Received: 2 October 2023 | Accepted: 4 October 2023

DOI: 10.1111/iji.12641

INTERNATIONAL JOURNAL OF IMMUNOGENETICS WILEY

ORIGINAL ARTICLE

BSHI and BTS UK guideline on the detection of alloantibodies in solid organ (and islet) transplantation

Richard Battle[1] | Deborah Pritchard[2] | Sarah Peacock[3] | Catherine Hastie[4] | Judith Worthington[5] | Sue Jordan[6] | Jennifer A McCaughlan[7] | Martin Barnardo[8] | Rebecca Cope[3] | Claire Collins[9] | Natalia Diaz-Burlinson[5] | Carla Rosser[6] | Luke Foster[9] | Delordson Kallon[10] | Olivia Shaw[11] | David Briggs[9] | David Turner[1] | Arthi Anand[12] | Arash Akbarzad-Yousefi[13] | Deborah Sage[6]

[1]Scottish National Blood Transfusion Service, Edinburgh, UK

[2]Welsh Blood Service, Pontyclun, UK

[3]Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

[4]Gartnavel General Hospital, Glasgow, UK

[5]Manchester Royal Infirmary, Manchester, UK

[6]National Blood Service Tooting, London, UK

[7]Northern Ireland Blood Transfusion Service, Belfast, UK

[8]Oxford University Hospitals NHS Foundation Trust, Oxford, UK

[9]Birmingham Blood Donor Centre, Birmingham, UK

[10]Barts Health Pathology Service, London, UK

[11]Guy's and St Thomas' NHS Foundation Trust, London, UK

[12]Imperial College Healthcare NHS Trust, London, UK

[13]National Blood Service Newcastle-upon-Tyne, Newcastle upon Tyne, UK

Correspondence
Richard Battle, Scottish National Blood Transfusion Service, Edinburgh, United Kingdom.
Email: Richard.Battle@nhs.scot

Abstract

Solid organ transplantation represents the best (and in many cases only) treatment option for patients with end-stage organ failure. The effectiveness and functioning life of these transplants has improved each decade due to surgical and clinical advances, and accurate histocompatibility assessment. Patient exposure to alloantigen from another individual is a common occurrence and takes place through pregnancies, blood transfusions or previous transplantation. Such exposure to alloantigen's can lead to the formation of circulating alloreactive antibodies which can be deleterious to solid organ transplant outcome. The purpose of these guidelines is to update to the previous BSHI/BTS guidelines 2016 on the relevance, assessment, and management of alloantibodies within solid organ transplantation.

KEYWORDS
allograft / allogeneic transplantation, histocompatibility, HLA, immunogenetics, medicine, transplantation

Table of Contents

EXECUTIVE SUMMARY OF RECOMMENDATIONS

Executive Summary of Renal Transplantation Recommendations

Level 1 (We recommend)	
Evidence GRADE -A	**Guideline section**
Laboratories must have procedures in place for the detection and characterisation of HLA Class I and II specific antibodies. [1A]	**1**
Laboratories must be able to define HLA-A, B, C, DRB1/3/4/5, DQA1, DQB1, DPA1 and DPB1 antibody specificities. [1A]	**1**
HLA antibody detection and identification techniques must be able to detect HLA IgG antibodies [1A]	**1**
HLA antibody detection and identification techniques should be able to exclude reactivity attributable to IgM antibodies. [1A]	**1**
Laboratory crossmatch techniques must be able to detect HLA IgG antibodies [1A]	**1**
Laboratory crossmatch testing must be able to exclude reactivity attributable to IgM antibodies. [1A]	**3**
Laboratories must employ methods to abrogate known causes of false positive or negative results (e.g., the additional of EDTA) [1A]	**1**
At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. [1A]	**1**

(Continues)

Level 1 (We recommend)	
Evidence GRADE -A	**Guideline section**
HLA-specific antibodies must be characterised at regular agreed intervals prior to transplantation in sensitised patients and whenever a change in HLA antibody profile is suspected e.g., following a sensitising event or following a change in the antibody screening test results. [1A]	1
For patients on the transplant list, regular samples must be sent to the histocompatibility laboratory for antibody testing. (3 monthly is suggested) [1A]	1
The clinical team must inform the laboratory of potential sensitisation events such as previous transplantation, skin grafting, transfusion of blood products, and pregnancy (including known miscarriage). [1A]	1
Serum samples must be stored for potential use in future antibody screening and crossmatch tests. [1A]	1
HLA donor and recipient typing, HLA antibody testing and crossmatch and vXM results must be assessed, reviewed and reported by Health and Care Professions Council (HCPC) registered biomedical / clinical scientists specifically trained in histocompatibility and immunogenetics. [1A]	1; 2; 3
A patient's HLA antibody profile must be assessed to determine the acceptable risk, and delineate the antigens regarded as unacceptable. A system should be in place to monitor changes in a patient's HLA antibody profile when listed as active for a deceased donor offer[1A]	1
Prior to issuing a vXM, it is recommended that antibody screening/ specificity analysis is performed from two separate samples obtained at different time points. [1A]	2
Full donor HLA typing (HLA-A, -B, -C, -DRB1/3/4/5, -DQA1, DQB1, DPA1 and DPB1) should be available for vXM interpretation. [1A]	2
Pretransplant assessment should be undertaken by carrying out a laboratory crossmatch test or, in selected cases, by performing a virtual crossmatch [1A]	2; 3
HLA antibody data should be used in conjunction with the crossmatch results for crossmatch interpretation [1A]	3
Patients with a complex antibody profiles or incompletely defined antibody profiles should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). [1A]	2
If a prospective crossmatch is omitted, confirmation of the HLA antibody status should be assessed with samples obtained at the time of transplant. This confirmation may be performed retrospectively by Luminex HLA antibody testing, Flow Cytometry crossmatch or CDC XM. [1A]	2

(Continues)

Level 1 (We recommend)	
Evidence GRADE -A	**Guideline section**
Laboratory crossmatch tests should distinguish donor T cell and B cell populations. [1A]	3
The crossmatch report must include appropriate interpretation of the crossmatch results in the context of the patient's antibody profile. [1A]	3
Post-transplant serum samples should be taken at regular intervals on an agreed basis (in view of the perceived immunological risk at the time of transplant). [1A]	4
In higher risk transplants (e.g., donor-specific antibody is present at the time of transplant) a timetable of post-transplant sampling must be agreed with the local transplant unit. [1A]	4
Post-transplant samples should be sent to the laboratory when graft rejection is suspected or antibody production a concern (e.g., following a change in immunosuppression or compliance issues). [1A]	5
The listing of unacceptable antigens with OTDT should reflect the centres locally accepted criteria regarding acceptable risk thresholds in relation to HLA-DSA. The acceptable risk may vary between patients. E.g., crossing a HLA-DSA maybe considered appropriate in a HSP but not in a moderately sensitised patient. [1A]	1
Patient HLA antibody profiles should be reviewed at agreed intervals. This is particularly important for HSP – whereby the review of antibody profiles should include identifying delisting opportunities. Such opportunities should be discussed with the clinical team. E.g., historic HLA antibody positive, current negative, which may be an acceptable level of risk for a patient that otherwise faces long wait and poorer long term outcomes associated with dialysis. [1A]	1
Evidence GRADE -B	**Guideline section**
A combination of tests should be considered in order to fully resolve complex antibody profiles. Using different manufacturers Luminex single antigen kits maybe beneficial during this assessment. [1B]	1
The clinical team must inform the laboratory of events that might influence the HLA antibody profile and send a serum sample 14 days after the event. These include the transfusion of blood products and treatment with therapeutic antibodies. [1B]	1
In cases where a patient has a stable HLA antibody profile or are consistently HLA antibody negative a vXM may be issued – in these cases the serum sample used to determine the absence of HLA-DSA should be less than 3 months old [1B]	2

(Continues)

Evidence GRADE -B	Guideline section
We recommended that a vXM crossmatch result is reported before an organ arrives at a transplant centre. [1B]	2
We recommend that confirmation a patient has had no sensitising events since the last sample tested should be sought from the clinical team at time of reporting a vXM. Where sensitisation has occurred, we suggest that prospective antibody characterisation is undertaken using a day of transplant sample [1B]	2
Where a recipient has uncharacterized HLA-specific antibodies, or has a defined antibody but there is an incomplete donor HLA type (in relation to a recipient's antibody specificities, e.g., recipient has allele-specific antibodies), or where the intended recipient has known donor HLA-specific antibodies, a pre-transplant XM must be performed [1B]	2
Reactivity attributable to autoantibodies can be excluded in crossmatch interpretation – performing an auto crossmatch at the time of allocrossmatch should be considered. [1B]	3
Where intermediate or high-risk cases are being considered discussion between HCPC registered biomedical / clinical scientist staff who hold FRCPath and the Clinical team is advised. This discussion should include the chances of the patient receiving a lower risk offer. [1B]	3
Level 2 (We suggest)	
Evidence GRADE A	**Guideline section**
N/A	
Evidence GRADE B	**Guideline section**
A risk assessment should be performed in conjunction with the transplanting centre to ensure the risks associated with a vXM are understood and mitigated. [2B]	1; 3
We recommend that the sample date of the serum assessed for vXM should be considered in relation to the patient's sensitising events. In regrafts an updated HLA antibody test should be performed - with a sample no older than 2 weeks before the transplant date. [2B]	3
Historic HLA-DSA should be considered during vXM and Crossmatching [2B]	1; 3
The use of single antigen bead MFIs to reflect changes in the level of DSA, is semi quantitative but may be beneficial in patient monitoring and assessing therapeutic interventions [2B]	5
Epitope analysis software may benefit HLA antibody profile interpretation - donor and recipient HLA typing to the second field are recommended for this process [2B]	1; 5; 6
Evidence GRADE B	**Guideline section**
We suggest that HNA antibodies may be investigated when crossmatch results and/or clinical outcome are not consistent with HLA specific antibody screening results [2B]	3; 6
Decisions to restrict the number of mismatches for paediatric patients awaiting deceased donor transplantation should be made in conjunction with the H&I laboratory [2B]	7

(Continues)

Executive Summary of Liver Transplantation Recommendations

Level 1 (We recommend)	
Evidence GRADE -A	**Guideline section**
Prospective HLA antibody definition is not indicated prior to primary deceased donor liver transplantation [1A]	8
Laboratories must have procedures in place for the detection and characterisation of HLA Class I and II specific antibodies. [1A]	12
Laboratories must be able to define HLA-A, B, C, DR, DQA1, DQB1 and DPA1 and DPB1 antibody specificities. [1A]	12
HLA antibody detection and identification techniques should be able to detect HLA IgG antibodies [1A]	12
HLA antibody detection and identification techniques should be able to exclude reactivity attributable to IgM antibodies. [1A]	12
Laboratories must employ methods to abrogate known causes of false positive or negative results. [1A]	12
At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. [1A]	12
H&I laboratories must store time of transplant samples for liver and SLK transplants (serum and DNA from patient, ideally DNA from donor, *although for most donors HLA typing data will be available via NHSBT*) to have material to assess baseline HLA antibody levels in the event of development of potential *de novo* HLA-DSA or declining graft function [1A]	8; 9; 13
Evidence GRADE -B	**Guideline section**
For living donor liver transplantation, especially in paediatric cases, full compatibility testing (HLA antibody screening and typing) of the patient and donor should be undertaken to aid patient management [1B].	11

(Continues)

Level 2 (We Suggest)	
Evidence GRADE-B	**Guideline section**
Where time permits, prospective antibody screening assays should be performed on patients awaiting re-transplantation and, to aid interpretation, both recipient and previous donor(s) HLA types obtained [2B].	**10**
IgG HLA specific antibodies that are circulating at levels likely to cause a positive crossmatch and are directed against known previous donor HLA mismatches associated with adverse events (e.g., rejection) warrant consideration for prospective avoidance in the liver re-transplantation setting. This decision should be taken with the clinical team and the risk of avoidance (and therefore the number of organs deemed not suitable) balanced against the risk of not transplanting [2B].	**10**
Where it is deemed appropriate to avoid certain HLA antigens for liver and SLK transplantation the laboratory must have the capacity to perform virtual crossmatches 24/7 to provide an individualised compatibility assessment for a given donor and recipient pair [2B].	**12**
Retrospective testing for the presence of HLA-DSA at the time of primary liver transplantation (by crossmatch or vXM) may be of use to aid post-transplant management [2B].	**8**
Prospective HLA antibody definition should be performed in patients listed for SLK in order to assess risk at the time of offer. We suggest that this decision be taken with the clinical team and the risk of antigen avoidance (and therefore the number of organs deemed not suitable) balanced against the risk of not transplanting [2B]	**9**

Executive Summary of Pancreas Transplantation Recommendations

Level 1 (We recommend)	
Evidence GRADE -A	**Guideline section**
Laboratories must have procedures in place for the detection and characterisation of HLA Class I and II specific antibodies. [1A]	**15**
Laboratories must be able to define HLA-A, B, C, DRB1/3/4/5, DQA1, DQB1, DPA1 and DPB1 antibody specificities. [1A]	**15**
HLA antibody detection and identification techniques must be able to detect HLA IgG antibodies [1A]	**15**
HLA antibody detection and identification techniques should be able to exclude reactivity attributable to IgM antibodies. [1A]	**15**

(Continues)

Level 1 (We recommend)	
Evidence GRADE -A	**Guideline section**
Laboratory crossmatch techniques must be able to detect HLA IgG antibodies [1A]	**17**
Laboratory crossmatch testing must be able to exclude reactivity attributable to IgM antibodies. [1A]	**17**
Laboratories must employ methods to abrogate known causes of false positive or negative results (e.g., the additional of EDTA) [1A]	**15**
At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. [1A]	**15**
HLA-specific antibodies must be characterised at regular agreed intervals prior to transplantation in sensitised patients and whenever a change in HLA antibody profile is suspected e.g., following a sensitising event or following a change in the antibody screening test results. [1A]	**15**
For patients on the transplant list, regular samples must be sent to the histocompatibility laboratory for antibody testing. (3 monthly is suggested) [1A]	**15**
The clinical team must inform the laboratory of potential sensitisation events such as previous transplantation, skin grafting, transfusion of blood products, and pregnancy (including known miscarriage). [1A]	**15**
Serum samples must be stored for potential use in future antibody screening and crossmatch tests. [1A]	**15**
HLA donor and recipient typing, HLA antibody testing and crossmatch and vXM results must be assessed, reviewed and reported by Health and Care Professions Council (HCPC) registered biomedical / clinical scientists specifically trained in histocompatibility and immunogenetics. [1A]	**15; 16; 17**
A patient's HLA antibody profile must be assessed to determine the acceptable risk, and delineate the antigens regarded as unacceptable. A system should be in place to monitor changes in a patient's HLA antibody profile when listed as active for a deceased donor offer[1A]	**15**
Prior to issuing a vXM, it is recommended that antibody screening/ specificity analysis is performed from two separate samples obtained at different time points. Including a recent sample [1A]	**16**
Full donor HLA typing (HLA-A, -B, -C, -DRB1/3/4/5, -DQA1, DQB1, DPA1 and –DPB1) should be available for vXM interpretation. [1A]	**16**
Pretransplant assessment should be undertaken by carrying out a laboratory crossmatch test or, in selected cases, by performing a virtual crossmatch [1A]	**16; 17**
HLA antibody data should be used in conjunction with the crossmatch results for crossmatch interpretation [1A]	**17**

(Continues)

Level 1 (We recommend)	
Evidence GRADE -A	**Guideline section**
Patients with a complex antibody profiles or incompletely defined antibody profiles should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). [1A]	**16; 17**
If a prospective crossmatch is omitted, confirmation of the HLA antibody status should be assessed with samples obtained at the time of transplant. This confirmation may be performed retrospectively by Luminex HLA antibody testing, Flow Cytometry crossmatch or CDC XM. [1A]	**16**
Laboratory crossmatch tests should distinguish donor T cell and B cell populations. [1A]	**17**
The crossmatch report must include appropriate interpretation of the crossmatch results in the context of the patient's antibody profile. [1A]	**17**
Post-transplant serum samples should be taken at regular intervals on an agreed basis (in view of the perceived immunological risk at the time of transplant). [1A]	**18**
CDC positive XM is a contraindication to pancreas transplantation, but lower levels of HLA-DSA that lead to a positive FCXM may on occasion be transplanted across in line with local policy.[1A]	**17**
In higher risk transplants (e.g., donor-specific antibody is present at the time of transplant) a timetable of post-transplant sampling must be agreed with the local transplant unit. [1A]	**18**
Post-transplant samples should be sent to the laboratory when graft rejection is suspected or antibody production a concern (e.g., following a change in immunosuppression or compliance issues). [1A]	**18**
The listing of unacceptable antigens with OTDT should reflect the centres locally accepted criteria regarding acceptable risk thresholds in relation to HLA-DSA. The acceptable risk may vary between patients. E.g., crossing a HLA-DSA maybe considered appropriate in a HSP but not in a moderately sensitised patient. [1A]	**15**
Patient HLA antibody profiles should be reviewed at agreed intervals. This is particularly important for HSP – whereby the review of antibody profiles should include identifying delisting opportunities. Such opportunities should be discussed with the clinical team. E.g., historic HLA antibody positive, current negative, which may be an acceptable level of risk for a patient that otherwise faces long wait. [1A]	**15**
Evidence GRADE -B	**Guideline section**
A combination of tests should be considered in order to fully resolve complex antibody profiles. Using different manufacturers Luminex single antigen kits maybe beneficial during this assessment. [1B]	**15**
The clinical team must inform the laboratory of events that might influence the HLA antibody profile and send a serum sample 14 days after the event. These include the transfusion of blood products and treatment with therapeutic antibodies. [1B]	**15**
In cases where a patient has a stable HLA antibody profile or are consistently HLA antibody negative a vXM may be issued – in these cases the serum sample used to determine the absence of HLA-DSA should be less than 3 months old [1B]	**16**
We recommended that a vXM crossmatch result is reported before an organ arrives at a transplant centre. [1B]	**16**
We recommend that confirmation a patient has had no sensitising events since the last sample tested should be sought from the clinical team at time of reporting a vXM [1B]	**16**
Where a recipient has uncharacterized HLA-specific antibodies, or has a defined antibody but there is an incomplete donor HLA type (in relation to a recipient's antibody specificities, e.g., recipient has allele-specific antibodies), or where the intended recipient has known donor HLA-specific antibodies, a pre-transplant XM must be performed [1B]	**16**
Reactivity attributable to autoantibodies can be excluded in crossmatch interpretation – performing an auto crossmatch at the time of allocrossmatch should be considered. [1B]	**17**
Where intermediate or high-risk cases are being considered discussion between HCPC registered biomedical / clinical scientists who hold FRCPath and the Clinical team is advised. This discussion should include the chances of the patient receiving a lower risk offer. [1B]	**16; 17**
Level 2 (We suggest)	
Evidence GRADE A	**Guideline section**
N/A	
Evidence GRADE B	**Guideline section**
A risk assessment should be performed in conjunction with the transplanting centre to ensure the risks associated with a vXM are understood and mitigated. [2B]	**16**
We recommend that the sample date of the serum assessed for vXM should be considered in relation to the patient's sensitising events. In regrafts an updated HLA antibody test should be performed - with a sample no older than 2 weeks before the transplant date. [2B]	**16**
Post-transplant sampling for anti-HLA-DSA in immunologically low risk patients and/or patients with stable function post-transplant might be undertaken at locally defined time points, to aid in patient management (2B)	**18**

(Continues)

(Continues)

Evidence GRADE B	Guideline section
Historic HLA-DSA should be considered during vXM and Crossmatching [2B]	**16; 18**
The use of single antigen bead MFIs to reflect changes in the level of DSA, is semi quantitative but may be beneficial in patient monitoring and assessing therapeutic interventions [2B]	**18**
Epitope analysis software may benefit HLA antibody profile interpretation - donor and recipient HLA typing to the second field are recommended for this process [2B]	**15; 18**

Executive Summary of Islet Transplantation Recommendations

Level 1 (We recommend)

Evidence GRADE -A	Guideline section
Laboratories must have procedures in place for the detection and characterisation of HLA Class I and II specific antibodies. [1A]	**20**
Laboratories must be able to define HLA-A, B, C, DRB1/3/4/5, DQA1, DQB1, DPA1 and DPB1 antibody specificities. [1A]	**20**
HLA antibody detection and identification techniques must be able to detect HLA IgG antibodies [1A]	**20**
HLA antibody detection and identification techniques should be able to exclude reactivity attributable to IgM antibodies. [1A]	**20**
Laboratory crossmatch techniques must be able to detect HLA IgG antibodies [1A]	**22**
Laboratory crossmatch testing must be able to exclude reactivity attributable to IgM antibodies. [1A]	**22**
Laboratories must employ methods to abrogate known causes of false positive or negative results (e.g., the additional of EDTA) [1A]	**20**
At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. [1A]	**20**
HLA-specific antibodies must be characterised at regular agreed intervals prior to transplantation in sensitised patients and whenever a change in HLA antibody profile is suspected e.g., following a sensitising event or following a change in the antibody screening test results. [1A]	**20**
For patients on the transplant list, regular samples must be sent to the histocompatibility laboratory for antibody testing. (3 monthly is suggested) [1A]	**20**
The clinical team must inform the laboratory of potential sensitisation events such as previous transplantation, skin grafting, transfusion of blood products, and pregnancy (including known miscarriage). [1A]	**20**

(Continues)

Level 1 (We recommend)

Evidence GRADE -A	Guideline section
Serum samples must be stored for potential use in future antibody screening and crossmatch tests. [1A]	**20**
HLA donor and recipient typing, HLA antibody testing and crossmatch and vXM results must be assessed, reviewed and reported Health and Care Professions Council (HCPC) registered biomedical / clinical scientists specifically trained in histocompatibility and immunogenetics. [1A]	**20; 21; 22**
A patient's HLA antibody profile must be assessed to determine the acceptable risk, and delineate the antigens regarded as unacceptable. A system should be in place to monitor changes in a patient's HLA antibody profile when listed as active for a deceased donor offer[1A]	**20**
Prior to issuing a vXM, it is recommended that antibody screening/ specificity analysis is performed from two separate samples obtained at different time points. Including a recent sample [1A]	**21**
Full donor HLA typing (HLA-A, -B, -C, -DRB1/3/4/5, -DQA1, DQB1, DPA1 and –DPB1) should be available for vXM interpretation. [1A]	**21**
Pretransplant assessment should be undertaken by carrying out a laboratory crossmatch test or, in selected cases, by performing a virtual crossmatch [1A]	**21;22**
HLA antibody data should be used in conjunction with the crossmatch results for crossmatch interpretation [1A]	**22**
Patients with a complex antibody profiles or incompletely defined antibody profiles should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). [1A]	**21; 22**
If a prospective crossmatch is omitted, confirmation of the HLA antibody status should be assessed with samples obtained at the time of transplant. This confirmation may be performed retrospectively by Luminex HLA antibody testing, Flow Cytometry crossmatch or CDC XM. [1A]	**21**
Laboratory crossmatch tests should distinguish donor T cell and B cell populations. [1A]	**22**
The crossmatch report must include appropriate interpretation of the crossmatch results in the context of the patient's antibody profile. [1A]	**22**
Post-transplant serum samples should be taken at regular intervals on an agreed basis (in view of the perceived immunological risk at the time of transplant). [1A]	**23**
CDC positive XM is a contraindication to islet cell transplantation, but lower levels of HLA-DSA that lead to a positive FCXM may on occasion be transplanted across in line with local policy.[1A]	**22**

(Continues)

