EM, et al. Liver transplantation with deceased ABO-incompatible donors is life-saving but associated with increased risk of rejection and post-transplant complications. Transpl Int 2015; 28: 800-12.

15. Srinivas Reddy M, Wilson C, Torpey N, Manas DM. ABO incompatible liver transplantation: a case of immediate need. Transpl Int 2007; 20: 904-5.
16. Gelas T, McKiernan PJ, Kelly DA, Mayer DA, Mirza DF, Sharif K. ABO-incompatible pediatric liver transplantation in very small recipients: Birmingham's experience. Pediatr Transplant 2011; 15: 706-11.
17. Wu J, Ye S, Xu X, Xie H, Zhou L, Zheng S. Recipient outcomes after ABO-incompatible liver transplantation: a systematic review and meta-analysis. PLoS One. 2011; 6: e16521.
18. Kluger M, Guarrera JV, Olsen SK, Brown RS Jr, Emond JC, Cherqui D. Safety of blood group A2-to-O liver transplantation: an analysis of the United Network of Organ Sharing database. Transplantation 2012; 94: 526-31.
19. Eckman PM, Hanna M, Taylor DO, Starling RC, Gonzalez-Stawinski GV. Management of the sensitized adult heart transplant candidate. Clin Transplant 2010; 24: 726-34.
20. Smith JD, Danskine AJ, Laylor RM, Rose ML, Yacoub MH. The effect of panel reactive antibodies and the donor specific crossmatch on graft survival after heart and heart-lung transplantation. Transpl Immunol 1993; 1: 60-5.
21. Smith JD, Ibrahim MW, Newell H, et al. Pre-transplant donor HLA-specific antibodies: characteristics causing detrimental effects on survival after lung transplantation. J Heart Lung Transplant 2014; 33: 1074-82.
22. Irving CA, Gennery AR, Carter V, et al. ABO-incompatible cardiac transplantation in pediatric patients with high isohemagglutinin titers. J Heart Lung Transplant 2015; 34, 1095-102.
23. Bergenfeldt H, Andersson B, Bu*f*áin D, et al. Outcomes after ABO-incompatible heart transplantation in adults: a registry study. J Heart Lung Transplant. 2015 Jan 16. pii: S1053-2498.
24. Snell GI, Holmes M, Levvey BJ, et al. Lessons and insights from ABO-incompatible lung transplantation. Am J Transplant 2013; 13: 1350-3.
25. Cai J. Intestine and multivisceral transplantation in the United States: a report of 20-year national registry data (1990-2009). Clin Transpl 2009: 83-101.
26. Fan DM, Zhao QC, Wang WZ, et al. Successful ABO-incompatible living-related intestinal transplantation: a 2-year follow-up. Am J Transplant 2015; 15: 1432-5.