Level 1 (We recommend)	
Evidence GRADE -A	**Guideline section**
In higher risk transplants (e.g., donor-specific antibody is present at the time of transplant) a timetable of post-transplant sampling must be agreed with the local transplant unit. [1A]	**23**
Post-transplant samples should be sent to the laboratory when graft rejection is suspected or antibody production a concern (e.g., following a change in immunosuppression or compliance issues). [1A]	**23**
The listing of unacceptable antigens with OTDT should reflect the centres locally accepted criteria regarding acceptable risk thresholds in relation to HLA-DSA. The acceptable risk may vary between patients. E.g., crossing a HLA-DSA maybe considered appropriate in a HSP but not in a moderately sensitised patient. [1A]	**20**
Patient HLA antibody profiles should be reviewed at agreed intervals. This is particularly important for HSP – whereby the review of antibody profiles should include identifying delisting opportunities. Such opportunities should be discussed with the clinical team. E.g., historic HLA antibody positive, current negative, which may be an acceptable level of risk for a patient that otherwise faces long wait and poorer long term outcomes associated with dialysis. [1A]	**20**
Evidence GRADE -B	**Guideline section**
A combination of tests should be considered in order to fully resolve complex antibody profiles. Using different manufacturers Luminex single antigen kits maybe beneficial during this assessment. [1B]	**20**
The clinical team must inform the laboratory of events that might influence the HLA antibody profile and send a serum sample 14 days after the event. These include the transfusion of blood products and treatment with therapeutic antibodies. [1B]	**20**
Between first and second (or subsequent) islet transplants patients should be regularly tested for HLA antibodies and unacceptable antigens updated as appropriate. [1B]	**20**
In cases where a patient has a stable HLA antibody profile or are consistently HLA antibody negative a vXM may be issued – in these cases the serum sample used to determine the absence of HLA-DSA should be less than 3 months old [1B]	**21**
We recommended that a vXM crossmatch result is reported before islets arrive arrives at a transplant centre. [1B]	**21**
We recommend that confirmation a patient has had no sensitising events since the last sample tested should be sought from the clinical team at time of reporting a vXM [1B]	**21**
Evidence GRADE -B	**Guideline section**
Where a recipient has uncharacterized HLA-specific antibodies, or has a defined antibody but there is an incomplete donor HLA type (in relation to a recipient's antibody specificities, e.g., recipient has allele-specific antibodies), or where the intended recipient has known donor HLA-specific antibodies, a pre-transplant XM must be performed [1B]	**21**
Reactivity attributable to autoantibodies can be excluded in crossmatch interpretation – performing an auto crossmatch at the time of allocrossmatch should be considered. [1B]	**22**
Where intermediate or high-risk cases are being considered discussion between HCPC registered biomedical / clinical scientist staff who hold FRCPath and the Clinical team is advised. This discussion should include the chances of the patient receiving a lower risk offer. [1B]	**22**
Level 2 (We suggest)	
Evidence GRADE A	**Guideline section**
N/A	
Evidence GRADE B	**Guideline section**
A risk assessment should be performed in conjunction with the transplanting centre to ensure the risks associated with a vXM are understood and mitigated. [2B]	**21**
We recommend that the sample date of the serum assessed for vXM should be considered in relation to the patient's sensitising events. In regrafts an updated HLA antibody test should be performed - with a sample no older than 2 weeks before the transplant date. [2B]	**21**
Post-transplant sampling for anti-HLA-DSA in immunologically low risk patients and/or patients with stable function post-transplant might be undertaken at locally defined time points, to aid in patient management (2B)	**23**
Post-transplant HLA-DSA testing (after all infusions for a patient have been completed) should be undertaken when graft dysfunction is suspected or when immunosuppression has been reduced, although early (~3 month post final graft) testing may provide a baseline for future DSA testing (2B).	**23**
Historic HLA-DSA should be considered during vXM and Crossmatching [2B]	**21; 22**
The use of single antigen bead MFIs to reflect changes in the level of DSA, is semi quantitative but may be beneficial in patient monitoring and assessing therapeutic interventions [2B]	**23**
Epitope analysis software may benefit HLA antibody profile interpretation - donor and recipient HLA typing to the second field are recommended for this process [2B]	**20; 23**
In simultaneous islet kidney transplants management according to local policy for renal transplant testing is recommended [2B]	**25**

(Continues)

Executive Summary of Intestinal Transplantation Recommendations

Level 1 (We recommend)	
Evidence GRADE -A	**Guideline section**
Laboratories must have procedures in place for the detection and characterisation of HLA Class I and II specific antibodies. [1A]	**26**
Laboratories must be able to define HLA-A, B, C, DRB1/3/4/5, DQA1, DQB1, DPA1 and DPB1 antibody specificities. [1A]	**26**
HLA antibody detection and identification techniques must be able to detect HLA IgG antibodies [1A]	**26**
HLA antibody detection and identification techniques should be able to exclude reactivity attributable to IgM antibodies. [1A]	**26**
Laboratory crossmatch techniques must be able to detect HLA IgG antibodies [1A]	**27**
Laboratory crossmatch testing must be able to exclude reactivity attributable to IgM antibodies. [1A]	**27**
Laboratories must employ methods to abrogate known causes of false positive or negative results (e.g., the additional of EDTA) [1A]	**26**
At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. [1A]	**26**
HLA donor and recipient typing, HLA antibody testing and crossmatch and vXM results must be assessed, reviewed and reported by Health and Care Professions Council (HCPC) registered biomedical / clinical scientists specifically trained in histocompatibility and immunogenetics. [1A]	**26;27**
Prior to issuing a vXM, it is recommended that antibody screening/ specificity analysis is performed from two separate samples obtained at different time points. Including a recent sample [1A]	**27**
Full donor HLA typing (HLA-A, -B, -C, -DRB1/3/4/5, -DQA1, DQB1, DPA1 and –DPB1) should be available for vXM interpretation. [1A]	**26**
HLA antibody data should be used in conjunction with the crossmatch results for crossmatch interpretation [1A]	**27**
Patients with a complex antibody profiles or incompletely defined antibody profiles should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). [1A]	**27**
If a prospective crossmatch is omitted, confirmation of the HLA antibody status should be assessed with samples obtained at the time of transplant. This confirmation may be performed retrospectively by Luminex HLA antibody testing, Flow Cytometry crossmatch or CDC XM. [1A]	**27**

(Continues)

Level 1 (We recommend)	
Evidence GRADE -A	**Guideline section**
Laboratory crossmatch tests should distinguish donor T cell and B cell populations. [1A]	**27**
The crossmatch report must include appropriate interpretation of the crossmatch results in the context of the patient's antibody profile. [1A]	**27**
Evidence GRADE -B	**Guideline section**
We recommend that during transplant assessment patients are screened for the presence of IgG HLA-specific antibodies using blood samples obtained on at least two separate occasions. [1B]	**26**
For patients on the transplant list, regular samples must be sent to the histocompatibility laboratory for antibody testing. (3 monthly is suggested) [1A]	**26**
In cases where a patient has a stable HLA antibody profile or are consistently HLA antibody negative a vXM may be issued – in these cases the serum sample used to determine the absence of HLA-DSA should be less than 3 months old [1B]	**27**
We recommend that confirmation a patient has had no sensitising events since the last sample tested should be sought from the clinical team at time of reporting a vXM. Otherwise, we suggest that prospective antibody characterisation is undertaken using a day of transplant sample [1B]	**27**
Reactivity attributable to autoantibodies can be excluded in crossmatch interpretation – performing an auto crossmatch at the time of allocrossmatch should be considered. [1B]	**27**
We recommend that samples should be taken for antibody screening at 2 and 4 weeks following a sensitising event (e.g blood transfusion). If the patient is having ongoing transfusion support, we recommend that the laboratory agrees a pragmatic approach to testing with the clinical team [1B]	**26**
Level 2 (We suggest)	
Evidence GRADE A	**Guideline section**
N/A	
Evidence GRADE B	**Guideline section**
A risk assessment should be performed in conjunction with the transplanting centre to ensure the risks associated with a vXM are understood and mitigated. [2B]	**27**
We recommend that the sample date of the serum assessed for vXM should be considered in relation to the patient's sensitising events. In regrafts an updated HLA antibody test should be performed - with a sample no older than 2 weeks before the transplant date. [2B]	**27**

(Continues)

Evidence GRADE B	Guideline section
For patients with pre-transplant donor specific antibodies (DSA) these should be reported to the clinical team. The clinical risk of undertaking transplantation should be assessed together with the risk of delaying transplantation and the likelihood of identifying an alternative suitable donor. We suggest that the overall degree of sensitisation should be reported as % cRF to aid in this assessment. **The following stratification according to organ type should be applied**: **Bowel with other organs including liver**: the transplanted liver is resilient to all but the highest levels of donor HLA class I specific antibodies (i.e., those likely to result in a positive CDC crossmatch) and concomitant transplantation of the liver together with other organs confers a degree of protection from acute antibody mediated rejection (AMR). HLA class I antibodies should generally **not** be included in the contraindicated list of specificities however all HLA class II antibodies should be considered and discussed with the clinical team. **Bowel with other organs excluding a liver**: The risks of transplanting against a known DSA should be balanced against the risks of not transplanting and the likelihood of the patient receiving an alternative donor with a lower immunological risk. Where bowel is transplanted in the absence of a liver, antibodies against all HLA loci should be considered equally. Antibodies to different specificities may differ in pathogenicity but there are insufficient peer-reviewed studies to define the magnitude of such differences.	**27**
Which specificities to list as UA will depend on factors including but not limited to the patient's overall level of sensitisation (expressed as calculated reaction frequency (%cRF)), clinical urgency and whether a liver is included in the allograft (broadly following the stratification outlined below). [2B] **Bowel with other organs including liver**: the transplanted liver is resilient to all but the highest levels of donor HLA class I specific antibodies (i.e., those likely to result in a positive CDC crossmatch) and concomitant transplantation of the liver together with other organs confers a degree of protection from acute antibody mediated rejection (AMR). HLA class I antibodies should generally **not** be included in the contraindicated list of specificities however all HLA class II antibodies should be considered and discussed with the clinical team. **Bowel with other organs excluding a liver**: The risks of transplanting against a known DSA should be balanced against the risks of not transplanting and the likelihood of the patient receiving an alternative donor with a lower immunological risk. Where bowel is transplanted in the absence of a liver, antibodies against all HLA loci should be considered equally. Antibodies to different specificities may differ in pathogenicity but there are insufficient peer-reviewed studies to define the magnitude of such differences.	**26**

(Continues)

Evidence GRADE B	Guideline section
We suggest that, when preparing to activate a patient on the transplant waiting list, if IgG HLA specific antibodies are detected in the patient serum, the patient should be discussed with the relevant clinician responsible for patient care in conjunction with the H & I laboratory to determine if/which HLA specificities should be recorded as unacceptable antigens (UA) with NHSBT-ODT [2B]	**26**
We suggest that, in the post-transplant period, testing for donor specific antibodies is performed at regular intervals (1, 3, 6, 9 and 12 months) and when there are clinical concerns of graft function [2B]	**27**

Executive Summary of Cardiothoracic transplantation Recommendations

Level 1 (We recommend) Evidence GRADE -B	Guideline section
That two independent samples are to be tested for HLA specific antibodies before listing a patient on the transplant waiting list. Exceptions to this due to the clinical urgency of the patient should be agreed locally.[1B]	**28**
Single antigen beads should be used to determine the HLA antibody profile in the presence of a positive antibody screening result. [1B]	**28**
Laboratories must be able to define HLA-A, B, C, DRB1/3/4/5, DQA1, DQB1, DPA1 and DPB1 antibody specificities. [1B]	**28**
HLA antibody detection and identification techniques should be able to exclude reactivity attributable to IgM antibodies. [1A] [1B]	**28**
The priming source (i.e., pregnancy; blood transfusion; previous transplant; insertion of ventricular assist device (VAD) in the presence of blood products) and the magnitude and duration of the HLA specific antibody response that may then develop should be taken into account when interpreting HLA antibody screening results. [1B]	**28**
Results from single antigen beads (SAB) should be used to determine the overall degree of sensitisation which should be reported as a calculated reaction frequency (cRF%). The cRF% should ideally be reported at the different levels of immunological risk defined in this guideline: [1B] • MFI 500 – 1999 • MFI 2000 – 4999 • MFI ≥ 5,000 (please note: the MFI levels stated above were derived from analysis using OneLambda Single Antigen Bead kits – alternative vendor kits may be used and equivalence to these MFI values determined)	**28**

(Continues)

Level 1 (We recommend)	
Evidence GRADE -B	**Guideline section**
Samples should be sent from patients on the waiting list for antibody screening at regular intervals, ideally at least three monthly for all patients. [1B]	**28**
Samples should be obtained for antibody screening at 2 and 4 weeks following a potential sensitising event. Where a patient is receiving ongoing transfusion support, the timing of testing should be agreed in a local policy. [1B]	**28**
When a patient has detectable HLA specific antibodies, HLA typing of the patient should be performed to aid interpretation of results. [1B]	**28**
All individual HLA antibodies detected by CDC (and C3d or C1q solid phase assays if validated by the laboratory) or at MFI level likely to cause a positive CDCXM, should be considered as representing the highest risk for development of antibody mediated rejection (AMR). [1B]	**28**
The transplant unit must confirm that no potential sensitising event has occurred since the last sample tested for HLA specific antibodies. Otherwise, prospective testing for HLA specific antibodies is suggested with omission subject to a documented risk assessment.[1B]	**29**
A vXM in a patient sensitised to HLA should utilise the results from the most recent sample to determine immunological risk. However, consideration should also be given to historical (i.e., over 6 months old) HLA sensitisation and prior sensitising events. There should be a locally agreed policy for defining the level of immunological risk in these "peak positive, current negative" patients. [1B]	**29**
Consideration should be given to the balance of transplanting versus not transplanting a patient when performing a vXM. This is of particular importance in those patients who are sensitised to HLA and/or are exhibiting clinical deterioration and listed on urgent or super-urgent waiting lists. [1B]	**29**
The laboratory should have an agreed strategy for managing offers for patients without fully defined HLA specific antibodies. [1B]	**29**
All vXM must be assessed and reported by Health and Care Professions Council (HCPC) registered biomedical / clinical scientists specifically trained in histocompatibility and immunogenetics. Consultant H&I advice should be available at all times and must be sought when the guidance in this document is deviated from. [1B]	**29**

(Continues)

Level 1 (We recommend)	
Evidence GRADE -B	**Guideline section**
Risk levels of donor-specific antibodies should be calculated at the time of an offer. The assignment of risk should include the following considerations: [1B] • Whether the donor is homozygous at a given loci (and MFI level of DSA doubled) • Presence of supertypic antibodies such as Bw4 • Cumulative versus highest MFI of donor mismatches. • All HLA loci should be treated as equal. Levels of immunological risk should be defined according to following levels: I. No DSA detected by Luminex™ we recommend this confers standard immunological risk II. DSA at a level that corresponds to a minimum risk of HAR but an increased risk of AMR. We recommend this is MFI 500 – 1999 and confers low immunological risk III. DSA at a level that corresponds to a low risk of HAR but a significant risk of early rejection and graft damage. We recommend this is MFI 2000 – 4999 and confers an intermediate immunological risk IV. DSA at a level which corresponds to a significant risk of HAR and a veto to transplantation apart from in exceptional cases. We recommend this is an MFI > 5,000 and confers a high immunological risk. **(please note: the MFI levels stated above were derived from analysis using OneLambda Single Antigen Bead kits – alternative vendor kits may be used and equivalence to these MFI values determined)**	**29**
Centres should have a locally agreed policy with regards to performing retrospective cell based (CDCXM or FCXM) crossmatches. There is no requirement to perform a retrospective crossmatch in a recipient who is negative for HLA specific antibodies, for other recipients, omission of a retrospective crossmatch can occur if supported by local audit evidence. [1B]	**30**
Each centre should have a procedure for informing the transplant unit in the event of a positive retrospective cell based crossmatch that is attributable to HLA donor specific antibodies. [1B]	**30**
Following transplantation, patients above standard risk should be tested for HLA-specific antibodies at 7 and 28 days; 3, 6, 9 and 12 months; and then as required. More frequent testing should be agreed as part of a local policy according to level of immunological risk, other risk factors and suspicion of rejection. [1B]	**30**
HLA antibody testing should be undertaken when antibody mediated rejection is suspected and when patients present with episodes of rejection associated with haemodynamic compromise. Further testing will depend on the course of the rejection episode. [1B]	**30**

(Continues)

Level 1 (We recommend)	
Evidence GRADE -B	Guideline section
There is a lack of consensus opinion from H& laboratories in the United Kingdom and a paucity of published evidence in the literature to suggest a change in approach of either timing of HLA specific antibody screening nor categorisation of level of immunological risk in patients awaiting retransplantation. We suggest that the transplant unit and H&I laboratory have an agreed policy for this scenario.[1B]	31
The use of dd-cfDNA assays to support the diagnosis of acute rejection in cardiothoracic transplantation is in its infancy therefore, no clinical recommendation can be made.	32
The use of assays detecting non-HLA antibodies to support the diagnosis of acute rejection in cardiothoracic transplantation is in its infancy therefore no clinical recommendation can be made.[1B]	33
That offers for highly sensitised patients that are not defined as high immunological risk are given the appropriate level of consideration taking into account the likelihood of receiving another suitable offer for that patient. [1B]	34

INTRODUCTION

Background

Solid organ transplantation represents the best (and in many cases only) treatment option for patients with end-stage organ failure. The effectiveness and functioning life of these transplants has improved each decade due to surgical and clinical advances, and accurate histocompatibility assessment. Patient exposure to alloantigen from another individual is a common occurrence and takes place through pregnancies, blood transfusions or previous transplantation. Such exposure to alloantigen's can lead to the formation of circulating alloreactive antibodies which can be deleterious to solid organ transplant outcome. The purpose of these guidelines is to update to the previous BSHI/BTS guidelines 2016 on the relevance, assessment, and management of alloantibodies within solid organ transplantation.

The Need for the Guidelines

The detection and identification of alloantibodies in a potential transplant recipient is a prerequisite prior to transplant in the majority of solid organ transplant settings. Guidelines to provide recommendations of UK best practice in alloantibody detection have previously been published by the British Society of Histocompatibility and Immunogenetics and the British Transplant Society. Significant changes in practice have occurred since these guidelines were last published (for example frequent use of virtual crossmatching in highly sensitised patients across the UK).

These guidelines make recommendations concerning the detection and identification of alloantibodies in the allotransplant setting and updates the previous version published in 2014 and updated in 2016.

Process of Writing, Methodology and grading of recommendations

This guideline was produced by the following actions:

(i) A main writing committee comprising Histocompatibility and Immunogenetics (H&I) Scientists who are Fellows of the Royal College of Pathologists, was convened under the auspices of the British Society of Histocompatibility and Immunogenetics (BSHI), the British Society of Transplantation (BTS) and the Royal College of pathologists. This group comprised a chair for each solid organ transplant type included in the guidelines.

(ii) Solid organ group chairs formed a writing committee for each organ type from HCPC registered professionals working within laboratory services supporting the organ transplant type

(iii) A search of peer-reviewed literature to 01/11/2022 was undertaken.

(iv) Recommendations were produced from evidence obtained from the literature search. Due to the specialist nature of histocompatibility testing, there are few large and/or multicentre studies. Some recommendations are based on both literature review and consensus of expert opinion.

(v) The evidence collected was evaluated using a modification of the GRADE nomenclature [https://www.gradeworkinggroup.org/]. 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)

Within each level, the quality of evidence has been graded as:

A (high)
B (low)

(i) Recommendations for Kidney, Kidney and Pancreas Transplantation, Pancreas, Islet Transplantation, Thoracic Organ Transplantation, Liver transplantation, Intestinal and Multi-Visceral Transplantation are grouped separately. Each organ type has been reviewed for the following guideline recommendations, HLA antibody detection pre-transplant (inc. listing unacceptable), virtual crossmatching, crossmatching, post-transplant HLA antibody detection and non-HLA antibodies. Not all sections may be appropriate for each organ type – in such cases this is acknowledged within the test.

(ii) When compiling the guidelines each section has been structured so that it can be read independently of the other sections, with cross-referencing between sections kept to a minimum. This was undertaken so that the reader can review the recommendations and rationale for each organ type separately. As such the guidelines can be considered as separate guidelines for each organ type. However, as a consequence of this approach some duplication of rationale and recommendations is observed between the sections. Furthermore, abbreviations will be given in full the first time they are used in each section.

Contributing Authors

Dr Richard Battle, Histocompatibility and Immunogenetics, Scottish National Blood Transfusion Service, Royal Infirmary of Edinburgh, Little France Crescent, Edinburgh **(Writing committee Chair)**

Mrs Deborah Pritchard, Welsh Transplantation Laboratory, Welsh Blood Service

Dr Martin Barnardo, Transplant Immunology Laboratory, Oxford University Hospitals Trust

Mrs Catherine Hastie, Histocompatibility and Immunogenetics, Laboratory Medicine Building, Gartnavel General Hospital, Glasgow

Dr Judith Worthington, Transplantation Laboratory, Manchester Royal Infirmary, Oxford Road, Manchester

Ms Sue Jordan, Histocompatibility and Immunogenetics, NHS Blood and Transplant Tooting, London

Dr Jennifer A McCaughlan, Regional Histocompatibility and Immunogenetics laboratory, Northern Ireland Blood Transfusion Building, Belfast City Hospital

Mrs Sarah Peacock, Tissue Typing Laboratory, Cambridge University Hospitals NHS Foundation Trust, Cambridge

Miss Rebecca Cope, Tissue Typing Laboratory, Cambridge University Hospitals NHS Foundation Trust, Cambridge

Mrs Clare Collins, Histocompatibility and Immunogenetics, NHSBT Birmingham

Dr Natalia Diaz-Burlinson, Transplantation Laboratory, Manchester Royal Infirmary, Oxford Road, Manchester

Dr Carla Rosser, Histocompatibility and Immunogenetics, NHS Blood and Transplant Tooting, London

Mr Luke Foster, Histocompatibility and Immunogenetics, NHSBT Birmingham

Dr Delordson Kallon, Histocompatibility and Immunogenetics, NHS East and South East London Pathology Partnership Barts Health NHS Trust, 3rd Floor Pathology & Pharmacy Building, 80 Newark Street London

Dr Olivia Shaw, Clinical Transplantation Laboratory, F03 Borough Wing, Synnovis, Guy's Hospital, London

Dr David Briggs, Histocompatibility and Immunogenetics, NHSBT Birmingham

Dr David Turner, Histocompatibility and Immunogenetics, Scottish National Blood Transfusion Service, Royal Infirmary of Edinburgh, Little France Crescent, Edinburgh

Dr Arthi Anand, Histocompatibility and Immunogenetics laboratory, Infection & Immunity sciences, North West London Pathology, Imperial College Healthcare NHS Trust, London

Dr Arash Akbarzad-Yousefi, Histocompatibility and Immunogenetics, NHSBT Newcastle

Dr Deborah Sage, Histocompatibility and Immunogenetics, NHS Blood and Transplant Tooting, London

Clinical review

For each organ type, the relevant section was submitted to the relevant Organ and Tissue Donation and Transplantation (OTDT) advisory group meeting for clinical review. E.g. – the renal guidelines section was submitted to the ODTD kidney advisory group (KAG) for comment.

Conflicts of Interest

None. Each writing group sought declarations of interest from their membership – none were declared.

Disclaimer

These recommendations represent consensus opinion from experts in the field of H&I within the United Kingdom. They represent a snapshot of the evidence available at the time of writing. This evidence may become superseded with time. It is recognised that recommendations have been made even when the evidence is weak. The British Society for Histocompatibility and Immunogenetics (BSHI), and the British Society of Transplantation, cannot attest to the accuracy, completeness or currency of the opinions and information contained herein and does 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 this guideline or as a result of any inaccurate or misleading opinion contained in the guideline.

Renal Transplant recommendations

1 | Pre-transplant testing

1.1 | Requirement of testing pre-transplant assays

1.1.1 Laboratories must have procedures in place for the detection and characterisation of HLA Class I and II specific antibodies. [1A]

1.1.2 Laboratories must be able to define HLA-A, B, C, DRB1/3/4/5, DQA1, DQB1, DPA1 and DPB1 antibody specificities. [1A]

1.1.3 HLA antibody detection and identification techniques must be able to detect HLA IgG antibodies [1A]

1.1.4 HLA antibody detection and identification techniques must be able to exclude reactivity attributable to IgM antibodies [1A]

1.1.5 Laboratories must employ methods to abrogate known causes of false positive or negative results (e.g., the addition of EDTA). [1A]

1.1.6 At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. [1A]

1.1.7 A combination of tests should be considered in order to fully resolve complex antibody profiles. Using different manufacturers Luminex single antigen kits maybe beneficial during this assessment. [1B]

1.2 | Pretransplant HLA antibody testing sampling requirements (excluding vXM)

1.2.1 HLA-specific antibodies must be characterised at regular agreed intervals prior to transplantation in sensitised patients and whenever a change in HLA antibody profile is suspected e.g., following a sensitising event or following a change in the antibody screening test results. [1A]

1.2.2 For patients on the transplant list, regular samples must be sent to the histocompatibility laboratory for antibody testing. (3 monthly is suggested) [1A]

1.2.3 The clinical team must inform the laboratory of events that might influence the HLA antibody profile and send a serum sample 14 days after the event. These include the transfusion of blood products and treatment with therapeutic antibodies. [1B]

1.2.4 Serum samples must be stored for potential use in future antibody screening and crossmatch tests. [1A]

1.3 | Reporting / interpretation of pretransplant HLA antibody detection

1.3.1 The clinical team must inform the laboratory of potential sensitisation events such as previous transplantation, skin grafting, transfusion of blood products, and pregnancy (including known miscarriage). [1A]

1.3.2 A patient's HLA antibody profile must be assessed to determine the risk, and delineate the antigens regarded as unacceptable which should be listed as such at OTDT. A system should be in place to monitor changes in a patient's HLA antibody profile when listed as active for a deceased donor offer [1A]

1.3.3 The listing of unacceptable antigens with OTDT should reflect the centres locally accepted criteria regarding acceptable risk thresholds in relation to HLA-DSA. The acceptable risk may vary between patients. E.g., crossing a HLA-DSA maybe considered appropriate in a HSP but not in a moderately sensitised patient. [1A]

1.3.4 HLA donor and recipient typing, HLA antibody testing and crossmatch and vXM results must be assessed, reviewed and reported by Health and Care Professions Council (HCPC) registered biomedical / clinical scientists specifically trained in histocompatibility and immunogenetics. [1A]

1.3.5 Epitope analysis software may benefit HLA antibody profile interpretation - donor and recipient HLA typing to the second field are recommended for this process [2B]

1.3.6 Patient HLA antibody profiles should be reviewed at agreed intervals. This is particularly important for HSP – whereby the review of antibody profiles should include identifying delisting opportunities. Such opportunities should be discussed with the clinical team. E.g., historic HLA antibody positive, current negative, which may be an acceptable level of risk for a patient that otherwise faces long wait and poorer long term outcomes associated with dialysis. [1A]

Rationale

Since the first use of cytotoxicity testing to define HLA antibodies (Patel & Terasaki, 1969), other methods (e.g. ELISA and flow cytometry based assays) have been developed which vary in their target, configuration, sensitivity and specificity (Tait et al., 2013). However, these have now mostly been superseded by Luminex based techniques. Therefore, the writing committee focused upon Luminex based assays as the dominant HLA antibody testing method in UK laboratories.

The committee agreed that a comprehensive programme for antibody detection and characterisation is an essential component of histocompatibility laboratory support for solid organ transplantation.

The aim of a laboratory's pre transplant HLA antibody testing strategy should be to support their clinical transplantation service by:

- Identifying HLA-specific antibodies in order to assess the immunological risk associated with transplantation from a given donor.
- Prevent shipping of an organ which is deemed incompatible by listing defined HLA antibody unacceptable antigens with OTDT. (Peacock et al., 2022)
- Ensuring known/suspected false positive HLA antibody data is not listed at OTDT so that patients are not disadvantaged by denying them the opportunity of an offer inappropriately.(Battle et al., 2022; Ziemann et al., 2022)
- In sensitised patients identifying HLA-specific antibody positive sera that can be used in the pre-transplant laboratory crossmatch test (i.e., historic serum).
- Allowing a pre-transplant virtual crossmatch assessment (where appropriate). (Taylor et al., 2000, 2010; Turner et al., 2019)
- Providing data to support a clinical antibody reduction protocol.

Many laboratories use a two-tier system for HLA antibody detection; an initial rapid primary screen to determine if a sample is HLA antibody positive or negative, followed by secondary testing of positive samples to define the antibodies present. Effort can then be focused on antibody definition in positive samples. The accuracy of initial antibody testing is key as many laboratories omit the pre-transplant crossmatch

in patients negative for HLA antibodies and those with well-defined antibody profiles.

While previous versions of these guidelines have recommended laboratories determine between IgG and IgM HLA antibodies, the writing committee's interpretation of the contemporary evidence led to a recommendation that IgM reactivity was excluded, due to the lack of evidence implicating HLA IgM DSA with AMR or graft loss.

The writing committee also considered the process for determining unacceptable antigen listing with OTDT. While it is widely acknowledged that several factors can influence reactivity levels within the Luminex assay and consequently when a patient is determined as positive for a particular HLA antibody (e.g., MFI levels being influenced by shared epitopes/large assay coefficient of variation/ high background reactivity etc), the writing committee also noted centres different acceptable thresholds for risk in terms of crossing HLA-DSA. This variation makes a universal recommendation for listing unacceptable antigens problematic. As such the writing committee recommend that the listing of unacceptable antigens with OTDT should reflect the centres locally accepted criteria regarding acceptable risk thresholds in relation to HLA-DSA. This acceptable risk may vary between patients. E.g., crossing a HLA-DSA maybe considered appropriate in a HSP but not in a moderately sensitised patient. In addition, the writing committee did not feel that listing a repeat mismatch as unacceptable was justified in the absence of a detected HLA-DSA. Indeed, recent evidence demonstrates that repeat mismatches were not associated with a risk of de novo DSA development, rejection or allograft loss (Lucisano et al., 2020).

The presence of inhibitory factors which negatively impacts the accurate determination of HLA antibody by SAB assays, have been widely reported in the literature (Tambur & Schinstock, 2022). Such inhibitory factors reported include presence of IgM antibodies (Kosmoliaptsis et al., 2009), high concentration HLA IgG (Tambur & Schinstock, 2022) and complement components (Schnaidt et al., 2011; Schwaiger et al., 2014; Weinstock & Schnaidt, 2013), the result of these reported inhibitions being a negative result or falsely low MFI readout. Although the term 'prozone' is often used to describe these inhibitions within the literature, this is an erroneous use of the term in this setting, and its use often causes confusion (Tambur & Schinstock, 2022). Prozone refers to agglutination / precipitation assays to describe a zone in which reactions do not happen. While the true cause of inhibition within SAB assays is most likely multifactorial, several studies have assessed mechanisms to remove assay inhibition and demonstrated that the most frequent cause of assay inhibition is due to complement components (Schwaiger et al., 2014; Weinstock & Schnaidt, 2013). Consequently the additional of EDTA into test serum is commonly used within H&I labs to present this inhibition (Schnaidt et al., 2011). While the writing committee agrees that this is the most effective mechanism of removing assay inhibition, several studies have shown dilutions may continue to be of benefit. Tambur and colleagues (Tambur & Schinstock, 2022) demonstrate that falsely low MFI values can still be observed in some sera treated with EDTA. Most recently the use of dilutions has been suggested to assist in the investigations of HSP sera, particularly where CDC XM assay may not be available (Daga & Briggs, 2023).

Some centres also incorporate the use of Luminex assays which detect either complement binding (C1q) or activation (C3d) HLA IgG. The writing committee felt the literature on the use of these assays was mixed. While several studies suggest an association with complement binding DSA detection in both the preformed and de novo settings (Lan & Tinckam, 2018), further literature demonstrates that this is strongly associated with high MFI levels/ antibody titres. Indeed low titre antibodies have been shown to be negative for complement binding but become positive when titres rise (Tambur et al., 2015; Tambur & Wiebe, 2018). As such careful interpretation of standard SAB assays appears to be able to generate the same information. Given the varied nature of the literature the writing committee did not feel the evidence supported a recommendation regarding the inclusion of SAB assays which identify complement fixing/binding detection within routine testing.

2 | Virtual crossmatching recommendations

2.1 | Assessing eligibility for a vXM

2.1.1 A risk assessment should be performed in conjunction with the transplanting centre to ensure the risks associated with a vXM are understood and mitigated. [2B]

2.1.2 Prior to issuing a vXM, it is recommended that antibody screening/ specificity analysis is performed from two separate samples obtained at different time points. [1A]

2.1.3 In cases where a patient has a stable HLA antibody profile or are consistently HLA antibody negative a vXM may be issued – in these cases the serum sample used to determine the absence of HLA-DSA should be less than 3 months old [1B]

2.1.4 Full donor HLA typing (HLA-A, -B, -C, -DRB1/3/4/5, -DQA1, DQB1, DPA1 and –DPB1) should be available for vXM interpretation. [1A]

2.1.5 Where a recipient has uncharacterized HLA-specific antibodies, or has a defined antibody but there is an incomplete donor HLA type (in relation to a recipient's antibody specificities, e.g., recipient has allele-specific antibodies), or where the intended recipient has known donor HLA-specific antibodies, a pre-transplant crossmatch must be performed [1B]

2.1.6 HLA donor and recipient typing, HLA antibody testing and crossmatch and vXM results must be assessed, reviewed and reported by Health and Care Professions Council (HCPC) registered biomedical / clinical scientists specifically trained in histocompatibility and immunogenetics. [1A]

2.1.7 Patients with complex antibody profiles or incompletely defined antibody profiles should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). [1A]

2.1.8 Historic HLA-DSA should be considered during vXM and Crossmatching [2B]

2.1.9 We recommend that the sample date of the serum assessed for vXM should be considered in relation to the patient's sensitising events. In regrafts an updated HLA antibody test should be

performed - with a sample no older than 2 weeks before the transplant date. [2B]

2.2 | Reporting of VXM

2.2.1 We recommended that a vXM crossmatch result is reported before an organ arrives at a transplant centre. [1B]

2.2.2 We recommend that confirmation a patient has had no sensitising events since the last sample tested should be sought from the clinical team at time of reporting a vXM. Where sensitisation has occurred, we suggest that prospective antibody characterisation is undertaken using a day of transplant sample [1B]

2.3 | Post - transplant testing when proceeding with vXM

2.3.1 If a prospective crossmatch is omitted, confirmation of the HLA antibody status should be assessed with samples obtained at the time of transplant. This confirmation may be performed retrospectively by Luminex HLA antibody testing, Flow Cytometry crossmatch or CDC XM. [1A]

Rationale

In deceased donor transplantation, the laboratory crossmatch was historically performed once the donor kidney, spleen and lymph nodes arrived in the transplanting centre which potentially delayed the transplant operation. This approach has been shown to prolong the cold ischaemic time (CIT) which is detrimental to graft outcomes, particularly for DCD and extended criteria organs where a CIT of less than 12h is a recognized determinant of outcome (Aubert et al., 2015; Summers et al., 2010). An alternate approach is to assess HLA antibody compatibility by predicting crossmatch results using a recipient's HLA-specific antibody data and donor HLA typing data. This virtual crossmatch (vXM) approach has been widely reported, (Taylor et al., 2010; Turner et al., 2019) and in most centres in the UK the majority of deceased donor pretransplant assessment is achieved via vXM. The use of vXM has been shown to reduce the aforementioned CIT and associated detrimental impact upon transplant outcome.(Rohan et al., 2020; Taylor et al., 2010; Turner et al., 2019).

In specific circumstances, vXM is safe and effective in allowing transplantation while minimising CIT.(Taylor et al., 2010) It is important to note that to maximise this impact upon CIT a vXM should ideally be reported prior to an organ arriving at a centre. There are risks associated with virtual crossmatching including the possibility of a donor mistype when performing a virtual crossmatch in a sensitised patient. In the UK, the donor mistype rate is consistently <1% and rarely impacts on allocation (Peacock et al., 2022). Consequently, the writing committee recommends that a risk assessment should be performed in conjunction with the transplanting centre to ensure the risks associated with a vXM are understood and mitigated. Inherent risks include changes to a HLA antibody profile since the last tested patient sample. Consequently, the committee's interpretation of the evidence for recommending the timing of samples led to two recommendations. Firstly, before a vXM is issued a patient should have been tested using a minimum of two samples collected at separate time points; secondly where a patient is negative or has a consistent HLA antibody profile a sample within 3 months of the transplant date can be used to assess vXM suitability. However, where a sensitisation event has occurred since the last test, or when the patient has had a previous transplant, an updated sample should be tested for HLA antibodies prior to transplant, or a prospective crossmatch should be performed to ensure no HLA antibodies have developed since the last test. The committee also recommended that historic HLA-DSA should also be considered during vXM assessment; while the presence of historic DSA in the absence of current DSA (DSA at time of transplant) may not be a contraindication to transplant the patient may be at increased risk of AMR (Rennie et al., 2022). This should be identified and communicated to the clinical team.

Clear communication with the clinical team is essential to ensure a vXM can be safely performed by ensuring no sensitising events since the last sample tested.

It is now recognised that alloantibodies can be stimulated by all the classical, polymorphic HLA proteins (HLA-A, -B, -C, DRB1/3/4/5, -DQA1, DQB1, -DPA1, DPB1), (Duquesnoy, Marrari, Mulder, et al., 2014; Duquesnoy, Marrari, Tambur, et al., 2014), this requirement aligns with the UK donor characterisation and commissioning service specification, document SPN1439/3). (NHSBT ODT 2022 SPN1439/3). The committee agreed that for safe vXM HLA typing at all the classical loci were required. If donor typing to this degree is not available, as a minimum HLA typing for HLA loci corresponding to those represented in the recipient's antibody profile must be available. Additionally, sharing of the raw HLA typing data between centres may aid the vXM process, as this information may enable the presence or absence of specific HLA alleles in the donor HLA type.

In order to assess the safety of proceeding with a vXM a comprehensive knowledge of the HLA system is required and should encompass a working knowledge of HLA linkage disequilibrium, rare alleles and HLA epitopes, consequently a suitably qualified and competent HCPC registered member of staff should be responsible for performing a vXM.

3 | Crossmatching recommendations

3.1 | Requirements of crossmatching assays

3.1.1 Laboratory crossmatch tests must distinguish donor T cell and B cell populations. [1A]

3.1.2 Laboratory crossmatch techniques must be able to detect HLA IgG antibodies [1A]

3.1.3 Laboratory crossmatch testing must be able to exclude reactivity attributable to IgM antibodies. [1A]

3.1.4 Historic HLA-DSA should be considered during vXM and Crossmatching [2B]

3.2 | Reporting and Interpretation of crossmatching assays

3.2.1 Pretransplant assessment should be undertaken by carrying out a laboratory crossmatch test or, in selected cases, by performing a virtual crossmatch [1A]

3.2.2 Reactivity attributable to autoantibodies can be excluded in crossmatch interpretation – performing an auto crossmatch at the time of allocrossmatch should be considered. [1B]

3.2.3 HLA antibody data should be used in conjunction with the crossmatch results for crossmatch interpretation [1A]

3.2.4 HLA donor and recipient typing, HLA antibody testing and crossmatch and vXM results must be assessed, reviewed and reported by Health and Care Professions Council (HCPC) registered biomedical / clinical scientists specifically trained in histocompatibility and immunogenetics. [1A]

3.2.5 The crossmatch report must include appropriate interpretation of the crossmatch results in the context of the patient's antibody profile. [1A]

3.2.6 Where intermediate or high-risk cases are being considered discussion between HCPC registered biomedical / clinical scientist staff who hold FRCPath and the Clinical team is advised. This discussion should include the chances of the patient receiving a lower risk offer. [1B]

3.2.7 We suggest that HNA antibodies may be investigated when crossmatch results and/or clinical outcome are not consistent with HLA specific antibody screening results [2B]

Rationale

Despite the widespread use of vXM within the UK, prospective laboratory crossmatching remains a pretransplant risk assessment tool, which can provide an important risk stratification (Bestard et al., 2021, 2022; Orandi et al., 2014), and assist in identifying reactivity towards denatured HLA which is thought to be clinically irrelevant (Visentin et al., 2014).

In general, a positive crossmatch in the presence of a donor specific HLA IgG antibody is considered a barrier to transplantation, except where additional treatment is planned, such as in the case of HLA incompatible transplantation.

Historically a complement-dependent-cytotoxic (CDC) crossmatch was used to identify donor specific HLA antibodies and inform risk of antibody mediated rejection, with a positive CDC XM due to HLA IgG being considered a contraindication to transplantation (Patel & Terasaki, 1969). Advances in HLA antibody detection techniques for the pretransplant detection of donor specific HLA IgG antibodies have enabled a degree of stratification of the immunological risk associated with a kidney transplant (Orandi et al). In general CDC positive crossmatches are thought to represent the highest risk threshold when attributable to HLA-IgG DSA (Bentall et al., 2013; Motter et al., 2021; Orandi et al., 2014) and are associated with inferior outcomes. Strong evidence also demonstrates flow cytometry crossmatch positive reactivity identifies higher risk transplants, when attributable to HLA-IgG DSA. FCXM is more sensitive than CDC and is able to detect non-complement fixing IgG subclasses (Bentall et al., 2013; Bestard et al., 2021; Roelen et al., 2012), however the association with positive FCXM and poorer outcome is dependent upon the presence of HLA-IgG DSA being present in the recipient serum at the time of crossmatch. In the absence of HLA-DSA being detected by Luminex single antigen bead assays a positive FCXM is not predictive of rejection (Bestard et al., 2021; Couzi et al., 2011; Eng et al., 2008). The incidence of B cell FCXM positivity in the absence of HLA-DSA is a frequent occurrence and has been observed in >60% of B cell positive FCXM in some reports (Eng et al., 2008).

Consequently, the committee recommended that crossmatch results should be interpreted in conjunction with Luminex based HLA antibody testing data, to aid in this risk assessment and ensure patients with a positive B cell crossmatch in the absence HLA-DSA are not disadvantaged.

T and B cell flow crossmatch positive results due to the presence of HNA3a antibodies have been reported at several centres in the UK (Day et al., 2014; Key et al., 2018, 2019) which are associated with detrimental outcomes. However, the literature is limited due to a small number of case reports. It is recommended that any T and B cell positive crossmatches in the absence of HLA-DSA are investigated, and any reactivity thought to be due to HNA3a is reported to the clinical team. The committee could not identify any evidence suggesting FCXM could detect any other antibody implicated in renal transplant outcome. Consequently, FCXM positivity in the absence of HLA or HNA3a antibodies should be considered as a standard risk transplant.

4 | Risk Stratification

4.1.1 Post-transplant serum samples should be taken at regular intervals on an agreed basis (in view of the perceived immunological risk at the time of transplant). [1A]

4.1.2 In higher risk transplants (e.g., donor-specific antibody is present at the time of transplant) a timetable of post-transplant sampling must be agreed with the local transplant unit. [1A]

Assigning universal risk categories is challenging, not least because of the variation in UK immunosuppression strategies (as in the rationale). To this end 4 broad risk categories have been defined. Veto, High, Intermediate and Standard. The criteria for inclusion are given below, with the assays and results available to stratify patients into these criteria are given in Table 4.1 and Table 4.2.

1) Veto -:

- High levels of circulating HLA-IgG antibodies specific for mismatched donor HLA present at the time of transplantation which

TABLE 4.1 Risk stratification based on CDC crossmatching results and HLA antibody Luminex testing.

CDC Crossmatch result	Current or historic DSA tested	Luminex HLA IgG antibody testing	Immunological risk
Positive B±T [1] (with DTT)	C	HLA DSA positive	Veto
Positive B±T [1] (with DTT)	H (Current Negative)	HLA DSA positive	High
Negative T & B (with DTT)	C	HLA DSA positive	CDC unable to stratify the risk - to be determined by Luminex &/or flow crossmatch results
Positive T &/or B [3] (without DTT)	C or H	No HLA DSA	Standard
Positive T &/or B [4] (with DTT)	C or H	No HLA DSA	Standard
Negative T & B	C or H	Negative	Standard

TABLE 4.2 Risk stratification based on Flow cytometry crossmatching results and HLA antibody Luminex testing.

Flow Crossmatch result	Current or historic DSA tested	Luminex HLA IgG antibody testing	Immunological risk
Strong Positive B±T	C	HLA DSA positive	High
Strong Positive B±T	H (Current Negative)	HLA DSA positive	High
Positive B±T	C	HLA DSA positive	High
Positive B±T	H (Current Negative)	HLA DSA positive	Intermediate
Negative B±T	C	HLA DSA positive [2]	Intermediate
Positive T &/or B [4]	C or H	No HLA DSA	Standard
Negative T & B	C or H	No HLA DSA	Standard

1. Significant risk of hyperacute rejection due to high titre HLA donor specific antibodies
2. Careful interpretation of Luminex only HLA DSA is required to exclude false positive reactivity
3. Suggestive of IgM reactivity
4. Unexpected T and B cell positive results should be investigated for the presence of HNA3a.

N.B. The additional risk associated with ABO incompatibility should also be considered.

produce a CDC positive XM (Some centres may have a threshold of 'Strong' FCXM positive which they consider a veto).

- The high risk of hyperacute rejection would constitute a veto to transplantation in all but exceptional circumstances.
- Alternative donor sources should be investigated; in cases of living donor transplantation this includes consideration of entering the incompatible donor and recipient into the UK National Kidney Sharing Schemes (UKNKSS).

2) **High immunological risk**:

- High levels of circulating antibodies for mismatches donor HLA present at the time of transplantation
- FCXM positive crossmatch due to HLA-IgG DSA
- The high risk of hyperacute rejection would normally constitute a veto to transplantation
- It may be justifiable to use pre-transplant desensitisation regimens to ameliorate this risk in some circumstances
- Alternative donor sources should be investigated, in cases of living donor transplantation this includes consideration of entering the incompatible donor and recipient into UK National Kidney Sharing Schemes (UKNKSS)

3) **Intermediate immunological risk**:

- Current FCXM negative
- Low level DSA present at the time of transplantation
- Historic DSA not detectable at the time of transplantation
- Patient at increased risk of humoral anamnestic response and AMR
- Consider augmented immunosuppression / alternative donor source, in cases of living donor transplantation this includes consideration of entering the incompatible donor and recipient into UK National Kidney Sharing Schemes (UKNKSS)

4) Standard immunological risk:

- The absence of current and/or historic HLA-DSA
- Patient has the lowest risk threshold for AMR

- However, the patient may be at risk of a memory response if they have received a sensitising event

Rationale

The aims of these guidelines are to enable risk stratification based upon the testing assays currently available to histocompatibility and Immunogenetics laboratories supporting renal transplantation. The acceptable levels of immunological risk differ between patients. This variation can be influenced by factors such as the patient's sensitisation level, wait time, window for transplantation and chances of receiving a better offer. As such, a clinical team may consider an intermediate risk offer for a highly sensitised patient with limited access to potential donors a reasonable level of risk for the patient and proceed to transplant with risk mitigation strategies such as augmented immunosuppression. Conversely the same risk level in a moderately sensitised patient who has numerous potential donors may be considered unsuitable to proceed to transplant – as the patient is likely to receive a standard risk transplant.

In order to help determine an appropriate level of risk for an individual patient, risk communication tools are available. For the H&I professional, input into clinical discussions around transplant risk stratification includes not only crossmatch and HLA antibody data interpretation but also the likelihood of a patient receiving a lower immunological risk transplant.

The Risk communication tools – ODT Clinical – NHS Blood and Transplant created by the NHSBT ODT statistical team allows patient characteristics to be entered and displays information on patients who have characteristics similar to these. Consequently, an estimate of wait time can be made. A more nuanced assessment can be made using the kidney calculated reaction frequency tool, Calculators – ODT Clinical – NHS Blood and Transplant. This enables the user to enter a defined HLA type and antibody specificity profile for a patient and provides a calculation of the number of donors deemed compatible from a pool of 10,000 UK donor HLA types. This can be particularly useful when determining how likely a patient is to receive a compatible organ. However, care should be taken when using these tools. In particular, the ability to define HLA antibody has reached a resolution that is not matched by the resolution of the UK register for defined unacceptable HLA antigens.

The numbers of HLA incompatible transplants have fallen in the UK in recent years with 11 HLAi transplants performed between April 2021 and March 2022 (Statistics and Clinical Research, 2022). The increased immunological risk associated with such transplants is not suitable for all patients and careful consideration of the appropriate level of risk deemed acceptable should be taken on an individual patient basis and involve a multidisciplinary team. While there is currently no recent UK data available to support a cRF cut-off in deciding if a high-risk transplant is appropriate for a potential transplant recipient, analysis of the kidney allocation scheme in the US following changes to benefit highly sensitised patients suggests that patients with a cRF of ≥99.9% may benefit from a higher risk transplant (Schinstock et al., 2019). While this data provides a good indicator of suitability for HLAi transplants in the US, similar data from a UK cohort would be valuable.

In cases where pretransplant FCXM is negative, but HLA-DSA is detected by Luminex SAB assays the associated risk is less clear. The literature associated with such transplants is mixed, with some studies suggesting HLA-DSA detected by Luminex only is not associated with an increased risk of AMR (Couzi et al., 2011). However, a meta-analysis of rejection rates and graft outcomes examining seven retrospective studies and including 1119 patients concluded AMR rates were significantly higher in Luminex only DSA groups in comparison to DSA negative patients (Mohan et al., 2012). This finding was seen even though only two of the included studies reached statistical significance as individual studies, a finding most likely related to the small number of patients included in some studies. Furthermore a significant decrease in allograft survival was observed by Mohan and colleagues when Luminex only HLA-DSA was detected pretransplant (Mohan et al., 2012). A study of 660 transplants performed at a single centre with a negative FCXM crossmatch but with HLA-DSA detected by Luminex and with no desensitisation therapy identified no increase in AMR when results were analysed in aggregate, however, an impact on AMR was observed when DSA ≥3000 MFI was assessed but this didn't translate into inferior graft survival in the intermediate term (Adebiyi et al., 2016). An assessment of MFI levels in Luminex only HLA-DSA transplants has also been undertaken. In a tightly controlled study examining DSA ranging from 800–3000 MFI in a cohort of 1318 patients, an increased risk of AMR was detected when class I and II HLA DSA were present (Morrison et al., 2019), the authors of this study also looked at post-transplant increases in MFI levels and found that increases >3000 MFI were associated with an increase in acute rejection. In an attempt to define which Luminex only HLA-DSA were associated with an increased risk, a pre– and post-transplant assessment of HLA-DSA was performed in 924 patients which found DSA resolved in 52.3% of cases. In the 47.7% of cases where DSA remained graft failure and increased incidence of AMR was observed (Senev et al., 2019). The resolving DSA was most often HLA class I. The impact of Luminex only DSA has also previously been postulated to vary between loci typically thought to have high expression on cells in comparison to those thought to be lower expressed, such as HLA-DP. Most recently a study examining transplants which crossed an isolated HLA-DP DSA, with a mean follow up of 1197 days, demonstrated that a pre-existing HLA-DP DSA was a significant risk factor for AMR. Furthermore they demonstrated that flow crossmatch positivity in these cases did not help inform on the risk of graft failure or AMR (Seitz et al., 2022). Thus, indicating a clear risk for pretransplant HLA-DP DSA regardless of if they are Luminex only, or capable of producing crossmatch positivity.

Members of the writing committee agreed that the presence of a Luminex only HLA-DSA indicated an increase level of risk in the literature when compared to when no HLA DSA was present. However, the writing committee acknowledged that a challenge exists in providing recommendations guiding interpretation of the risk associated

with the presence of pretransplant HLA-DSA when the FCXM is negative. This challenge is associated with the varying practice across the UK, and in the literature used to support this practice. The UK renal transplant centres do not share common immunosuppression thresholds for when augmented immunosuppression should be considered in higher risk cases and practice varies between centres. ATG, for example being indicated in some UK centres when a cRF value reaches a specific threshold despite a lack of HLA-DSA, when Luminex only HLA-DSA is detected in others, and in some centres when the transplant is a regraft. These differences are also observed in the literature (Mohan et al., 2012), which can make interpretation of the literature and data comparison between centres challenging. Furthermore, variation is observed in HLA antibody detection techniques. There is no standard positive cut-off threshold associated with a FCXM for example, testing laboratories validate their own thresholds against local data. This is also true of MFI cut-offs used in Luminex HLA antibody testing. While attempts have been made to standardise Luminex assays to provide cut-offs which can be applied universally between centres, this practice is not recommended due to significant assay coefficients of variation (% CV). Indeed, a comparison of SAB results from 7 centres (Reed et al., 2013) using the same SAB reagents lots demonstrated that while the centres detected the same HLA specificities the variation in the MFI levels were high (CV = 62%). When attempts to standardise the assays were made through universal protocols and reagent lots this was reduced (CV = 25%) but remained too high for standardisation of MFI cut-offs. Additionally, centres may also use different manufacturers Luminex SAB assay kits. These kits have been demonstrated to detect denatured HLA at different frequencies (Jucaud et al., 2017; J.-H. Lee, 2019), and such denatured HLA has been demonstrated to be non-clinically relevant (Cai et al., 2009; Visentin et al., 2014, 2015). Exclusion of denatured HLA reactivity in patients HLA antibody profiles is challenging (Battle et al., 2020; Visentin et al., 2014). This variation and complexity suggest different centres may detect non clinically relevant denatured HLA at different frequencies depending upon manufactures kit used, which again impacts upon a comparison of the impact of Luminex only HLA-DSA between centres. Furthermore, the presence of reactivity towards denatured HLA detected by the Luminex single antigen bead assays to some degree confounds the assessment of reactivity towards HLA-DSA within the literature; there is only limited data available which seeks to exclude reactivity towards denatured HLA when assessing the impact of Luminex only HLA-DSA on allograft outcome. Where this has been performed reactivity on HLA-DSA detected on SAB which was due to denatured HLA was not detrimental to outcome, only the reactivity identified to be against native HLA were associated with inferior outcome (Visentin et al., 2015). Consequently, when analysing SAB data the likelihood a Luminex only HLA-DSA is directed towards native conformation HLA rather than denatured HLA should be considered by reviewing sensitising events, epitope grouping within SAB data, reactivity against self, comparing results from different time points and using different manufactures kits / allele panels (Tambur et al., 2018).

There are many nuances to immunological risk assessment with factors such as priming sensitising event type and complement fixation (amongst others) being suggested to influence risk. Most recently the ENGAGE guidelines have ascribed risk in broad terms (Bestard et al., 2021), including risk stratification not only for patients who have defined HLA DSA detected, either by CDC XM, FCXM or Luminex only, but for memory responses when no HLA DSA is detected. These guidelines identify a risk of a memory response when the patient has had a sensitising event when no DSA has been detected. The STAR working group (Tambur et al., 2018) have also previously described a latent potential for an alloimmune memory response when a patient has a history of a sensitising event. However, a further report by the same working group (Tambur et al., 2020) identifies a need to develop the assays used to characterise such memory responses and design studies to assess the predictive value of such antibodies on transplant outcome. The reports which are available in the literature have identified antibody production towards paternal antigens being restimulated via a non-specific stimulus (Billen et al., 2009). While the assays used to detect humoral memory responses have been shown to detect humoral memory directed towards mismatched paternal antigens in patients whose sera was HLA antibody negative (Karahan et al., 2021), the only clinical data available assessing memory B cells using these assays examines patients with known HLA-DSA rather than those who are negative at time of transplant ((Wehmeier, Karahan, et al., 2020)). Further work is therefore required to assess this risk further.

While the writing committee acknowledge that while ABO incompatibility (ABOi) has historically been considered an absolute contraindication to transplantation, this is now no longer the case, as desensitisation techniques enable this barrier to be crossed in some selected cases. The British Transplantation Society has previously produced guidance on antibody incompatible transplantation (https://bts.org.uk/wpcontent/uploads/2016/09/02_BTS_Antibody_Guidelines-1.pdf) which outline the strategies, risks, rejection treatment options and outcomes for ABOi - consequently the writing committee did not produce guidance within this document.

5 | Post-transplant antibody detection

5.1.1 Post-transplant samples should be sent to the laboratory when graft rejection is suspected or antibody production a concern (e.g., following a change in immunosuppression or compliance issues). [1A]

5.1.2 The use of single antigen bead MFIs to reflect changes in the level of DSA, is semi quantitative but may be beneficial in patient monitoring and assessing therapeutic interventions [2B]

5.1.3 Epitope analysis software may benefit HLA antibody profile interpretation – donor and recipient HLA typing to the second field are recommended for this process [2B]

Rationale

The development of donor HLA specific antibodies following kidney transplantation has been implicated in acute and chronic antibody

mediated rejection and poor graft outcome (R. Zhang, 2018). Circulating *de novo* DSA (dnDSA) are considered bio markers for antibody mediated rejection, the early detection of which enables early therapeutic intervention and increased chance of prolonged graft survival.

dnDSAs have long been implicated as risk factors of antibody mediated injury and allograft failure. In order to identify these potentially deleterious antibodies post-transplant it is important to specify reactivity against mismatched donor antigens. The presence of these antibodies is routinely confirmed using Luminex® Single Antigen Beads (SABs); whereas once the flow cytometric crossmatch would have been repeated, the need for viable donor lymphocytes and the persistence of therapeutic antibodies has favoured the solid phase assay.

The proportion of previously antibody negative patients developing *dn*DSA post-transplant is reportedly up to 30% within 10 years from transplantation, while 40% of patients are estimated to lose their grafts within 5 years of developing *dn*DSA (Tambur et al., 2021). The time taken to detection of HLA antibody varies considerably (Lionaki et al., 2013).

The high number of shared epitopes within HLA antigens mean that *dn*DSA have the potential to cross react with antigens in addition to those expressed by the allograft. Patients losing their graft due to *dn*DSA are likely to develop humoral alloreactivity broader than the original immunising donor antigen; such increases in sensitisation will impact directly on the likelihood of future transplants (Duquesnoy, 2011).

The prevalence of HLA Class II *dn*DSA is approximately three times that of HLA Class I (Bouatou et al., 2018). The role of DSA directed against HLA-A, HLA-B and HLA-DR is well understood and reflected in our current national allocation scheme. Despite this, HLA-DQ are the most common *dn*DSA detected and known to have a deleterious effect on graft survival and an increased risk of graft loss. This higher prevalence is attributed to fact that the HLA-DQ molecule is comprised of two chains, DQα_1, DQβ_1 and the particularly polymorphic nature of the DQβ_1 chain (Chowdhry et al., 2019). Between 54% and 77% of *dn*DSA are due to antibodies directed against HLA-DQ (DeVos et al., 2012; Willicombe et al., 2012). Unique to these DSAs is their strength, persistence, and apparent resistance to treatment. The greater the degree of HLA-DQ mismatch the greater the associated risk of rejection; the concept of eplet mismatch and the impact of eplet load on *dn*DSA development is not a new one but the effect is most clearly demonstrated by the HLA-DQ loci (Senev et al., 2020). Whether the influence of epitope load is a cumulative one or, rather, an increased likelihood that a more highly immunogenic eplet is amongst the mismatches is not yet known. Current evidence identifies some HLA-DQB1 and DQA1 combinations as being more immunogenic than others (McCaughan et al., 2018), however further exploration of this concept is required.

The prevalence of *dn*DSA directed against either HLA-Cw or HLA-DP is unclear. As a result of lower levels of expression on renal endothelial cells, HLA-Cw and HLA-DP were assumed to be less immunogenic, however the contribution of these DSA, in particular HLA-DP DSA on both graft outcome and rejection risk is increasingly recognised (Bachelet et al., 2016).

The writing committee agreed that post-transplant samples should be taken from transplant recipients at regular intervals on an agreed basis and in view of perceived immunological risk, when AMR is suspected or in cases of delayed graft function. Serum samples may be tested at the time of receipt in those patients where there is cause for concern or frozen for testing at a later date if there is no ongoing clinical concern. For those patients that have undergone transplants associated with intermediate or higher degrees of immunological risk e.g., HLA incompatible, more intense monitoring is necessary. The risk of rejection is greatest during the first two weeks' post-transplant so the frequency of testing must reflect this; exact regimens will depend on both the clinical situation of the patient and local policies agreed with the Renal Unit. Even in those patients considered as low risk with no evidence of DSA at the time of transplant it is recommended that DSA testing be performed when antibody production is a concern (following a change in immunosuppression or compliance issues) (Wiebe et al., 2012).

Testing of these samples at the time of graft loss will assist in defining those antigens that should be listed as unacceptable for future transplants.

6 | Non-HLA antibodies

6.1.1 We suggest that HNA antibodies may be investigated when crossmatch results and/or clinical outcome are not consistent with HLA specific antibody screening results [2B]

Rationale

Non-HLA antibodies develop either to donor epitopes of polymorphic antigens not present in the recipient, or to epitopes of self-antigens that become exposed on the cell surface in response to various mechanisms such as apoptosis or ischaemic damage. It is thought that there may be many unidentified non-HLA antigens that could cause allograft rejection. However, without identification of the target antigen, antibody specificity screening is practically impossible (Li et al., 2009). Despite this challenge the current version of the Banff Classification suggests testing for non-HLA antibodies in the absence of DSA-HLA antibodies (Haas et al., 2018). Therefore, the interest of non-HLA antibodies in transplantation recipients is a developing area, but their relevance has been limited by a lack of techniques available for reliable detection and definition. Most of the techniques used in the literature have been assays developed in-house. Some commercial kits have been developed including single plex ELISA based kits for the detection of angiotensin II type 1 receptor (AT1R) and endothelin receptor type A (One Lambda Inc) and multi-plex solid phase assays covering a broad range of targets (LABScreen Autoantibody and Lifecodes Non-HLA Antibody kit). Due to the recognition of non-HLA antibodies within the Banff Classification the writing committee thought it appropriate to review the non-HLA antibodies implicated within the renal transplant setting. However, the evidence is limited – the writing

committee felt that insufficient evidence existed to support a recommendation of testing for non-HLA antibodies within the routine H&I laboratory supporting renal transplantation in the UK, with the exception of investigating HNA in unexpected T and B cell positive crossmatch cases.

The Human Neutrophil Antigen (HNA) system consists of five groups (HNA 1–5) with limited polymorphism. HNA-3 is expressed on neutrophils, lymphocytes, platelets, and endothelial cells. There have been small studies suggesting that HNA-3a antibodies may be associated with antibody mediated rejection ((Key et al., 2018, 2019)). The detection of HNA-3 antibodies in the pre and post-transplant setting has resulted from the investigation of unexpected FCXM T and B cell positive crossmatches (when no HLA-DSA was detected). The case reports available describe strong FCXM reactivity in both the B and T cell crossmatch. B cell or T cell positivity in isolation has not been demonstrated as indicative of HNA reactivity. Furthermore, the sensitising event leading to the production of HNA antibodies is commonly pregnancy. While the evidence base remains small the writing committee felt that unexpected positive crossmatch demonstrating the above characteristics should be investigated.

The MHC I-related (MIC) gene family includes seven genes (MICA to MICG). MICA and MICB are functional genes with numerous alleles for each gene being identified.

A retrospective study of 727 kidney recipients, with a MICA antibody pre-transplant incidence of 7.1%, found that the presence of MICA specific antibodies and HLA specific antibodies was significantly associated with decreased allograft survival and that MICA specific antibodies were associated with early graft rejection (Sánchez-Zapardiel et al., 2013). A similar study by Chowdhry et al suggested an independent role for MICA specific antibodies (Chowdhry et al., 2018). Conversely other groups did not find significant associations between pre-transplant MICA antibodies and graft outcome. Lemy et al found no association with rejection at 1 year nor with graft outcome at 10 years (Lemy et al., 2010)

The studies looking at de novo production post-transplant of MICA antibodies also demonstrate cases where development is associated with higher incidence of rejection (He et al., 2013; Panigrahi et al., 2007) and those where no impact on graft survival (Ciszek et al., 2017; Lemy et al., 2012). Most recently Carapito and colleagues reported a multicentre cohort of 1356 kidney transplants, demonstrating that MICA mismatches were associated with decreased graft survival and that pre and post-transplant MICA DSA was strongly associated with AMR, with post-transplant DSA being associated with reduced graft survival. The group were able to demonstrate MICA-DSA was independently associated with AMR (Carapito et al., 2022). The writing committee acknowledged the developing evidence base assessing the impact of MIC within the renal transplant setting, however they did not feel the evidence justified a recommendation of routine testing within the H&I lab at present.

Angiotensin II type 1 receptor (AT1R) and endothelin type A receptor (ETAR) are G protein-coupled receptors expressed on endothelial cells. Unlike HLA, AT1R has limited polymorphism and antibodies targeting this antigen are auto-antibodies capable of binding both recipient and allograft antigens.

Pre-transplant AT1R antibodies have been found to be significantly associated with both acute antibody mediated rejection (Min et al., 2018; Philogene et al., 2018) and acute cellular rejection (J. Lee et al., 2017).

The association with graft failure however is less clear with studies demonstrating an association between de novo production and early and long-term graft failure (Banasik et al., 2014; Giral et al., 2013). Whilst a multi-centre study of 940 recipients was unable to detect any association (Deltombe et al., 2017).

A group from the Johns Hopkins Hospital, Maryland, recognising the low "cost vs benefit ratio" sought to define characteristics of kidney transplant recipients who may benefit from screening for non-HLA antibodies by testing patients transplanted over a 5-year period at their centre. Pre-transplant antibody levels were compared to clinical and biopsy indications of graft dysfunction. They found that re-transplanted patients ($p < 0.0001$), males ($p = 0.008$) and those with Focal Segmental Glomerulosclerosis ($p = 0.04$) and younger ($p = 0.04$) at time of transplantation were more likely to be positive for AT1R antibody prior to transplantation. Recipients who were positive for AT1R prior to transplantation had increases in serum creatinine within 3 months post-transplantation ($p < 0.0001$) and developed abnormal biopsies earlier than did AT1R antibody negative patients (126 days versus 368 days respectively; $p = 0.02$) (Philogene et al., 2018). The writing committee did not feel the evidence justified testing within the routine H&I lab.

Perlecan is a major component of vessels' walls and is involved in modulation of cell growth. The C-terminal fragment of perlecan, LG3, can be released during apoptosis and lead to antibody production. Cardinal et al showed that higher levels of LG3 specific antibodies were associated with vascular rejection (Cardinal et al., 2013). More recently in a study with 172 kidney recipients the pre-transplant LG3 antibody titres were shown to be associated with a risk of delayed graft function (Yang et al., 2016). The writing committee did not feel the evidence justified testing within the routine H&I lab.

Agrin is a heparan sulphate proteoglycan expressed in the glomerular basement membrane. Antibodies against agrin have been identified in patients with transplant glomerulopathy (TG) and therefore, they may be associated with the pathogenesis of TG (Joosten et al., 2005). It has been found that renal transplant recipients with TG generally have a higher concentration of agrin specific antibodies, which suggests that patients with these antibodies have a greater risk of allograft rejection and graft failure (Cardinal et al., 2017). The writing committee did not feel the evidence justified testing within the routine H&I lab.

Vimentin is a type III intracellular intermediate filament protein that is found in cells of mesenchymal origin, such as neutrophils, macrophages, leukocytes, and endothelial cells; it is the crucial cytoskeletal component of these cells. Vimentin, however, can sometimes be expressed on the surface of apoptotic cells or secreted under specific conditions; therefore, it is thought to play a role as an antigen which can initiate an immune response (Rose, 2013).

Besarani *et al* investigated the production of vimentin specific antibodies in primary kidney transplant patients. It was found that levels of IgG vimentin specific antibodies in serum were proven to be associated with Interstitial fibrosis/tubular atrophy (Besarani et al., 2014). These findings have been supported by several other groups (Fhied et al., 2014; Lopez-Soler et al., 2016) especially in chronic rejection.

Peroxisomal Trans-2-Enoyl-CoA Reductase (PECR) catalyses the reduction of trans-2-enoyl-CoAs as part of lipid metabolism and is NADPH-specific. Dinavahi *et al* showed that the pre-transplantation detection of antibodies against the kidney-expressed target, PECR, has a strong association with the late development of transplant glomerulopathy (TG) (Dinavahi et al., 2011). Patients with TG can experience a variety of symptoms ranging from proteinuria, hypertension, rising creatinine, and declining glomerular filtration rate. TG can be silent in many cases, making it difficult to detect, therefore the presence of PECR specific antibodies may be a marker of TG. The writing committee did not feel the evidence justified testing within the routine H&I lab.

7 | Special considerations for paediatric renal patients

7.1.1 Decisions to restrict the number of mismatches for paediatric patients awaiting deceased donor transplantation should be made in conjunction with the H&I laboratory [2B]

Rationale

The impact of ESRD during childhood has a significant effect on life development as measured using Health Related Quality of Life (HQRL) tools such as PedsQL (Varni et al., 1999). Consequently, paediatric renal transplantation aims to provide a transplant which provides good patient and graft survival rates without unnecessarily prolonged wait times, whilst protecting the chance for re-transplantation in the future.

Data from national and multi-national registries shows the benefit on graft survival of pre-emptive transplantation (Amaral et al., 2016).

The writing committee considered the impact of HLA mismatching within a first transplant and the subsequent access to a graft if retransplant is required. The committee felt the current data was mixed. With UNOS data demonstrating that HLA mismatch is a risk factor for decreased allograft survival with a 30% reduction for 1 HLA mismatch and almost two-fold reduction for 6 mismatches (Williams et al., 2018), while concluding that in the modern era, the effect of HLA mismatching is additive and is not influenced by which loci is mismatched, which is in contrast to the older studies which showed the deleterious effect of HLA-DR mismatching (Connolly et al., 1996).

Many paediatric units in the UK adopt strategies aimed at minimising the risk of sensitisation that might preclude future donation.

Including:

- Registering parental mismatches as unacceptable antigens in a deceased donor
- Restricting the match grade to minimise the number of antigens mismatched
- Restricting the match grade to avoid 2 HLA-DR mismatches

When adopting this approach, it is essential that these decisions are made in the context of;

- the patient's chance of an offer – using the NHS BT ODT risk communication tool and the matchability calculator
- living donor options available – including registration in the UKLKSS
- the clinical status of the patient – such as dialysis access

Whilst the evidence for prioritising matching at HLA-DR is not demonstrated in recent studies, many centres still avoid 2 HLA-DR mismatches as this has been shown to be associated with sensitisation, rejection and malignancies which all impact negatively on the chance of re-transplantation (Gralla et al., 2013). The writing committee felt that care needs to be taken when considering restricting mismatches so that a patient is not unfairly disadvantaged. For example, the frequency of a patient's HLA antigens in the UK population will impact upon the number of potential donors which will be matched at HLA-DR. Furthermore, if a patient is homozygous, they will also have fewer donors who would be matched at HLA-DR. Restricting mismatches when a patient has a less frequent type my disadvantage them unfairly. If a patient is already sensitised consideration should also be given to the appropriateness of restricting mismatches. A sensitised patient will have fewer potential donors and mismatch restrictions will further deplete the potential donor pool.

Due to the complexity of the HLA system the writing committee felt that any decision to restrict mismatches for paediatric patients should be made on an individual basis and in collaboration with the H&I laboratory. Tools which access the impact of sensitisation and match grades may facilitate this decision. Calculators – ODT Clinical – NHS Blood and Transplant.

Liver Transplant recommendations

The liver is generally perceived as at a lower risk of HLA antibody mediated damage when compared to other solid organ transplants. This protection from rejection is thought to be mediated by a number of liver specific factors, including high secretion of soluble HLA class I antigens leading to elimination of resultant antigen-antibody complexes by Kupffer cells; a large endothelial surface; potential for regeneration and diminished endothelial expression of HLA class II antigens (Demetris et al., 2016). Despite this protection, the relevance of preformed and *de-novo* HLA donor specific antibody has been well studied in liver transplant alone and simultaneous liver-kidney (SLK) recipients.

8 | Primary deceased donor pretransplant recommendations

8.1.1 Prospective HLA antibody definition is not indicated prior to primary deceased donor liver transplantation [1A]

8.1.2 H&I laboratories must store time of transplant samples for liver and SLK transplants (serum and DNA from patient, ideally DNA from donor, *although for most donors HLA typing data will be available via NHSBT*) to have material to assess baseline HLA antibody levels in the event of development of potential *de novo* HLA-DSA or declining graft function [1A]

8.1.3 Retrospective testing for the presence of HLA-DSA at the time of primary liver transplantation (by crossmatch or vXM) may be of use to aid post-transplant management [2B].

Rationale

Evidence has accumulated over many years that preformed IgG HLA donor specific antibodies (HLA-DSA) increase the risk of deleterious outcomes in liver transplant recipients, although most of this data comes from small single centre retrospective analyses. In early studies, patients with a positive complement dependent cytotoxic crossmatch (CDC-XM) were shown to have a reduced 1 year graft survival (Bathgate et al., 1998; Takaya et al., 1992), although other groups did not see such an association (Gordon et al., 1986). In a prospective study of 109 consecutive liver transplant patients, preformed HLA-DSA, defined by flow cytometry, were associated with clinically significant acute cellular rejection (Musat et al., 2013). This contrasted with an earlier study that showed no association with outcome at one year when 90 consecutive recipients were tested for HLA-DSA pre and post-transplant (Taner et al., 2012). This latter study highlighted one of the consistent observations with liver transplantation; levels of HLA-DSA are often significantly reduced immediately post-transplant, presumably as a result of antibodies binding to the graft or being phagocytosed as complexes after binding to soluble HLA molecules.

In a large retrospective study of over 1000 patients by O'Leary *et al*, preformed HLA-DSA reduced significantly after liver transplant, although to a lesser extent with high level HLA class II antibodies. In this study the presence of HLA class II antibodies at the time of transplant was associated with an increased risk of rejection and even impacted patient survival, as did preformed HLA class I HLA-DSA (O'Leary, Gebel, et al., 2013; O'Leary, Kaneku, et al., 2013). In an update to this initial study the impact of preformed HLA-DSA on patient and graft survival was refined by stratifying according to IgG subclass and C1q positivity in bead based assays (O'Leary et al., 2015). Preformed high level HLA-DSA (>10,000 median fluorescent intensity (MFI) in a standard Luminex bead based assay) were also associated with recipient mortality in a study of 459 liver transplant recipients from Scotland (McCaughan et al., 2016). Another analysis of a mix of living and deceased donor liver transplant recipients showed that preformed DSA were associated with graft failure in deceased donor recipients, again with higher level DSA leading to lower graft survival (Levitsky et al., 2016). A single centre analysis from France of 297 adult liver transplant recipients showed only 4.7% had preformed DSA, lower than in other studies and likely due to the mostly male, non-transfused patient cohort. Patients with persistent preformed DSA, when analysed with those who developed *de novo* DSA in the first year, had increased acute rejection, but patient and graft survival were not significantly different at 1 year (Vandevoorde et al., 2018). For paediatric liver transplantation, in a review of 31 patients from UCLA, early AMR cases associated with the presence of HLA-class I and/or class II DSA, either pre-formed or defined as *de novo* (Wozniak et al., 2017).

While the writing committee acknowledge the results above linking the presence of preformed HLA-DSA with liver outcome, in the UK, listing of 'unacceptable antigens' for liver patients is not part of the NHSBT liver allocation system (NHSBT ODT allocation Liver policy, 2018)Therefore, the presence of HLA antibodies is not usually assessed whilst patients are listed for deceased donor primary liver transplantation or even at the time of transplant via crossmatch. While the writing committee thought in some circumstances pretransplant assessment may be warranted e.g., in SLK or retransplant cases (see below), a recommendation was not made for pretransplant assessment in primary deceased donor liver transplantation.

9 | Simultaneous liver-kidney (SLK)

9.1.1 H&I laboratories must store time of transplant samples for liver and SLK transplants (serum and DNA from patient, ideally DNA from donor, *although for most donors HLA typing data will be available via NHSBT*) to have material to assess baseline HLA antibody levels in the event of development of potential *de novo* HLA-DSA or declining graft function [1A]

9.1.2 Prospective HLA antibody definition should be performed in patients listed for SLK in order to assess risk at the time of offer. We suggest that this decision be taken with the clinical team and the risk of antigen avoidance (and therefore the number of organs deemed not suitable) balanced against the risk of not transplanting [2B]

Rationale

For patients being listed for simultaneous liver-kidney (SLK) transplant, the writing committee felt that understanding the antibody profile of patients may be important for assessing AMR risk to the kidney graft, given that the transplanted liver may not always provide immunological protection against high levels of preformed HLA-DSA. Investigations of the kinetics of HLA antibody changes pre, peri and post SLK transplant show that both HLA class I and II DSA are reduced after reperfusion of the liver allograft, albeit with more pronounced changes to HLA class I DSA (Kueht et al., 2021).

As with the liver transplant data, contradictory studies have been published on the relevance of preformed HLA-DSA in recipients of SLK transplants. In a study of 30 SLK patients, the presence of preformed HLA class II DSA, but not class I, gave an increased risk of renal AMR, liver rejection and both renal and liver graft loss (O'Leary, Gebel, et al., 2013). Multiple HLA-DSA (>10,000 MFI) that resulted in strongly positive FCXM have also been shown to increase the rate of kidney and liver graft rejection in SLK recipients (Ong et al., 2016). A recent study of SLK patients from Spain (n = 88) analysed HLA-DSA and patient/graft survival in conjunction with other risk factors such as comorbidities, rejection, immunosuppression and infections (Piñeiro et al., 2020). Patients were divided into high immunological risk and low risk groups based on CDC-XM and/or FCXM positivity and presence of DSA. The high risk group had more episodes of kidney rejection although eGFR was similar between the two groups at 3 years post-transplant. These results were similar to those from Leca *et al*, who in an analysis of 56 SLK transplants categorised 15 patients into a high sensitisation group which had more renal rejection episodes, but no change to graft function at 1 year (Leca et al., 2013). A larger retrospective multicentre study of SLK outcomes in 166 recipients from eight European centres, including 46 with preformed DSA, again showed that kidney rejection was increased in the patients with HLA-DSA, but this did not influence long term kidney function, with similar eGFR rates observed at 5 years in those with and without HLA-DSA at the time of transplant (Del Bello et al., 2020). This latter study did, however, show that SLK recipients with preformed HLA-DSA had reduced patient survival, likely due to differences in induction and maintenance immunosuppression given to the higher immunological risk patients. Recently, in an observational study, Shah *et al* have reported on outcomes of 27 SLK transplants. Patients classified as higher risk, with class II DSA >10,000 MFI by Luminex, exhibited kidney AMR even with increased immunosuppression, but these cases were successfully treated by plasma exchange and IvIg. Interestingly this data also indicated that HLA class I DSA may have an impact on kidney rejection with the liver not always abrogating the impact of preformed HLA class I antibodies. In common with other studies, graft function at 1 year post transplant was not compromised by the presence of time of transplant HLA-DSA or AMR (Shah et al., 2020).

Overall, studies of the effect of preformed HLA-DSA in SLK recipients show that the liver provides a protective effect in relation to alloreactivity directed towards the kidney graft. It is supposed that this is mediated by the ability of the liver to reduce levels of circulating alloantibody, but recent data indicates that the liver may also impact on alloreactivity by skewing T cell phenotypes to donor-specific hypo-alloresponsiveness (Taner et al., 2018). However, given the observations of high level DSA, particularly HLA class II, impacting on AMR in the kidney, knowledge of HLA antibodies present pre-transplant in SLK patients may be of use to guide decisions on acceptance of offers and for post-transplant patient management (Das et al., 2021).

10 | Liver Re-transplantation

10.1.1 Where time permits, prospective antibody screening assays should be performed on patients awaiting re-transplantation and, to aid interpretation, both recipient and previous donor(s) HLA types obtained [2B].

10.1.1 IgG HLA specific antibodies that are circulating at levels likely to cause a positive crossmatch and are directed against known previous donor HLA mismatches associated with adverse events (e.g., rejection) warrant consideration for prospective avoidance in the liver re-transplantation setting. This decision should be taken with the clinical team and the risk of avoidance (and therefore the number of organs deemed not suitable) balanced against the risk of not transplanting [2B].

Rationale

Re-transplantation is the only life-saving option for patients with primary graft failure, however it comes with significant ethical and economic issues due to factors such as lower patient and graft survival compared to primary transplantation and the use of organs from a scarce donor pool (Zahr Eldeen et al., 2014; Zakaria et al., 2020). The incidence of re-transplantation is typically quoted as between 5% - 22% of total liver transplants numbers (Zahr Eldeen et al., 2014). With the increasing use of marginal and DCD donors for transplantation it is likely that the incidence of primary graft loss may increase resulting in a concomitant increase in re-transplantation (Zahr Eldeen et al., 2014; Zakaria et al., 2020). Re-transplantation is generally divided into early (within 30 days of primary transplant) and late (after 30 days of primary transplant) (Shamsaeefar et al., 2021). The two main indications for early re-transplantation are hepatic artery thrombosis (HAT) and primary non function (PNF), (Zahr Eldeen et al., 2014) with HAT increased in paediatric recipients and adults receiving a partial graft (Shamsaeefar et al., 2021). The indications for later re-transplantation typically show a more mixed picture, including chronic rejection, vascular complications and disease reoccurrence (Shamsaeefar et al., 2021).

The UK data from the NHSBT Annual report on liver transplantation 2019/2020 summarises transplants taking place between 2010 to 2020 including both adult and paediatric transplantation (NHSBT liver transplantation report 2020, 2021). During this time period there were a total of 8,502 transplants (excluding atypical or multiorgan) and of these 821 (9.7%) were re-transplants. There was no information available on the timing of re-transplant from primary transplant. In the year 2019/2020 there were 46/979 (5%) adults listed for a regraft and the number of liver only retransplants from donors after brain death (DBD) ranged between 51 in 2010/2011 and 98 in 2013/2014 with 86 performed in 2019/2020 (NHSBT liver transplantation report 2020, 2021)

There is very limited published literature on the role of HLA specific antibodies in the re-transplant setting. There may be a role of HLA in the late graft failure setting with chronic rejection being a factor for later graft loss (Shamsaeefar et al., 2021). This, together with the overall lower graft and patient survival in the re-transplant setting, means the writing committee thought it may be prudent to consider HLA-DSA for patients requiring re-transplantation. In the early re-transplant setting, immediately after initial transplant, this may not be possible nor necessary due to time constraints, but there is time for consideration of HLA in the late re-transplant setting. We suggest, where time permits, that prospective antibody screening assays are performed on patients awaiting re-transplantation and, to aid interpretation, both the recipient and previous donor(s) HLA types should be obtained.

We suggest that IgG HLA specific antibodies that are circulating at levels likely to cause a positive FCXM or CDC-XM and are directed against known previous donor HLA mismatches and have been associated with adverse events (e.g., rejection) warrant consideration for prospective avoidance in the re-transplantation setting. We suggest that this decision be taken with the clinical team and the risk of avoidance (and therefore the number of organs deemed not suitable) balanced against the risk of not transplanting.

Where it is deemed appropriate to avoid certain HLA antigens the laboratory must have the capacity to perform vXM to provide an individualised compatibility assessment for a given donor and recipient pair.

11 | Living liver transplantation

11.1.1 For living donor liver transplantation, especially in paediatric cases, full compatibility testing (HLA antibody screening and typing) of the patient and donor should be undertaken to aid patient management [1B].

Rationale

In the financial year to 31 March 2020, living donor liver transplantation represented a small proportion (18/942 (1.9%)) of transplants performed in 2019/2020 in the UK (NHSBT liver transplantation report 2020, 2021). Living donor liver transplants are performed in both adult and paediatric recipients but are more common in paediatric recipients where the left lateral lobe is transplanted (NHSBT liver transplantation report 2020, 2021). The presence of HLA-DSA has been investigated in the living liver transplant setting. Tamura *et al* demonstrated that in their series of transplants between 2001 and 2015 in Japan that preformed HLA-DSA had a detrimental impact on survival, with the 90-day survival rate of DSA-positive patients (50%) being significantly lower than that of DSA-negative patients (84.4%). This was also seen on univariate analysis with the HLA-DSA positive rate being significantly higher in the 90-day mortality group (Tamura et al., 2019). Conversely, Badaway *et al* showed that a positive lymphocytotoxic crossmatch and HLA mismatches between donor and recipient did not affect the overall graft survival after adult to adult living donor liver transplantation and this group suggest that HLA incompatibility should not be considered as a contraindication for liver transplantation (Badawy et al., 2018). However, this group did not perform HLA antibody screening using more sensitive solid phase assays so it may be that HLA-DSA levels were underreported by this group. Overall, the writing committee recommend that given the living liver donor transplant operation is planned, full work up prior to transplantation, including consideration of HLA compatibility through antibody screening and HLA typing, is performed prior to transplantation.

12 | Crossmatching and Virtual Crossmatching

12.1.1 Where it is deemed appropriate to avoid certain HLA antigens for liver and SLK transplantation the laboratory must have the capacity to perform virtual crossmatches 24/7 to provide an individualised compatibility assessment for a given donor and recipient pair [2B].

12.1.2 Laboratories must have procedures in place for the detection and characterisation of HLA Class I and II specific antibodies. [1A]

12.1.3 At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. [1A]

12.1.4 Laboratories must be able to define HLA-A, B, C, DR, DQA1, DQB1 and DPA1 and DPB1 antibody specificities. [1A]

12.1.5 HLA antibody detection and identification techniques should be able to detect HLA IgG antibodies [1A]

12.1.6 HLA antibody detection and identification techniques should be able to exclude reactivity attributable to IgM antibodies. [1A]

12.1.7 Laboratories must employ methods to abrogate known causes of false positive or negative results. [1A]

Rationale

In the absence of patient HLA antibody data in primary liver transplantation, a prospective virtual crossmatch (vXM) approach is not possible, nor necessary. However, in SLK or liver re-transplantation, in patients identified to have high levels of HLA antibodies, attempts at pre-Tx assessment by vXM may enable decisions on acceptance of donor offers and patient management.

As described above, in liver transplantation historic data shows that patients with positive CDC-XM and FCXM results are at a higher risk of rejection and graft loss. A *retrospective* crossmatch or antibody test (enabling a retrospective vXM) may be used in some centres to inform patient management, but it is unclear how clinically useful this information is.

13 | Post-transplant HLA antibody detection

13.1.1 H&I laboratories must store time of transplant samples for liver and SLK transplants (serum and DNA from patient, ideally DNA from donor, *although for most donors HLA typing data will be available via NHSBT*) to have material to assess baseline HLA antibody levels in the event of development of potential *de novo* HLA-DSA or declining graft function [1A]

Rationale

Many studies have shown the relationship between development of *de novo* HLA-DSA and acute or chronic AMR and graft failure in liver transplantation (Beyzaei et al., 2020; Sultani et al., 2021; Vandevoorde et al., 2018). The rate of positivity for *de novo* HLA-DSA is comparable to rates observed after kidney transplantation and a prevalence of class II, particularly HLA-DQ directed HLA-DSA, has been described (Sultani et al., 2021; Vandevoorde et al., 2018). Knowledge of the presence of HLA-DSA post-transplant can help in diagnosis of rejection when recipients are being investigated for declining graft function. It is therefore recommended that the H&I laboratory has arrangements in place to test for HLA-DSA when indicated post-transplant. Samples taken at the time of transplant (patient serum and DNA samples) will enable investigation of the presence of *de novo* HLA-DSA at the time of graft dysfunction or a biopsy indicating AMR.

As in other forms of solid organ transplantation a case can be made for regular post-transplant DSA monitoring of liver transplant and SLK patients as a non-invasive biomarker measure of graft rejection risk. A recent meta-analysis of over 2000 liver transplant recipients showed that the development of *de novo* HLA-DSA gave an OR of 6.43 for allograft rejection leading the authors to argue that HLA antibody screening could be of use in identifying patients requiring changes to immunosuppression or informing decisions about immunosuppression withdrawal (Beyzaei et al., 2020).

Whilst it is recorded that pre-formed DSA is not uncommon in the SLK setting, data relating to the incidence and clinical significance of *de novo* HLA-DSA in SLK recipients is limited in the main to small single centre studies. Parajuli *et al* recently reported a series of 83 SLK recipients, transplanted between 2005 and 2017, who were monitored for the production of *de novo* DSA post SLK transplant (Parajuli, Aziz, et al., 2021a). From this cohort 23/83 patients (28%) produced *de novo* DSA, mainly against HLA class II mismatches, including 6 patients who had additional, pre-existing, DSA at the time of transplant. Even though at last follow up the presence of *de novo* DSA was not associated with failure of either the kidney or the liver, protocol and 'for-cause' biopsies suggested a higher incidence of subclinical rejection of the kidney in the *de novo* DSA positive group. The group note that these patients received additional immunosuppression at the time of the subclinical diagnosis, which may have limited the impact of the antibody on outcome.

Similar to the advice for liver transplantation, monitoring HLA antibody in SLK recipients transplanted across known preformed DSA, and for *de novo* DSA at times of organ dysfunction, is recommended to assist in decisions relating to treatment and biopsy.

14 | Non-HLA

14.1.1 No recommendation made

Rationale

The presence of non-HLA antibodies and their impact on graft and patient outcomes has also been investigated in liver transplantation. O'Leary *et al* studied both HLA and non-HLA autoantibodies (against angiotensin II type-1 receptor and endothelin-1 type A receptor) in 1269 recipients and described an increased risk of death in the presence of combined HLA-DSA and non-HLA antibodies as well as effects on rejection and fibrosis of *de novo* non-HLA antibodies alone (O'Leary, Kaneku, et al., 2013). In another study, following immunosuppression withdrawal in paediatric living donor liver transplant recipients, post-transplant detection of both HLA class II and angiotensin II type-1 receptor antibodies was seen more frequently in patients defined as having advanced fibrosis compared to controls, leading to speculation that antibodies to both HLA and non-HLA targets may be important in graft fibrosis (Ohe et al., 2014). Investigations in this area are limited, so the writing committee did not feel the evidence justified recommendations. Therefore, no recommendation that routine testing of non-HLA antibodies in the context of liver transplant or SLK is indicated.

Pancreas Transplant recommendations

A successful pancreas transplant in patients with type 1 diabetes may prolong life, reduce many complications associated with prolonged hyperglycaemia, and improve the quality of life (F. Aziz et al., 2020a). Pancreas transplant is now a frequently performed procedure that dramatically improves quality of life and may prolong survival expectancy of selected diabetic recipients. Pancreas transplantation is frequently performed in patients with insulin-dependent diabetes and advanced diabetic nephropathy in the context of a simultaneous pancreas and kidney (SPK) transplantation. It may also be performed as a sequential pancreas after kidney (PAK), in patients with a functioning kidney graft, or as a pancreas transplant alone (PTA) in patients without overt diabetic nephropathy and poor metabolic control leading to hypoglycaemia unawareness (Vistoli et al., 2021).

Pancreas graft survival has improved significantly over the past decades, mainly due to better immunosuppression protocols and lower technical failure rates. However, immunological factors continue to play an important role in pancreas graft loss beyond the first 3 months

after transplantation. Antibody-mediated rejection of the pancreas graft has gained attention over the past 10 years and is now considered a key factor in long-term pancreas graft failure (Uva et al., 2020). Multiple studies have shown that acute and chronic pancreas allograft rejection is a significant prognostic factor for long-term pancreas graft failure (F. Aziz et al., 2020a). Both pre-transplant DSA, and post-transplant de-novo DSA (dnDSA) which specifically refers to DSA development after transplantation, are associated with acute antibody-mediated rejection (ABMR) and T-cell-mediated rejection (TCMR) of the pancreas allograft, and any rejection episode is associated with reduced long-term allograft survival (Khan et al., 2021).

Pancreas allograft function is clinically most often monitored by measuring hyperglycaemia, serum amylase, serum lipase, or (when bladder drained) amylasuria . More accurate diagnosis is made by tissue evaluation, enabling the distinction between non-immune and immune injury to the graft and T-cell mediated versus antibody-mediated injury. Pancreas biopsy is however, perceived as having more risks for complications than in other transplanted organs and availability of serial donor specific antibody data may therefore improve pancreas transplant patient management.

Histocompatibility testing for kidney transplantation can be applied to pancreas transplantation and, by inference, the same histocompatibility criteria can also be applied to PTA and PAK transplantation in that, the presence of HLA antibodies is investigated at the time of listing and regularly whilst awaiting an offer, with unacceptable antigens listed to avoid donor HLA that may pose an immunological risk. Consequently, much of the rationale in renal sections of these guidelines (sections 1–7) can be applied to the pancreas section. As such to avoid duplication, rationale relating to methodology and sample timing is not reproduced here.

15 | Pre-transplant testing recommendations

15.1 | Requirement of testing pre-transplant assays

15.1.1 Laboratories must have procedures in place for the detection and characterisation of HLA Class I and II specific antibodies. [1A]

15.1.2 Laboratories must be able to define HLA-A, B, C, DRB1/3/4/5, DQA1, DQB1, DPA1 and DPB1 antibody specificities. [1A]

15.1.3 HLA antibody detection and identification techniques must be able to detect HLA IgG antibodies [1A]

15.1.4 HLA antibody detection and identification techniques should be able to exclude reactivity attributable to IgM antibodies. [1A]

15.1.5 Laboratories must employ methods to abrogate known causes of false positive or negative results (e.g., the additional of EDTA) [1A]

15.1.6 At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. [1A]

15.1.7 HLA-specific antibodies must be characterised at regular agreed intervals prior to transplantation in sensitised patients and whenever a change in HLA antibody profile is suspected e.g., following a sensitising event or following a change in the antibody screening test results. [1A]

15.1.8 For patients on the transplant list, regular samples must be sent to the histocompatibility laboratory for antibody testing. (3 monthly is suggested) [1A]

15.1.9 The clinical team must inform the laboratory of potential sensitisation events such as previous transplantation, skin grafting, transfusion of blood products, and pregnancy (including known miscarriage). [1A]

15.1.10 Serum samples must be stored for potential use in future antibody screening and crossmatch tests. [1A]

15.1.11 HLA donor and recipient typing, HLA antibody testing and crossmatch and vXM results must be assessed, reviewed and reported by Health and Care Professions Council (HCPC) registered biomedical / clinical scientists specifically trained in histocompatibility and immunogenetics. [1A]

15.1.12 A patient's HLA antibody profile must be assessed to determine the acceptable risk, and delineate the antigens regarded as unacceptable. A system should be in place to monitor changes in a patient's HLA antibody profile when listed as active for a deceased donor offer [1A]

15.1.13 The listing of unacceptable antigens with OTDT should reflect the centres locally accepted criteria regarding acceptable risk thresholds in relation to HLA-DSA. The acceptable risk may vary between patients. E.g., crossing a HLA-DSA maybe considered appropriate in a HSP but not in a moderately sensitised patient. [1A]

15.1.14 Patient HLA antibody profiles should be reviewed at agreed intervals. This is particularly important for HSP – whereby the review of antibody profiles should include identifying delisting opportunities. Such opportunities should be discussed with the clinical team. E.g., historic HLA antibody positive, current negative, which may be an acceptable level of risk for a patient that otherwise faces long wait and poorer long term outcomes associated with dialysis. [1A]

15.1.15 A combination of tests should be considered in order to fully resolve complex antibody profiles. Using different manufacturers Luminex single antigen kits maybe beneficial during this assessment. [1B]

15.1.16 The clinical team must inform the laboratory of events that might influence the HLA antibody profile and send a serum sample 14 days after the event. These include the transfusion of blood products and treatment with therapeutic antibodies. [1B]

Rationale

The writing committee felt the majority of renal transplant recommendations aligned with those of pancreas transplantation. In a concise summary, the committee recommends testing two independent clotted blood samples (obtained on two separate occasions) before a patient

is registered for transplantation to ensure confirmation of the HLA antibody profile. It is suggested that patients are screened for HLA antibodies every three months and two weeks after known sensitising events while waiting for a pancreas transplant. Careful selection of patients for PAK transplantation is recommended where a history of renal allograft rejection is present. Optimal HLA matching and avoidance of donor-specific antibodies are both expected to mitigate the risk of post-PAK rejection (Boggi et al., 2021).

The inaugural world consensus conference on pancreas transplant held in 2021 recommends that donor specific antibodies (DSA) with a mean fluorescence intensity (MFI) of up to 5000 may not be an absolute contraindication to pancreas transplantation if the T and B cell laboratory crossmatch is negative. The recommendation was subject to clinical and methodological limitations, with MFI values acknowledged as method dependent and hence centre specific (Boggi et al., 2021).

Considering recent developments in analyses of both B cell and T cell epitopes (i.e., eplet mismatches and predicted indirectly recognisable HLA epitopes (PIRCHE) scores, respectively), more sophisticated approaches may become possible in pancreas transplant that lead to personalised predictions with respect to post-transplant dnDSA production and tailored post-transplant immunosuppression. Evaluating mismatches of HLA eplets,—small configurations of surface-exposed amino acids of the HLA molecule—instead of antigen mismatches might offer an improved approach to prediction of dnDSA. Since not all eplets are equally capable of inducing an immune response, antibody verification to confirm their ability to be bound by antibodies could enhance the clinical relevance of eplet mismatches that are considered (Meneghini et al., 2021; A. Russo et al., 2018; Senev et al., 2020).

Post-transplant risk prediction strategies may also benefit from the use of the PIRCHE II algorithm which is able to predict HLA-mismatch derived T-cell epitopes by quantifying the number of mismatched donor HLA-derived peptides that can be presented on HLA class II molecules of the recipient. PIRCHE-II scores have been shown to be related to HLA antibody formation after kidney transplantation and also had positive predictive capabilities with respect to de novo DSA production in pancreas and islet transplantation (Nakamura & Shirouzu, 2021). Implementation of these risk prediction strategies may be especially important for young recipients, in whom repeat transplantations are anticipated and the goal is to minimize HLA sensitization.

16 | Virtual crossmatching Recommendations

16.1.1 HLA donor and recipient typing, HLA antibody testing and crossmatch and vXM results must be assessed, reviewed and reported by Health and Care Professions Council (HCPC) registered biomedical / clinical scientists specifically trained in histocompatibility and immunogenetics. [1A]

16.1.2 Prior to issuing a vXM, it is recommended that antibody screening/ specificity analysis is performed from two separate samples obtained at different time points. Including a recent sample [1A]

16.1.3 In cases where a patient has a stable HLA antibody profile or are consistently HLA antibody negative a vXM may be issued – in these cases the serum sample used to determine the absence of HLA-DSA should be less than 3 months old [1B]

16.1.4 Full donor HLA typing (HLA-A, -B, -C, -DRB1/3/4/5, -DQA1, DQB1, DPA1 and –DPB1) should be available for vXM interpretation. [1A]

16.1.5 Pretransplant assessment should be undertaken by carrying out a laboratory crossmatch test or, in selected cases, by performing a virtual crossmatch [1A]

16.1.6 Patients with complex antibody profiles or incompletely defined antibody profiles should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). [1A]

16.1.7 If a prospective crossmatch is omitted, confirmation of the HLA antibody status should be assessed with samples obtained at the time of transplant. This confirmation may be performed retrospectively by Luminex HLA antibody testing, Flow Cytometry crossmatch or CDC XM. [1A]

16.1.8 A risk assessment should be performed in conjunction with the transplanting centre to ensure the risks associated with a vXM are understood and mitigated. [2B]

16.1.9 We recommend that the sample date of the serum assessed for vXM should be considered in relation to the patient's sensitising events. In regrafts an updated HLA antibody test should be performed - with a sample no older than 2 weeks before the transplant date. [2B]

16.1.10 Historic HLA-DSA should be considered during vXM and Crossmatching [2B]

Rationale

The writing committee recognized the benefit of the VXM being its ability to provide an assessment of donor-recipient compatibility without subjecting the pancreas to additional cold ischemia time (CIT) required for laboratory crossmatching. The ability to reduce CIT may not only contribute to improved outcomes by reducing ischemia-reperfusion injury, but also may increase the overall number of pancreases transplanted (Eby et al., 2016)

The rationale relating to recommendations for vXM in pancreas transplantation mirrors that within the renal transplantation. Where practice in a centre differs between pancreas and kidney vXM protocols these should be captured with the risk assessment relating to vXMs within the unit and clinical teams should be made aware of the limitations of the antibody screening methods available and the potential for errors in donor HLA typing that could lead to an unexpectedly positive retrospective crossmatch post-transplant.

17 | Crossmatching recommendations

17.1.1 HLA donor and recipient typing, HLA antibody testing and crossmatch and vXM results must be assessed, reviewed and reported by Health and Care Professions Council (HCPC) registered biomedical / clinical scientists specifically trained in histocompatibility and immunogenetics. [1A]

17.1.2 Pretransplant assessment should be undertaken by carrying out a laboratory crossmatch test or, in selected cases, by performing a virtual crossmatch [1A]

17.1.3 HLA antibody data should be used in conjunction with the crossmatch results for crossmatch interpretation [1A]

17.1.4 Patients with complex antibody profiles or incompletely defined antibody profiles should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). [1A]

17.1.5 Laboratory crossmatch tests should distinguish donor T cell and B cell populations. [1A]

17.1.6 The crossmatch report must include appropriate interpretation of the crossmatch results in the context of the patient's antibody profile. [1A]

17.1.7 CDC positive XM is a contraindication to pancreas transplantation, but lower levels of HLA-DSA that lead to a positive FCXM may on occasion be transplanted across in line with local policy.[1A]

17.1.8 Where a recipient has uncharacterized HLA-specific antibodies, or has a defined antibody but there is an incomplete donor HLA type (in relation to a recipient's antibody specificities, e.g., recipient has allele-specific antibodies), or where the intended recipient has known donor HLA-specific antibodies, a pre-transplant XM must be performed [1B]

17.1.9 Reactivity attributable to autoantibodies can be excluded in crossmatch interpretation – performing an auto crossmatch at the time of allocrossmatch should be considered. [1B]

17.1.10 Where intermediate or high-risk cases are being considered discussion between HCPC registered biomedical / clinical scientist staff who hold FRCPath and the Clinical team is advised. This discussion should include the chances of the patient receiving a lower risk offer. [1B]

17.1.11 Historic HLA-DSA should be considered during vXM and Crossmatching [2B]

Rationale

As with renal transplantation, a number of factors determine the clinical significance of a laboratory crossmatch. The writing committee recommend the reader reads the rationale regarding crossmatching in renal transplantation when considering these guidelines. These include: specificity and immunoglobulin class of the antibodies, timing of patient samples in relation to sensitisation event(s) and sensitisation history. As with kidney transplantation both complement-dependent cytotoxicity (CDC) and flow cytometry crossmatches may be performed for pancreas transplantation. In general, a positive crossmatch contraindicates pancreas transplantation. Limited evidence shows that pre-transplant B cell crossmatch positivity does not affect patient and pancreas graft survival but is associated with higher rates of antibody-mediated rejection. In addition, a few solitary pancreas transplants have also been reported that have proceeded with a positive crossmatch with reported good outcomes (Vistoli et al., 2021)

18 | Post-transplant HLA antibody detection

18.1.1 Post-transplant serum samples should be taken at regular intervals on an agreed basis (in view of the perceived immunological risk at the time of transplant). [1A]

18.1.2 In higher risk transplants (e.g., donor-specific antibody is present at the time of transplant) a timetable of post-transplant sampling must be agreed with the local transplant unit. [1A]

18.1.3 Post-transplant samples should be sent to the laboratory when graft rejection is suspected or antibody production a concern (e.g., following a change in immunosuppression or compliance issues). [1A]

18.1.4 Post-transplant sampling for anti-HLA-DSA in immunologically low risk patients and/or patients with stable function post-transplant might be undertaken at locally defined time points, to aid in patient management (2B)

18.1.5 The use of single antigen bead MFIs to reflect changes in the level of DSA, is semi quantitative but may be beneficial in patient monitoring and assessing therapeutic interventions [2B]

18.1.6 Epitope analysis software may benefit HLA antibody profile interpretation - donor and recipient HLA typing to the second field are recommended for this process [2B]

Rationale

The prognostic significance of de novo DSA (dnDSA) on transplant outcomes has been demonstrated in solid organ transplantation including kidney, heart and lung; however, evidence in pancreas transplantation is still emerging (Mittal et al., 2014). Biopsies are the gold standard for the diagnosis of pancreas graft rejection, with an available international standardized rejection-grading schema. However, pancreas graft biopsies are invasive and not performed in every centre.

Antibody-mediated rejection (ABMR) of the pancreas allograft is a serious condition, and when it occurs it is likely to be detrimental to the graft (De Kort et al., 2013). Pre-transplant DSA and dnDSA are important markers to predict pancreas allograft rejection and are risk factors for worse graft survival. In patients with antibody-mediated rejection (ABMR), dnDSA appears to have greater negative effect than pre-existing DSA (F. Aziz et al., 2020a) (Parajuli et al., 2019)(Parajuli, Aziz, et al., 2021b). In a large homogenous cohort of patients,

analysis of DSA specificities showed all loci to be represented, although recipients who developed both class I and class II DSA were more likely to have pancreas or pancreas and kidney graft failure than recipients who developed either class I or class II only (Mittal et al., 2014). Post-transplant monitoring of donor-specific antibodies is now more widely recommended as consensus evidence shows that the detection of dnDSA is strongly associated with worse outcomes (F. Aziz et al., 2020b; Khan et al., 2021; Parajuli, Aziz, et al., 2021c).

A 2021 meta-analysis by Khan et al., evaluating the efficacy of anti-HLA dnDSA in predicting pancreatic allograft rejection and failure that included eight studies and over 1400 patients, showed that patients with positive anti-HLA dnDSA are at significantly increased risk of both graft rejection and graft failure. Subgroup comparisons between anti-HLA dnDSA and other de novo anti-HLA antibodies confirmed that anti-HLA antibodies without donor specificity are of little significance consistent with similar observations in kidney and lung transplantation (Khan et al., 2021) . Other studies on association between de novo HLA antibodies following pancreas transplant and graft outcomes also demonstrate a strong association between development of DSA and graft dysfunction leading to recommendations of post –transplant antibody monitoring as part of routine follow-up (De Kort et al., 2013; Mittal et al., 2014; Uva et al., 2020)

In common with renal and liver transplantation a major proportion of dnDSA detected have been against class II antigens, especially DQ (Ladowski et al., 2021). The results of a recent meta-analysis show that anti-HLA dnDSA is strongly associated with pancreas graft failure and rejection. The ability of anti-HLA dnDSA to bind C1q and activate the complement cascade are mechanisms of ABMR, and previous studies document deposition of both DSA and complement components in rejecting grafts. Even in the absence of complement binding, dnDSA can mediate graft damage via antibody-dependent cellular cytotoxicity (Khan et al., 2021). Surveillance for HLA dnDSA is recommended in the setting of recipients with pre-transplant DSA, immunosuppression reduction, patient non-adherence, or a rejection episode occurrence, with close allograft function surveillance when detected (Drachenberg et al., 2011).

19 | Non-HLA antibodies

19.1.1 We suggest that HNA antibodies may be investigated when crossmatch results are not consistent with HLA specific antibody screening results [2B]

Rationale

Type 1 diabetes (T1D) recurrence has been documented in simultaneous pancreas–kidney transplants (SPKT), but this diagnosis may be underestimated. It is reported that recurrent T1D explains 50% of the immunologic failures, while the other 50% is attributed to chronic graft rejection. A recent study found that approximately 7% to 8% of all recipients develop T1D recurrence particularly those carrying HLA DR3/DR4 genotype and sharing HLA-DR alleles with the donor (Vendrame et al., 2016a), but other studies did not find this association significant (Anteby et al., 2021a) (Martins et al., 2014).There is growing interest in the use of pancreatic autoantibodies, anti-islet cell (ICA), antieglutamic acid decarboxylase (GAD), anti-insulin autoantibodies (IAA), anti-tyrosine phosphatase (IA2) and anti-zinc transporter 8 (anti-ZnT8) as possible early detection markers for T1DR after pancreas or islet transplantation. The writing committee acknowledged this is a developing area but did not belive sufficient evidence was available to support a recommendation.

Several autoantibodies against non-HLA targets have been implicated in allograft rejection, such as angiotensin II type 1 receptor (AT1R), endothelin-1 type A receptor (ETAR), agrin, myosin, perlecan, vimentin and tubulin. Some of these autoantibodies might also influence pancreas transplant outcomes, but currently data on this is limited (Jackson et al., 2020). While the writing committee also aknowledged that no data is available relating to HNA antibodies in pancreas transplantation, the committee non-the-less recommended HNA antibodies are investigated as a possible cause of unexplained crossmatch positive. This is recommended to determine the cause of the unexplained positity rather than confer a risk stratification on the transplant – as the impact of HNA within pancreas transplantation is unknown.

Islet Transplant recommendations

Islet transplantation is established as a therapy for selected patients with type 1 diabetes mellitus (T1D). Patients usually require more than one allograft from different donors to achieve metabolic success. The more limited pool of donors suitable for islet isolation and the small number of patients on the transplant list precludes significant HLA matching between donors and recipients and therefore recipients may be exposed to multiple mismatched antigens during treatment. Transplants may be performed as islet alone, islet after kidney or as simultaneous islet and kidney.

As with other tissue and cell transplantation, loss of function after allogeneic islet transplantation is a multifactorial process. In islet transplantation the re-emergence of autoimmune reactivity may be as relevant as alloimmune responses directed against donor HLA and non-HLA targets. In addition, when considering the relevance of auto and alloimmune elements of the response to transplanted islets, both cellular and antibody reactivity needs to be considered (Buron et al., 2021).

20 | Pre-transplant testing recommendations

20.1.1 Laboratories must have procedures in place for the detection and characterisation of HLA Class I and II specific antibodies. [1A]

20.1.2 Laboratories must be able to define HLA-A, B, C, DRB1/3/4/5, DQA1, DQB1, DPA1 and DPB1 antibody specificities. [1A]

20.1.3 HLA antibody detection and identification techniques must be able to detect HLA IgG antibodies [1A]

20.1.4 HLA antibody detection and identification techniques should be able to exclude reactivity attributable to IgM antibodies. [1A]

20.1.5 Laboratories must employ methods to abrogate known causes of false positive or negative results (e.g., the additional of EDTA) [1A]

20.1.6 At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. [1A]

20.1.7 HLA-specific antibodies must be characterised at regular agreed intervals prior to transplantation in sensitised patients and whenever a change in HLA antibody profile is suspected e.g., following a sensitising event or following a change in the antibody screening test results. [1A]

20.1.8 For patients on the transplant list, regular samples must be sent to the histocompatibility laboratory for antibody testing. (3 monthly is suggested) [1A]

20.1.9 Between first and second (or subsequent) islet transplants patients should be regularly tested for HLA antibodies and unacceptable antigens updated as appropriate. [1B]

20.1.10 The clinical team must inform the laboratory of potential sensitisation events such as previous transplantation, skin grafting, transfusion of blood products, and pregnancy (including known miscarriage). [1A]

20.1.11 Serum samples must be stored for potential use in future antibody screening and crossmatch tests. [1A]

20.1.12 HLA donor and recipient typing, HLA antibody testing and crossmatch and vXM results must be assessed, reviewed and reported by Health and Care Professions Council (HCPC) registered biomedical / clinical scientists specifically trained in histocompatibility and immunogenetics. [1A]

20.1.13 A patient's HLA antibody profile must be assessed to determine the acceptable risk, and delineate the antigens regarded as unacceptable. A system should be in place to monitor changes in a patient's HLA antibody profile when listed as active for a deceased donor offer [1A]

20.1.14 The listing of unacceptable antigens with OTDT should reflect the centres locally accepted criteria regarding acceptable risk thresholds in relation to HLA-DSA. The acceptable risk may vary between patients. E.g., crossing a HLA-DSA maybe considered appropriate in a HSP but not in a moderately sensitised patient. [1A]

20.1.15 Patient HLA antibody profiles should be reviewed at agreed intervals. This is particularly important for HSP – whereby the review of antibody profiles should include identifying delisting opportunities. Such opportunities should be discussed with the clinical team. E.g., historic HLA antibody positive, current negative, which may be an acceptable level of risk for a patient that otherwise faces long wait and poorer long term outcomes associated with dialysis. [1A]

20.1.16 A combination of tests should be considered in order to fully resolve complex antibody profiles. Using different manufacturers Luminex single antigen kits maybe beneficial during this assessment. [1B]

20.1.17 The clinical team must inform the laboratory of events that might influence the HLA antibody profile and send a serum sample 14 days after the event. These include the transfusion of blood products and treatment with therapeutic antibodies. [1B]

Rationale

There are limited studies in the literature addressing the question as to whether the presence of HLA-DSA at the time of transplant has a deleterious impact on islet graft outcome. In 2006 data in just seven patients was published describing outcomes in patients with HLA antibody positivity defined by CDC, ELISA and Flow bead methods and FCXM positivity, with the conclusion that "pre-existing HLA sensitization may be one of the factors that could be detrimental to the function of transplanted islets" (Mohanakumar et al., 2006). Another early study from the Edmonton group (n = 81), using Flow Beads for antibody analysis, indicated that the presence of HLA-DSA at the time of transplant led to a reduced C-peptide survival, although this study did not see an effect of a positive FCXM on outcome (Campbell et al., 2007). A 2016 study of 18 islet patients had 5 cases with moderate levels of class I and II DSA, measured by Luminex SAB, at the time of transplant, which were not associated with reduced graft function (Chaigne et al., 2016). Work from an Italian group looking at the relevance of changes to both HLA and Type 1 diabetes (T1D) associated autoantibodies even showed that patients having IgG and or/IgM HLA-DSA had improved islet transplant outcome (Piemonti et al., 2013). However, elegant mouse experiments investigating the relevance of HLA DSA in islet transplantation support the impact of pre-formed DSA by showing that the pre-transfer of donor specific alloimmune serum into mice led to a reduction in graft function when allogeneic donor islets were injected into the portal vein (Chen et al., 2018).

Based on this clinical and experimental data it has become practice for islet transplant recipients to be treated in a similar fashion to renal transplant recipients in that the presence of HLA antibodies is investigated at the time of listing and regularly whilst awaiting an offer, with unacceptable antigens listed to avoid donor HLA that may pose an immunological risk. In previous UK Islet Consortium H&I Subgroup guidelines, published in 2009, it was recommended that patients should be tested at 2 weeks, 4 weeks and then monthly following the first islet transplant, to identify *de novo* DSA that needed to be listed as unacceptable for a second or subsequent graft. However, as there is limited *de novo* HLA antibody production in islet recipients whilst they remain immunosuppressed (see below), less frequent testing may be indicated as long as antibodies are reassessed regularly after the first procedure and unacceptable antigens updated if required.

21 | Virtual crossmatching

21.1.1 HLA donor and recipient typing, HLA antibody testing and crossmatch and vXM results must be assessed, reviewed and reported by Health and Care Professions Council (HCPC) registered biomedical / clinical scientists specifically trained in histocompatibility and immunogenetics. [1A]

21.1.2 Prior to issuing a vXM, it is recommended that antibody screening/ specificity analysis is performed from two separate samples obtained at different time points. Including a recent sample [1A]

21.1.3 In cases where a patient has a stable HLA antibody profile or are consistently HLA antibody negative a vXM may be issued – in these cases the serum sample used to determine the absence of HLA-DSA should be less than 3 months old [1B]

21.1.4 Full donor HLA typing (HLA-A, -B, -C, -DRB1/3/4/5, -DQA1, DQB1, DPA1 and –DPB1) should be available for vXM interpretation. [1A]

21.1.5 Pretransplant assessment should be undertaken by carrying out a laboratory crossmatch test or, in selected cases, by performing a virtual crossmatch [1A]

21.1.6 Patients with a complex antibody profiles or incompletely defined antibody profiles should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). [1A]

21.1.7 If a prospective crossmatch is omitted, confirmation of the HLA antibody status should be assessed with samples obtained at the time of transplant. This confirmation may be performed retrospectively by Luminex HLA antibody testing, Flow Cytometry crossmatch or CDC XM. [1A]

21.1.8 We recommended that a vXM crossmatch result is reported before islets arrive at a transplant centre. [1B]

21.1.9 We recommend that confirmation a patient has had no sensitising events since the last sample tested should be sought from the clinical team at time of reporting a vXM [1B]

21.1.10 A risk assessment should be performed in conjunction with the transplanting centre to ensure the risks associated with a vXM are understood and mitigated. [2B]

21.1.11 We recommend that the sample date of the serum assessed for vXM should be considered in relation to the patient's sensitising events. In regrafts an updated HLA antibody test should be performed - with a sample no older than 2 weeks before the transplant date. [2B]

21.1.12 Historic HLA-DSA should be considered during vXM and Crossmatching [2B]

Rationale

Virtual crossmatching is acceptable for islet transplantation in line with local policies. As with pancreas translantation the recommendations align with those of renal transplantation, often however, given the time taken for islet isolation, updating HLA antibody testing or even wet crossmatching might be possible within normal working hours without impacting on the transplant timing. It should be noted that patients for initial islet transplantation often receive lymphocyte depleting agents at induction (e.g. Alemtuzumab) which interfere with the wet crossmatch, therefore a vXM may be the only option for assessing compatibility for second and subsequent islet grafts.

22 | Crossmatching

22.1.1 HLA donor and recipient typing, HLA antibody testing and crossmatch and vXM results must be assessed, reviewed and reported by Health and Care Professions Council (HCPC) registered biomedical / clinical scientists specifically trained in histocompatibility and immunogenetics. [1A]

22.1.2 Pretransplant assessment should be undertaken by carrying out a laboratory crossmatch test or, in selected cases, by performing a virtual crossmatch [1A]

22.1.3 HLA antibody data should be used in conjunction with the crossmatch results for crossmatch interpretation [1A]

22.1.4 Patients with a complex antibody profiles or incompletely defined antibody profiles should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). [1A]

22.1.5 Laboratory crossmatch tests should distinguish donor T cell and B cell populations. [1A]

22.1.6 Laboratory crossmatch techniques must be able to detect HLA IgG antibodies [1A]

22.1.7 Laboratory crossmatch testing must be able to exclude reactivity attributable to IgM antibodies. [1A]

22.1.8 The crossmatch report must include appropriate interpretation of the crossmatch results in the context of the patient's antibody profile. [1A]

22.1.9 CDC positive XM is a contraindication to islet cell transplantation, but lower levels of HLA-DSA that lead to a positive FCXM may on occasion be transplanted across in line with local policy.[1A]

22.1.10 Where a recipient has uncharacterized HLA-specific antibodies, or has a defined antibody but there is an incomplete donor HLA type (in relation to a recipient's antibody specificities, e.g., recipient has allele-specific antibodies), or where the intended recipient has known donor HLA-specific antibodies, a pre-transplant XM must be performed [1B]

22.1.11 Reactivity attributable to autoantibodies can be excluded in crossmatch interpretation – performing an auto crossmatch at the time of allocrossmatch should be considered. [1B]

22.1.12 Where intermediate or high-risk cases are being considered discussion between HCPC registered biomedical / clinical scientist staff who hold FRCPath and the Clinical team is advised. This discussion should include the chances of the patient receiving a lower risk offer. [1B]

22.1.13 Historic HLA-DSA should be considered during vXM and Crossmatching [2B]

Rationale

In line with the renal transplant recommendations crossmatching prior to islet transplantation may be required depending on the listing of unacceptable antigens and the presence of potential DSA. To aid interpretation of testing at the time of transplant, it is important to distinguish between alloreactivity and autoreactivity in the islet patient group by undertaking both allo and auto crossmatches.

23 | Post-transplant HLA antibody detection

23.1.1 Post-transplant serum samples should be taken at regular intervals on an agreed basis (in view of the perceived immunological risk at the time of transplant). [1A]

23.1.2 Post-transplant HLA-DSA testing (after all infusions for a patient have been completed) should be undertaken when graft dysfunction is suspected or when immunosuppression has been reduced, although early (~3 month post final graft) testing may provide a baseline for future DSA testing (2B).

23.1.3 In higher risk transplants (e.g., donor-specific antibody is present at the time of transplant) a timetable of post-transplant sampling must be agreed with the local transplant unit. [1A]

23.1.4 Post-transplant sampling for anti-HLA-DSA in immunologically low risk patients and/or patients with stable function post-transplant might be undertaken at locally defined time points, to aid in patient management (2B)

23.1.5 Post-transplant samples should be sent to the laboratory when graft rejection is suspected or antibody production a concern (e.g., following a change in immunosuppression or compliance issues). [1A]

23.1.6 The use of single antigen bead MFIs to reflect changes in the level of DSA, is semi quantitative but may be beneficial in patient monitoring and assessing therapeutic interventions [2B]

23.1.7 Epitope analysis software may benefit HLA antibody profile interpretation – donor and recipient HLA typing to the second field are recommended for this process [2B]

Rationale

Many studies have shown that islet transplants can induce *de novo* HLA antibody production, particularly after immunosuppression withdrawal (Chaigne et al., 2016; Hilbrands et al., 2013; Rios et al., 2021). However, there is still no consensus as to whether the production of *de novo* HLA-DSA in immunosuppressed patients affects graft survival, with some studies supporting a role for HLA antibody mediated rejection of islets (Brooks et al., 2015; Kessler et al., 2009) and others finding no association between HLA-DSA formation and graft loss (Chaigne et al., 2016; Pouliquen et al., 2017). Unpublished data from NHSBT shows that out of 39 patients receiving two islet donations, 11 (28%) produced HLA-DSA to either the first, second or both grafts. In 6 patients with DSA against both grafts, 3 lost function at a time point after or coincident with DSA being identified against both donors. However, data on the immunosuppression in these patients at the time of *de novo* DSA production is limited, so it is unclear whether antibody production caused rejection or was a result of immunosuppression tapering when the grafts were deemed to have failed.

A potential explanation for a limited impact of de novo HLA-DSA on islet graft survival is the observation that revascularisation of islets derives mainly from recipient cells, thereby limiting the frequency of donor HLA target antigens on the surface of endothelial cells. In a mouse model of allogeneic islet transplantation, the infusion of DSA twice weekly for 30 days post-transplant did not lead to microvascular lesions, which were seen in a comparable heart transplant model (Chen et al., 2018). This latter study also observed that vascular sequestration of immunoglobulin and complement components may also help to limit the damage caused by HLA-DSA, by preventing the migration of these immune mediators into tissues.

The 2009 UK Islet Consortium H&I Subgroup guidelines recommended that post second islet transplant samples should be sent to the lab at 2 weeks, 4 weeks and monthly up to 6 months and thereafter quarterly up to 5 years post-transplant. In the absence of any clear data indicating the benefit of post-transplant monitoring to predict islet allograft loss, it may not be appropriate to maintain this frequency of sampling, but to align with other forms of transplant and only request samples are sent for testing when rejection/graft loss is suspected. However, regular sampling between first and second (and subsequent) procedures is required to ensure the HLA antibody profile of the patient is clear to facilitate vXM where used.

24 | Non-HLA antibodies

24.1.1 No recommendation made

Rationale

Islet transplant recipients are at risk of recurrence of T1D, which would be observed as a loss of graft function. This would be caused by the re-emergence of autoreactive T cells and might be expected to be seen more frequently in well matched class II recipients given the known importance of class II as a target of autoimmune T cells, especially if donors expressed the T1D associated HLA class II proteins. In a study of pancreas transplant recipients, T1D recurrence occurred more often when donor and recipient were matched for HLA-DRB1, especially HLA-DR3 (Vendrame et al., 2016b). In the absence of routine methods for T cell monitoring, a number of studies have investigated autoantibodies associated with T1D, including anti-insulin, anti-GAD65, anti-IA2 and anti-ZnT8, pre and post islet

transplantation. Some studies have suggested that changes in autoantibodies may be associated with graft outcome (Lablanche et al., 2014; Piemonti et al., 2013), although this has not been confirmed in a more recent study (Anteby et al., 2021b). The writing committee did not feel that the evidence in the literature supported a recommendation regarding non-HLA antibodies in the islet transplant setting.

25 | Simultaneous islet kidney transplant recipients

25.1.1 Management according to local policy for renal transplant testing is recommended [2B]

Rationale

For patients being listed for simultaneous islet kidney (SIK) transplant, antibodies should be investigated and unacceptables listed as for other kidney transplant recipients in the centre. Islets will normally be transplanted 12–24 hours after the kidney procedure, therefore there will be little risk of immune consequences for the islet graft if the kidney transplant has been deemed immunologically safe to proceed.

Intestinal Transplant recommendations

Intestinal transplantation (Itx) is relatively new in the field of transplantation, with the first UK transplant taking place in Cambridge in 1988, ((Grant et al., 1990)) and few Itxs performed worldwide each year compared to other types of solid organ transplant (SOT). For example, in the UK 134 adult Itxs were performed between 2010–2020, compared to 3,190 adult kidney transplants in year 2019–2020 alone (NHSBT, 2020, 2021). It is partly due to the novelty of the field, as well as the unique immunological and surgical challenges with Itx itself, why outcomes following Itx are inferior compared to other SOT types. The Intestinal Transplant Registry (ITR) analysed outcomes of 2887 transplants in 2699 patients across the world, and found that survival rates were 76%, 56% and 43% at 1, 5 and 10 years post-transplant respectively (Grant et al., 2015). This compares to kidney transplantation in the UK where patient survival is 98%, 88% and 75% at 1, 5 and 10 years post-transplant respectively. It is evident that patient survival in Itx remains a challenge. Improvements in the first year following transplant have been observed in recent years due to the advances in immunosuppressive protocols, surgical techniques, and antimicrobial therapy, however these effects are not seen long-term. Achieving long-term patient and graft survival following Itx is now the focus of the next era of Itx.

The ITR report stated that practices and outcomes across the world are now similar, however we recognise that centre variability still exists, for example at Cambridge they prefer not include the stomach in Itx as previous experience has resulted in poorer outcomes (due to gastroparesis). (Rutter et al., 2016) The report also highlighted a worldwide decline in patients undergoing Itx, which is in contrast to the UK where an increase in adult Itx has been observed in the past 10 years (NHSBT, 2020; Rutter et al., 2016). It is important to consider this variation between centres, as generalised outcome data from multiple centres may not reflect individual practices.

The nature of ITx itself presents a number of unique challenges, particularly regarding the immunological feasibility of transplantation (S. J. Middleton & Jamieson, 2005)

Graft Rejection in Intestinal transplantation

The intestinal graft comprises the largest mass of lymphoid tissue in the human body (the gut-associated lymphoid tissue (GALT)), expression of HLA class II on intestinal epithelial cells is high, and the graft has good blood supply. This means that circulating antigen is readily presented. The graft is highly immunogenic, rendering itself susceptible to rejection (Berger et al., 2012) Additionally, patients are often highly sensitised prior to Itx adding further risk of rejection to transplant (Hawksworth & Matsumoto, 2019)

Rejection involves both cellular and humoral arms of the adaptive immune response and is provoked when mismatches between donor and recipient human leucocyte antigen (HLA) occur.

The cellular response, mediated by CD8+ T cell recognition of donor HLA and subsequent destruction of graft cells, can occur within hours or days following transplant and is termed acute cellular rejection (ACR). Despite advances in immunosuppressive regimes targeting ACR which have improved short-term success of Itx, ACR is still the primary cause of intestinal graft failure in the first two months post-transplant, suggesting that the optimal immunosuppressive regime for Itx is still unknown (Farmer et al., 2001; Koo & Wang, 2016).

The humoral response is referred to as antibody mediated rejection (AMR). In AMR, antigen presenting cells (APCs) capable of processing and displaying mismatched donor-derived peptide in HLA class II to CD4+ T cells, activate CD4+ T cells, which then stimulates the formation of class switched, high affinity IgG antibodies from plasma cells. To mount a *de novo* donor specific antibody (DSA) response via this mechanism takes some time, days-years in some cases (Gerlach et al., 2014), but if preformed DSA is already circulating in the patient at the time of transplant or a rapid memory response is generated, hyperacute rejection becomes a major risk and immediate graft loss may result (G.-S. Wu, 2016). DSA can trigger activation of the classical complement cascade via C1q, resulting in leukocyte recruitment, cell-mediated cytotoxicity and formation of membrane attack complexes on the surface of donor cells, culminating in graft cell damage (van Erp et al., 2019). For assessment of ACR in Itx, there are specific histological criteria and some endoscopic guidance, but findings are similar in AMR and there are no specific clinical features to single out an AMR diagnosis (Crismale et al., 2021; Ruiz et al., 2004). There is uncertainty around the relevance of C4d deposition (used in renal transplantation) as a marker for AMR in Itx; studies have produced conflicting findings (de Serre et al., 2008; Rabant et al., 2018). Clear-cut histopathologic guidelines for AMR, noting the relevance of DSA

in Itx, that are comparable to the likes of Banff criteria in renal transplantation would be ideal (Loupy et al., 2020). There is now increasing evidence of DSA involvement in long-term chronic rejection, (K. M. Abu-Elmagd et al., 2012; Berger et al., 2012; G.-S. Wu, 2016),which is a significant contributor to long-term graft failure, occurring in up to 10% of Itx patients (Lauro et al., 2018). Some individuals require eventual enterectomy and/or re-transplantation. This highlights the need for improved understanding of immune mechanisms in Itx, including the relevance of DSA, in order to improve long-term success in absence of rejection.

Liver Inclusion in Intestinal Transplantation

Multiple accounts of the liver providing protective effect in Itx have been reported in single-centre studies and international registries. Notably, the ITR reported significantly better graft survival outcomes with LITx (Grant et al., 2015), likewise to outcomes reported from Europe alone (Clarysse et al., 2020).

The liver is unique compared to other organs of the human body as it receives blood from the systemic circulation as well as the portal circulation linking to the gastrointestinal tract, and it also houses specialist immune cells. This facilitates a default tolerogenic environment so that when foreign antigen is brought from the gastrointestinal tract, activation of the immune system is avoided. The mechanisms of immunomodulation by the liver are not fully elucidated but it is acknowledged that the organ has immunological privilege. In the UK, isolated liver transplantation takes place without any pre transplant compatibility assessment for determination of immunological risk and cases of AMR are extremely rare (Taner, 2017). Studies have shown that HLA class I DSA in particular is preferentially cleared by the liver (Cheng et al., 2017), reasoning that the liver has strong expression of HLA class I, is able to secrete HLA class I antigens and absorb away any circulating HLA class I DSA (Abrol et al., 2019). This is reflected in current UK immunological risk guidelines for Itx which state that for LITx, HLA class I antibodies (unless at extremely high level) should not be included in the contraindicated list of specificities that would preclude transplantation (NHSBT, 2012)

Results from Pittsburgh in a cohort of 194 adults indicated better clearance of pre-transplant DSA and lower incidence of *de novo* DSA development and better graft survival with LITx (K. M. Abu-Elmagd et al., 2012). In a more recent study by the same centre with 212 adults, LITx proved more tolerable to rejection, but increased the risk of acquiring infection post-transplant (G. Wu & Cruz, 2018). At this centre however, recipients receive adjunct intravenous infusion of donor bone marrow cells and simultaneous *ex-vivo* graft irradiation which is not common practice in the UK. There are concerns regarding graft radiation damage (S. J. Middleton & Jamieson, 2005; Murase et al., 2000). Contextualising these results in the scope of UK practice should be approached with caution (S. J. Middleton & Jamieson, 2005). Similar findings were reported from a smaller study with 30 adults where, in the 33% of patients who produced *de novo* DSA post-transplant, fewer LITx recipients (n = 3) produced *de novo* DSA than LETx (n = 7) (Gerlach et al., 2014). This was speculated to result from the immunoprotection provided by the liver. LITx recipients also had reduced AMR risk in this study (Gerlach et al., 2014).

Conversely, in the Indiana study in 79 adults, LITx showed no benefit in clearing preformed DSA or preventing *de novo* DSA, but significantly more LETx patients experienced acute rejection (Kubal et al., 2015). However, authors speculated that differences in immunosuppression between LITx and LETx groups may have contributed towards this effect. Elsewhere, LITx has shown protection against rejection-related graft loss but not when persistent DSA is present (K. M. Abu-Elmagd et al., 2012; Cheng et al., 2017). In terms of overall patient survival, results have been conflicting. ITR data and data from a sizable Itx study from California found that LITx was a significant predictor of better survival outcomes (Farmer et al., 2010; Grant et al., 2015). However Pittsburgh data indicated poorer overall survival statistics for LITx patients, despite death-censored graft survival data for LITx showing superior outcomes (G. Wu & Cruz, 2018). Although there are mixed reports and differences in practices at Itx centres across the world, the general consensus appears to be that LITx is beneficial to post-Itx outcomes.

This seeming advantage of a concomitant liver transplant is restricted to patients listed for Itx with advanced liver disease only, and could not benefit all Itx patients due to the need for deceased donor livers to alleviate the high death rates worldwide in patients awaiting isolated liver grafts (Grant et al., 2015). Paradoxically, if a liver is required as part of the graft (applicable to approximately half of all Itx listed patients), patients will typically wait much longer for a transplant offer which may result in their condition worsening, but waiting for a liver may achieve better outcomes long-term. In time, we may better understand the immunomodulatory effects of the liver to be able to replicate the mechanisms by which it is able to harness the immune system.

26 | Pretransplant testing in Intestinal transplantation and transplant Immunological Risk Management

26.1.1 Laboratories must have procedures in place for the detection and characterisation of HLA Class I and II specific antibodies. [1A]

26.1.2 Laboratories must be able to define HLA-A, B, C, DRB1/3/4/5, DQA1, DQB1, DPA1 and DPB1 antibody specificities. [1A]

26.1.3 HLA antibody detection and identification techniques must be able to detect HLA IgG antibodies [1A]

26.1.14 HLA antibody detection and identification techniques should be able to exclude reactivity attributable to IgM antibodies. [1A]

26.1.5 Laboratories must employ methods to abrogate known causes of false positive or negative results (e.g., the additional of EDTA) [1A]

26.1.6 At least one solid phase assay should be used to detect and characterise HLA class I and II specific antibodies. [1A]

26.1.7 We recommend that during transplant assessment patients are screened for the presence of IgG HLA-specific antibodies using blood samples obtained on at least two separate occasions. [1B]

26.1.8 We suggest that, when preparing to activate a patient on the transplant waiting list, if IgG HLA specific antibodies are detected in the patient serum, the patient should be discussed with the relevant clinician responsible for patient care in conjunction with the H & I laboratory to determine if/which HLA specificities should be recorded as unacceptable antigens (UA) with NHSBT-OTDT [2B]

26.1.9 Which specificities to list as UA will depend on factors including but not limited to the patient's overall level of sensitisation (expressed as calculated reaction frequency (%cRF)), clinical urgency and whether a liver is included in the allograft (broadly following the stratification outlined as below). [2B]

Bowel with other organs including liver: the transplanted liver is resilient to all but the highest levels of donor HLA class I specific antibodies (i.e., those likely to result in a positive CDC crossmatch) and concomitant transplantation of the liver together with other organs confers a degree of protection from acute antibody mediated rejection (AMR). HLA class I antibodies should generally **not** be included in the contraindicated list of specificities however all HLA class II antibodies should be considered and discussed with the clinical team.

Bowel with other organs excluding a liver: The risks of transplanting against a known DSA should be balanced against the risks of not transplanting and the likelihood of the patient receiving an alternative donor with a lower immunological risk. Where bowel is transplanted in the absence of a liver, antibodies against all HLA loci should be considered equally. Antibodies to different specificities may differ in pathogenicity but there are insufficient peer-reviewed studies to define the magnitude of such differences.

26.1.10 For patients on the transplant list, regular samples must be sent to the histocompatibility laboratory for antibody testing. (3 monthly is suggested) [1B]

26.1.11 We recommend that samples should be taken for antibody screening at 2 and 4 weeks following a sensitising event (e.g., blood transfusion). If the patient is having ongoing transfusion support, we recommend that the laboratory agrees a pragmatic approach to testing with the clinical team [1B]

Rationale

Identification and characterisation of preformed DSA in the pre-transplant setting is crucial when evaluating patients for transplant. As patients are often highly sensitised prior to Itx (Hawksworth & Matsumoto, 2019), understanding the impact of pre-transplant DSA is important to establish so that the appropriate level of caution is applied to safeguard and not hinder access to transplantation.

Luminex X-Map technology is a semi-quantitative, solid-phase binding assay commonly used to identify HLA antibodies, including DSA, in patient sera pre- and post-transplant. HLA antibodies in the patient sera are detected via their specific binding to SABs of a unique HLA specificity which comprise a unique dye signature, as well as a secondary antibody-conjugate which fluoresces. Testing using SABs produces MFI values relative to the amount of IgG HLA antibody bound to beads. This is an extremely sensitive assay, not without its limitations, namely interferences of non-specific binding, cryptic epitopes, high dose hook effect and manufacturer differences (D. Middleton et al., 2014; South & Grimm, 2016). Over time, centres have gained more confidence understanding these limitations, and with expertise interpretation, the value of DSA detection in predicting graft outcomes has increased, facilitating virtual crossmatching.

Which specificities to list as unacceptable antigens with NHSBT-ODT depend upon several factors, including but not limited to, the patient's overall level of sensitisation (expressed as calculated reaction frequency (%cRF)), clinical urgency and whether a liver is included in the allograft (broadly following the stratification outlined as below).

Bowel with other organs including liver: the transplanted liver is resilient to all but the highest levels of donor HLA class I specific antibodies (i.e., those likely to result in a positive CDC crossmatch) and concomitant transplantation of the liver together with other organs confers a degree of protection from acute antibody mediated rejection (AMR). HLA class I antibodies should generally **not** be included in the contraindicated list of specificities however all HLA class II antibodies should be considered and discussed with the clinical team.

Bowel with other organs excluding a liver: The risks of transplanting against a known DSA should be balanced against the risks of not transplanting and the likelihood of the patient receiving an alternative donor with a lower immunological risk. Where bowel is transplanted in the absence of a liver, antibodies against all HLA loci should be considered equally. Antibodies to different specificities may differ in pathogenicity but there are insufficient peer-reviewed studies to define the magnitude of such differences.

With still very few transplants taking place worldwide, there are few centres able to comment on the implications of pre-transplant DSA in Itx. The outcomes from six major recent Itx studies are summarised (table 26.1). Evident from all studies is that presence of pre-transplant DSA is common, detected in 7.6-38% of patients, indicating that sensitisation events prior to Itx are common. Pittsburgh and Miami studies clearly indicated that pre-transplant DSA results in higher incidences of acute rejection and graft loss (K. M. Abu-Elmagd et al., 2012; Tsai et al., 2011). The Pittsburgh study cohort is of reasonable size, with considerable follow-up period and a breadth of pre-transplant DSA to analyse, therefore conclusions drawn seem credible. As previously discussed however, the Pittsburgh study data should be interpreted with caution as their pre-transplant conditioning practices are quite different to the UK. The Miami study is less reliable, being smaller and with only five patients harbouring pre-transplant DSA. In 2010 the California group stated that pre-transplant DSA was the most powerful multivariate predictor for both graft loss and patient survival, with poorer outcomes in LETx, however pre-transplant DSA status was

TABLE 26.1 Summary of literature examining pre-transplant DSA and the protective effects of the liver in Itx.

Summary of recent studies noting outcomes of pre-transplant DSA and liver protective effects in Itx									
					Outcome of pre-transplant DSA:			Outcome of including the liver in Itx:	
Study (year), location/group	Cohort size (N = number of patients)	LITx %	Method of assessment of DSA	Pre-transplant DSA prevalence	Rejection	Graft Loss	Patient survival	Liver protective against pre-transplant DSA clearance?	Liver protective against *de novo* DSA development?
Tsai *et al.* (2011) Miami[63]	N = 13 (5 adults)	69%	Luminex SAB >3000 MFI	38%	Increased	Increased	Decreased	Not reported	Not tested
Abu-Elmagd *et al.* (2012) Pittsburgh[29]	N = 194 adults	37%	ELISA or Luminex SAB >1000 MFI	31%	Increased	Increased	Not reported	Yes	Yes
Gerlach *et al.* (2014) Germany[21]	N = 30 adults	40%	ELISA or Luminex SAB >1000 MFI	0%	No patients transplanted with pre-transplant DSA.			Not reported	Yes
Kubal *et al.* (2015) Indiana[47]	N = 79 (59 adults)	51%	Luminex SAB >1000 MFI	13%	No impact	No impact	Not reported	No impact But significantly fewer LITx patients had acute rejection and acute rejection-related graft loss	No impact
Cheng *et al.* (2017) California[42]	N = 95 (34 adults)	73%	Luminex SAB >1000 MFI	11%	No impact	Increased	Not reported	Not reported But LITx lowered risk of rejection-related graft loss, but not when persistent DSA present	No impact
Farmer *et al.* (2010) California[48]	N = 88 (28 adults)	76%	PRA screening – by CDC or Luminex	7.6%	Not reported	Increased	Decreased	Not reported But LITx was a significant predictor of survival	Not reported

unknown in 43.3% of patients in this study due to evolving practices during the study period (Farmer et al., 2010). In their later 2017 study involving further SAB testing of all patients with positive PRA, they concluded that although pre-transplant DSA did increase the cumulative incidence of acute rejection slightly, this was not significantly different when compared to patients without pre-transplant DSA (Cheng et al., 2017). Similar outcomes from the Indiana study showed that pre-transplant DSA did not impact graft rejection or survival (Kubal et al., 2015). Also from the California study, conversely, earlier graft failure and poorer graft survival outcomes were associated with patients with pre-transplant DSA. The conflicting significance of findings from the California group may be as a result of small sample size, as only 11% of patients had pre-transplant antibody DSA (Cheng et al., 2017). Data from the 2010 California study showed early evidence that pre-transplant DSA was a significant dominant risk factor for mortality after Itx (Farmer et al., 2010). This was also demonstrated in the Miami study where pre-transplant DSA was associated with greater mortality (Tsai et al., 2011). Finally, a UK study awaiting publication examined a retrospective cohort of 95 adult Itx patients transplanted between 2007–2019 at Cambridge. 54 (57%) Itx cases contained a liver, and 28 (29%) harboured pre-transplant DSA. Using the Kaplan-Meier survival method, pre-transplant DSA at any level greater than 500 MFI as identified by Luminex single antigen beads, seemed to negatively affect post-Itx survival and rejection outcomes. Additionally, liver-inclusive allografts seemed to show particular resistance to HLA class I DSA. Evidently, findings are inconsistent between centres and there is no consensus on the role of pre-transplant DSA influencing post-Itx patient outcomes. This could be reflective of the different practices at each centre, including differences in: immunosuppressant strategies, surgical preferences, experience, level of risk/severity of disease burden at transplant, and methods/definitions of DSA assessment. Also, as most studies report results surmised from both paediatric and adult cohorts, results may not translate directly into adults. Paediatric Itx typically includes a smaller volume lymphoid tissue, and a younger, immature immune system may be more immunologically malleable, potentially resulting in seemingly better Itx outcomes compared to adults (Selvaggi et al., 2007; G. Wu & Cruz, 2018).

Immunological risk is currently interpreted from guidelines developed in 2013 by the MCTAG on behalf of NHSBT OTDT (table 26.2)).The level of immunological risk assigned to a donor/recipient pair, in addition to consideration of other clinical risk factors, helps inform clinicians on whether to proceed to transplant versus holding out for a potentially more suitable offer in the future. Patient management post-transplant can be tailored according to immunological risk and clinical status, highlighting the importance of providing an accurate assessment of pre-transplant DSA.

As previously mentioned, in LITx, current guidance is to include only HLA class II and not HLA class I antibodies (unless at extremely high level) in the list of contraindicated specificities, as the liver has shown resilience to most HLA class I antibodies. In LETx however, all HLA class I and II antibodies should be considered. This is in line with current literature (Matsumoto & Rosen-Bronson, 2021).

TABLE 26.2 NHSBT ODT MCTAG criteria for defining immunological risk based on pre-transplant Luminex SAB and crossmatch results.

NHSBT ODT MCTAG criteria for defining immunological risk based on pre-transplant Luminex SAB and crossmatch results		
DSA MFI cut-offs	**Crossmatch results**	**Immunological Risk**
0	Flow cytometry and CDC crossmatch likely negative	Standard risk
<2000	Flow cytometry and CDC crossmatch likely negative	Low risk
2000-8000	Flow cytometry crossmatch likely positive	Intermediate risk
>8000	CDC crossmatch likely positive	High risk

It is important to note, as Luminex SAB assay results are only semi-quantitative (that is, the MFI level of an antibody for a particular epitope is not precise and is only ever an estimation of how much antibody is actually present), DSA MFI cut-offs are somewhat arbitrary. DSA MFI cut-offs in an immunological risk assessment have evolved through clinical necessity as a means to evaluate DSA somewhat objectively in the context of a donor offer. They are not hard cut-offs applied in a strict fashion, but are more fluid, to allow for variation in batches/lots (e.g., due to fluctuating negative control values) and variation between centres (Sullivan et al., 2017). The clinical context of SAB results with knowledge of the patient's sensitisation history is important to consider and will affect overall interpretation of results. Other factors, such as epitope sharing (which may dilute the apparent strength of an antibody over multiple beads), and variable amounts of HLA molecules on beads will also affect the non-precise MFI output (Tait et al., 2013). It is acknowledged that there are inherent problems associated with this technology, however it is the best technology available at present for an assessment of HLA antibodies via virtual crossmatch. Where it is warranted, a "wet" pre-transplant crossmatch could be performed which would help evaluate the status of HLA antibodies present to establish their clinical relevance, although in practice this rarely occurs primarily due to the complex logistics of Itx.

27 | Crossmatching and vXM recommendations

27.1.1 Laboratory crossmatch tests should distinguish donor T cell and B cell populations. [1A]

27.1.2 Laboratory crossmatch techniques must be able to detect HLA IgG antibodies [1A]

27.1.3 Laboratory crossmatch testing must be able to exclude reactivity attributable to IgM antibodies. [1A]

27.1.4 HLA antibody data should be used in conjunction with the crossmatch results for crossmatch interpretation [1A]

27.1.5 Reactivity attributable to autoantibodies can be excluded in crossmatch interpretation – performing an auto crossmatch at the time of allocrossmatch should be considered. [1B]

27.1.6 The crossmatch report must include appropriate interpretation of the crossmatch results in the context of the patient's antibody profile. [1A]

27.1.7 In cases where a patient has a stable HLA antibody profile or are consistently HLA antibody negative a vXM may be issued – in these cases the serum sample used to determine the absence of HLA-DSA should be less than 3 months old [1B]

27.1.8 We recommend that confirmation a patient has had no sensitising events since the last sample tested should be sought from the clinical team at time of reporting a vXM. Otherwise, we suggest that prospective antibody characterisation is undertaken using a day of transplant serum sample.[1B]

27.1.9 We recommend that the sample date of the serum assessed for vXM should be considered in relation to the patient's sensitising events. In regrafts an updated HLA antibody test should be performed - with a sample no older than 2 weeks before the transplant date. [2B]

27.1.10 HLA donor and recipient typing, HLA antibody testing and crossmatch and vXM results must be assessed, reviewed and reported by Health and Care Professions Council (HCPC) registered biomedical / clinical scientists specifically trained in histocompatibility and immunogenetics. [1A]

27.1.11 Prior to issuing a vXM, it is recommended that antibody screening/ specificity analysis is performed from two separate samples obtained at different time points. Including a recent sample [1A]

27.1.12 Full donor HLA typing (HLA-A, -B, -C, -DRB1/3/4/5, -DQA1, DQB1, DPA1 and –DPB1) should be available for vXM interpretation. [1A]

27.1.13 Patients with complex antibody profiles or incompletely defined antibody profiles should be prospectively crossmatched using flow cytometric techniques and/or complement dependent cytotoxicity (CDC). [1A]

27.1.14 If a prospective crossmatch is omitted, confirmation of the HLA antibody status should be assessed with samples obtained at the time of transplant. This confirmation may be performed retrospectively by Luminex HLA antibody testing, Flow Cytometry crossmatch or CDC XM. [1A]

27.1.15 A risk assessment should be performed in conjunction with the transplanting centre to ensure the risks associated with a vXM are understood and mitigated. [2B]

27.1.16 For patients with pre-transplant donor specific antibodies (DSA) these should be reported to the clinical team. The clinical risk of undertaking transplantation should be assessed together with the risk of delaying transplantation and the likelihood of identifying an alternative suitable donor. We suggest that the overall degree of sensitisation should be reported as %cRF to aid in this assessment. The following stratification according to organ type should be applied:

Bowel with other organs including liver: the transplanted liver is resilient to all but the highest levels of donor HLA class I specific antibodies (i.e., those likely to result in a positive CDC crossmatch) and concomitant transplantation of the liver together with other organs confers a degree of protection from acute antibody mediated rejection (AMR). HLA class I antibodies should generally **not** be included in the contraindicated list of specificities however all HLA class II antibodies should be considered and discussed with the clinical team.

Bowel with other organs excluding a liver: The risks of transplanting against a known DSA should be balanced against the risks of not transplanting and the likelihood of the patient receiving an alternative donor with a lower immunological risk. Where bowel is transplanted in the absence of a liver, antibodies against all HLA loci should be considered equally. Antibodies to different specificities may differ in pathogenicity but there are insufficient peer-reviewed studies to define the magnitude of such differences.

27.1.17 We suggest that, in the post-transplant period, testing for donor specific antibodies is performed at regular intervals (1, 3, 6, 9 and 12 months) and when there are clinical concerns of graft function (Grade 2B).

Rationale

The relevance of DSA and a CDC positive crossmatch in Itx is not clear. Historically, the approach at some centres has been to perform Itx irrespective of DSA presence and a positive CDC crossmatch status, however it is from these studies that increased frequency and severity of rejection episodes, as well as graft loss in CDC crossmatch positive patients, has been observed (K. Abu-Elmagd et al., 1998; Bond et al., 2000).

Some early research reported severe perioperative observations of spasming of the allograft with cyanotic discoloration, haemorrhaging, and severe mucosal congestion, which were later attributed to CDC crossmatch positive patients (T. Wu et al., 2004). Since these early studies, centres across the world appear to be more cautious with regards to taking the decision to transplant in the presence of pre-transplant DSA, although practices are still variable. The Pittsburgh group still do not consider a CDC positive crossmatch a contraindication to transplant (G. Wu & Cruz, 2018), as they have previously found positive T and B cell CDC results not to impact overall or allograft survival (K. M. Abu-Elmagd et al., 2009). Whereas some centres, e.g., Germany, hold a more conservative approach, transplanting only in complete absence of DSA and with a negative CDC crossmatch (Gerlach et al., 2014). At other centres, clinicians tend to evaluate more on a patient-by-patient basis, where immunological risk is considered in the context of the patient's clinical status; urgency for transplant may be prioritised, and the importance placed on pre-transplant DSA downgraded. A similar approach is taken at some centres in the United States but the

acceptance level of DSA MFIs with respect to future potential donor offers is evaluated prospectively (Matsumoto & Rosen-Bronson, 2021).

"Wet" crossmatching using either/both Complement Dependent Cytotoxicity (CDC) and/or Flow Cytometry techniques are currently utilised by some centres. Both methods involve an assessment of reactivity occurring between donor lymphocytes and recipient sera containing possible IgG HLA antibodies. The CDC assay is a functional test, providing evidence of clinically relevant, complement fixing DSA in the patient serum (Patel & Terasaki, 1969). Typically, IgG DSA at high MFI level fixes complement, however complement fixing autoantibody (usually IgM) could give false-positive CDC results. Addition of dithiothreitol (DTT) is used to discriminate IgM antibodies, which have limited pathological relevance in transplantation, from IgG . Flow Cytometry is a more sensitive crossmatching technique, capable of detecting non-complement fixing antibodies, non-HLA antibodies and low level DSA, as well as autoantibodies. Despite differences in crossmatching practices in UK H&I laboratories, all results contribute to informing a level of immunological risk for a specific donor offer.

Cardiothoracic transplantation guidelines

Cardiothoracic organ allocation in the UK is based on blood group and size match, taking into account clinical urgency and HLA antibody risk (NHSBT ODT POL 228/13, 2023; NHSBT ODT POL 230, 2023). IgG HLA specific antibodies present in the recipient, directed against mismatched donor antigens remain a serious cause of morbidity and mortality for patients following cardiothoracic transplantation (Kobashigawa et al., 2018). The development of antibody-mediated rejection (AMR) is responsible for up to 40% of deaths before 5 years in cardiac transplant patients (Barten & Zuckermann, 2019). This has been linked to development of DSA to the graft, but there is no established threshold level between AMR development and DSA levels, as well as no clear treatment strategies for when DSA has developed (Su et al., 2019).

28 | Pre-Transplant Testing recommendations

28.1.1 That two independent samples are to be tested for HLA specific antibodies before listing a patient on the transplant waiting list. Exceptions to this due to the clinical urgency of the patient should be agreed locally.[1B]

28.1.2 Single antigen beads should be used to determine the HLA antibody profile in the presence of a positive antibody screening result. [1B]

28.1.3 Laboratories must be able to define HLA-A, B, C, DRB1/3/4/5, DQA1, DQB1, DPA1 and DPB1 antibody specificities. [1B]

28.1.4 HLA antibody detection and identification techniques should be able to exclude reactivity attributable to IgM antibodies. [1B]

28.1.5 The priming source (i.e., pregnancy; blood transfusion; previous transplant; insertion of ventricular assist device (VAD) in the presence of blood products) and the magnitude and duration of the HLA specific antibody response that may then develop should be taken into account when interpreting HLA antibody screening results. [1B]

28.1.6 Results from single antigen beads (SAB) should be used to determine the overall degree of sensitisation which should be reported as a calculated reaction frequency (cRF%). The cRF% should ideally be reported at the different levels of immunological risk defined in this guideline: [1B]

- MFI 500 – 1999
- MFI 2000 – 4999
- MFI ≥ 5,000

(please note: the MFI levels stated above were derived from analysis using OneLambda Single Antigen Bead kits – alternative vendor kits may be used and equivalence to these MFI values determined)

28.1.7 Samples should be sent from patients on the waiting list for antibody screening at regular intervals, ideally at least three monthly for all patients. [1B]

28.1.8 Samples should be obtained for antibody screening at 2 and 4 weeks following a potential sensitising event. Where a patient is receiving ongoing transfusion support, the timing of testing should be agreed in a local policy. [1B]

28.1.9 When a patient has detectable HLA specific antibodies, HLA typing of the patient should be performed to aid interpretation of results. [1B]

28.1.10 All individual HLA antibodies detected by CDC (and C3d or C1q solid phase assays if validated by the laboratory) or at MFI level likely to cause a positive CDCXM, should be considered as representing the highest risk for development of antibody mediated rejection (AMR). [1B]

Rationale

Since Patel and Terasaki demonstrated a correlation between a positive pre-transplant complement-dependent cytotoxicity crossmatch (CDCXM), hyperacute rejection (HAR) and graft loss (Patel & Terasaki, 1969), detection and characterisation of HLA specific antibodies using CDC together with the pre-transplant crossmatch became the standard approach for defining immunological risk in transplantation. However, despite a very strong association with clinical outcome, as technologies advanced, it became apparent that detection of HLA specific antibodies solely by CDC was neither sensitive nor specific enough to detect all pathogenic antibodies. This led to the development of the more sensitive flow cytometry crossmatch (FCXM) and solid phase assays (SPA) such as ELISA and bead-based Luminex™ assays.

Currently, the main method of antibody detection and characterisation used in laboratories in the UK is the solid-phase bead assay, and more specifically Luminex™ based testing. This consists of incubating patient serum with fluorochrome impregnated beads displaying HLA antigens, staining with a fluorescence-conjugated secondary antibody, and then running these in single file past two lasers, one of which identifies the specific bead due to its unique fluorochrome signature, and the second which detects the amount of bound antibody in a semi-quantitative manner, giving this amount as median fluorescence intensity (MFI). The beads can either contain multiple HLA antigens giving an indication of whether a person has antibodies to HLA class I or II (antibody detection or screening), or individual HLA antigens, allowing characterisation of the specific HLA antibodies present, termed single antigen beads (SAB). The results from the SAB are typically expressed in the UK as a calculated reaction frequency (cRF %), which is the antibody reactivity against 10,000 consecutive deceased organ donors. NHSBT-OTDT have provided a tool that can be used to calculate this.

The high specificity of Luminex™ assays (and resultant high negative predictive value) allowed for the development of the virtual crossmatch (vXM) which has been in routine clinical practice in the UK for over 20 years (Taylor et al., 2000, 2010). The vXM assesses immunological compatibility between a recipient and potential donor by analysing the results of the recipient HLA specific antibody screening tests and the donor HLA type. To be able to utilise the solid-phase bead assay for vXM, detailed knowledge of the recipient's sensitisation history is extremely important when interpreting HLA antibody screening results and a single cut-off value cannot accurately be applied to all tests. The high sensitivity of SAB has led to a limited positive predictive value (50.0% and 51.6% for positive CDCXM and FCXM respectively) (Morris et al., 2010), suggesting that low level antibodies detected by Luminex™ alone may not be clinically relevant.

The MFI result generated from the SAB assay in itself is not a true representation of antibody load (Levine et al., 2016) as the assay is semi-quantitative and MFI is not a true representation of the strength of an antibody. MFI levels are affected by antigen density on any given bead and its numerical value can neither be compared across different beads or different batches of kits, nor is it an accurate representation of actual antibody titre (Wehmeier, Hönger, et al., 2020). In addition, studies show an inter-laboratory coefficient variation (CV) of 65% (Reed et al., 2013) and there are inter-batch variations, potentially making a difference of up to 50% in MFI clinically insignificant (Tambur et al., 2018). There are also variations reported in MFI levels when the same samples are tested with kits from different Luminex™ suppliers (Tait et al., 2013) and the HLA antigen representation on the single antigen bead panels varies between suppliers.

MFI levels are also influenced by the epitope that the antibody recognises. For instance, an antibody reacting with an epitope that is present on several beads can be falsely underestimated. Although the most obvious example of this is the case of a patient sensitised against Bw4 or Bw6 epitopes, other shared epitopes such as those on HLA-DP antigens should not be ignored as the sensitisation against HLA-DP antigens is predominantly due to reactivity against a limited number of cross-reactive immunogenic epitopes (Cano & Fernández-Viña, 2009; Simmons et al., 2016).

Another reason for the lack of association between Luminex™ detected DSA and clinical outcome may be the presence of antibody reacting with denatured HLA antigens on Luminex™ SAB kits (El-Awar et al., 2009). While patients receiving kidney transplants with DSA reacting to native HLA antigens had significantly lower graft survival ($P = 0.007$) compared to those without DSA, reactivity with denatured HLA antigens was not associated with decreased graft survival (Otten et al., 2013). The presence of antibodies reacting with cryptic epitopes on denatured antigens can result in a positive vXM in the absence of a positive CDCXM or FCXM (Jacob et al., 2011), resulting in denial of an organ transplantation or unnecessary desensitisation treatment. However, it should be noted that denatured HLA antigens may be present on activated T- and B-cells (Matko et al., 1994) and may result in positive crossmatches (Oaks et al., 2014). SAB kits are marketed by two vendors (One Lambda Inc., Canoga Park, CA, USA and Immucor, Life Codes Corporation, Stamford, CT, USA). Although both SAB kits are affected by the presence of denatured HLA antigens, the frequency and the beads that are affected differ (Battle et al., 2022) and using both kits may assist in detecting potentially clinically irrelevant reactivity to the denatured beads.

Utilising epitope analysis is another approach that may help with identifying such reactivity as the specificities that do not fit with a clear epitope reactivity may be false positive due to denatured antigens. Programs such as HLA matchmaker (Duquesnoy, 2008) can be utilised clinically to facilitate transplantation, as demonstrated with its use in the Eurotransplant acceptable mismatch program for highly sensitised patients (Heidt et al., 2018). Much work has been undertaken to publish known HLA epitopes with both the HLA matchmaker and Terasaki epitope groups creating registries. Regardless of the source of the definition of the epitope, there is a growing body of evidence that utilizing an epitope based approach to antibody analysis will be a useful addition to determining HLA profiles in patients. A recent study used HLAMatchmaker, PIRCHE-II and HLA epitope mismatch algorithm (HLA-EMMA) to calculate eplet mismatch (EpMM) loads, T-cell epitope mismatch (TEpMM) loads and solvent accessible amino acid mismatch (SAMM) loads, respectively and showed the benefit of epitope based assignment of risk in the heart transplant setting (Bedford et al., 2022).

The standard Luminex™ assay has been further modified to detect C3d and C1q binding antibodies and therefore has an ability to distinguish between complement fixing and non-complement fixing antibodies. These assays have superior specificity when compared to CDCXM but inferior sensitivity with C1q being more sensitive than C3d (Tait et al., 2013). The C3d and C1q assays have been shown to detect a sub-set of antibodies capable of fixing complement and which are predictive of AMR early after transplant (Chin et al., 2011a; Kang et al., 2022; Smith et al., 2007).

Risk factors for the development of HLA specific antibodies include pregnancy; previous transplant; transfusions and implantation of human homograft tissue or ventricular assist devices (VAD) with the concomitant administration of blood products (Kobashigawa et al.,

2018). In one kidney transplantation study (Higgins et al., 2015), the largest increase from pre-transplant HLA DSA MFI levels to peak post-transplant MFI level were detected in patients where the DSA also correlated with previous pregnancy sensitisation. The next highest increases were seen for patients sensitised by transplant with repeat HLA epitope mismatches, followed by transfusion-induced sensitisation.

Development of sensitisation to HLA associated with ventricular assist device (VAD) implantation appears to pose a low risk (Shankar et al., 2013). Studies suggested that reactivity detected by Luminex™ in VAD patients was due to production of anti-albumin antibodies, which could bind beads on SAB kits (Newell et al., 2006) but did not lead to positive FCXM results or adverse clinical outcomes (Nikaein et al., 2012). However, if a VAD implant occurs with the concomitant administration of blood products there is evidence that sensitisation to HLA can occur in this scenario (Halpin et al., 2019).

There is little data on the relevance of historic transient HLA antibodies versus HLA antibodies detectable on the day of transplant. In one case report, accelerated acute severe antibody mediated graft failure was reported in a 31 year old male heart transplant patient with no pre-transplant circulating HLA antibodies. The transplant took place 17 years following a heart valve replacement at which time transient HLA antibodies were reported (Andreas et al., 2018) but beyond this there is a scarcity of published literature on the relevance of transient HLA specific antibodies. UK practice is to treat historic transient antibodies as non-relevant if not detectable on the day of transplant dependent on the priming source.

While a clear and strong association exists between antibodies detected by CDC with graft loss (Patel & Terasaki, 1969), and an increased risk of cellular and vascular rejection and poor graft survival with a positive FCXM (S. Aziz et al., 1998; McCarthy et al., 1998), studies into the clinical impact of DSA detected by Luminex™ alone have produced conflicting results. Studies in kidney transplantation demonstrated that these antibodies were associated with a higher incidence of acute antibody mediated rejection (AMR) but had no effect on acute cell-mediated rejection (ACR) and produced conflicting results on their impact on graft survival (Caro-Oleas et al., 2012; Kannabhiran et al., 2015; Kwon et al., 2018; Lefaucheur et al., 2010; Parajuli, Aziz, et al., 2021a; Salvadé et al., 2016; Thammanichanond et al., 2012; Wehmeier et al., 2021; Ziemann et al., 2019). Thammanichanond and colleagues (Thammanichanond et al., 2012) showed that recipients with a negative CDCXM but positive DSA detected by Luminex™ had a higher incidence of AMR but with no statistically significant impact on delayed graft function, patient survival or 1- and 5-year graft survival, when compared with DSA negative patients. Similarly, (Parajuli, Bath, et al., 2021) did not see any effect of DSA on graft or patient survival. In contrast, Caro-Oleas et al. (2012) and Lefaucheur et al. (2010) reported a lower graft survival in patients with pre-formed DSA detected by Luminex™ despite a negative CDCXM. The presence of DSA was not associated with decreased patient survival (Lefaucheur et al., 2010). A CDCXM negative, FCXM positive result in the presence of DSA is not a contraindication to cardiothoracic transplantation but has been shown to be associated with AMR (Couzi et al., 2011).

There are several methods available for the definition of an individual's HLA type currently in clinical use in the UK and these vary between different laboratories. For solid organ transplantation, DNA based typing that allows "split" antigens to be defined is required for deceased donors. Although the recipient HLA type is not required pre-transplant for cardiothoracic transplants as it is not considered in the organ allocation process, it is essential for the interpretation of HLA antibody detection and characterisation results as well as establishing compatibility between a donor and HLA sensitised patients. Recipient HLA typing to the second field level is not required but is helpful in instances where patients have allelic antibodies, in order to distinguish whether a donor with a particular allele may be compatible. The main methods used in deceased donor typing are polymerase chain reaction (PCR) sequence-specific primers, PCR sequence-specific oligonucleotides or real-time PCR, which can provide a low to intermediate resolution HLA type within the 4 hours timeframe required by NHSBT-OTDT. High resolution HLA typing can be achieved by next and third generation sequencing, but the current turnaround time exceeds that required for deceased donors, although improvements with this technology and turnaround times are expected.

29 | Recommendations at the time of an organ offer

29.1.1 The transplant unit must confirm that no potential sensitising event has occurred since the last sample tested for HLA specific antibodies. Otherwise, prospective testing for HLA specific antibodies is suggested with omission subject to a documented risk assessment.[1B]

29.1.2 Patients that are either negative for HLA specific antibodies or have fully defined HLA specific antibodies tested within the last 3 months using single antigen beads can be transplanted on the basis of a pre-transplant vXM. Deviation from this should be subject to a documented risk assessment. [1B]

29.1.3 A vXM in a patient sensitised to HLA should utilise the results from the most recent sample to determine immunological risk. However, consideration should also be given to historical (i.e., over 6 months old) HLA sensitisation and prior sensitising events. There should be a locally agreed policy for defining the level of immunological risk in these "peak positive, current negative" patients. [1B]

29.1.4 Consideration should be given to the balance of transplanting versus not transplanting a patient when performing a vXM. This is of particular importance in those patients who are sensitised to HLA and/or are exhibiting clinical deterioration and listed on urgent or super-urgent waiting lists. [1B]

29.1.5 The laboratory should have an agreed strategy for managing offers for patients without fully defined HLA specific antibodies. [1B]

29.1.6 All vXM must be assessed and reported by Health and Care Professions Council (HCPC) registered biomedical / clinical scientists specifically trained in histocompatibility and

immunogenetics. Consultant H&I advice should be available at all times and must be sought when the guidance in this document is deviated from. [1B]

29.1.7 Risk levels of donor-specific antibodies should be calculated at the time of an offer. The assignment of risk should include the following considerations: [1B]

- Whether the donor is homozygous at a given loci (and MFI level of DSA doubled)
- Presence of supertypic antibodies such as Bw4
- Cumulative versus highest MFI of donor mismatches.
- All HLA loci should be treated as equal.

Levels of immunological risk should be defined according to following levels:

V No DSA detected by Luminex™ we recommend this confers **standard immunological risk**

VI DSA at a level that corresponds to a minimum risk of HAR but an increased risk of AMR. We recommend this is MFI 500 – 1999 and confers **low immunological risk**

VII DSA at a level that corresponds to a low risk of HAR but a significant risk of early rejection and graft damage. We recommend this is MFI 2000 – 4999 and confers an **intermediate immunological risk**

VIII DSA at a level which corresponds to a significant risk of HAR and a veto to transplantation apart from in exceptional cases. We recommend this is an MFI > 5,000 and confers a **high immunological risk**.

(please note: the MFI levels stated above were derived from analysis using OneLambda Single Antigen Bead kits – alternative vendor kits may be used and equivalence to these MFI values determined)

Rationale

For cardiothoracic transplants the total ischemic time has been shown to affect transplant outcome (Banner et al., 2008). For donation after brainstem death (DBD) donors 4 hours cold ischemia time (CIT) is thought to be acceptable (Vandendriessche et al., 2021) and an extended CIT (>3.5h) has been shown to be detrimental to survival (M. J. Russo et al., 2007). There have been recent advances organ preservation such as machine perfusion allowing for a greater flexibility in donor management which may alleviate the time pressures associated with cardiothoracic transplantation.

Risk stratification based on a prospective positive CDCXM or FCXM would be relatively straightforward, but due to the time constraints, risk stratification is performed using a vXM based on the MFI results obtained from SAB. It is generally accepted that a positive CDCXM would be a contraindication to transplantation while a positive FCXM would not be a contraindication to transplant but would represent an increased risk of cellular and humoral rejection. Although it has been reported that an MFI less than 2000 will result in negative CDCXM and FCXM and an MFI greater than 8000 will result in positive CDC XM (Colvin et al., 2019; Tambur & Lavee, 2016), identifying an MFI cut-off point that will translate to a positive cell-based crossmatch and defined clinical significance is not a straightforward task. In one small single centre study which considered FCXM results in 55 patients, DSA with MFI less than 2000 was predictive of a negative crossmatch; DSA greater than 5000 MFI was predictive of a positive crossmatch, while DSA between 2000 and 5000 was likely, though not certain to be associated with a negative crossmatch. In a larger retrospective study of 102 crossmatches, DSA MFI > 2215 was significantly associated with a positive flow cytometric crossmatch ($p<0.001$) while DSA MFI > 4689 was significantly associated with a positive CDC crossmatch ($p<0.001$) (Pandey et al., 2021)

Many studies use an MFI of 2000 as a cut-off value for positivity, as lower levels are generally considered to be not clinically relevant or nonspecific binding due to the sensitivity of the assay. However, lack of or true low level antibodies could still indicate potential for a memory response if there has been previous exposure to the specific antigen (Karahan et al., 2017; Njue & Chih, 2019). Conversely, low level antibodies have poor correlation with positivity in CDCXM, indicating that they do not carry a high risk of rejection, so using these to define risk may result in over cautious compatibility assessment (Hachem & Reinsmoen, 2015; Setia et al., 2021). Setia et al (2021) studied 864 adult heart transplant recipients and reported a reduced freedom from AMR in the patients with pre-existing DSA at low (MFI<5,000) or moderate (MFI 5,000-10,000) levels in the first year post-transplant but with no effect on 3-year survival, freedom from cardiac allograft vasculopathy (CAV) and non-fatal major adverse cardiac events (NF-MACE). 86.6% of patients with pre-existing DSA received induction immunosuppression with anti-thymocyte globulin (ATG). This was 75% and 40.3% for sensitised patients with no DSA and unsensitised patients, respectively.

In a single centre study (Olymbios, 2017), out of 514 transplanted patients, 63 and 36 patients were transplanted against DSA at MFI<5000 and MFI 5,000-10,000, respectively. No difference in 3-year survival was detected between patients with DSA and those with no DSA. However, the presence of DSA resulted in a higher incidence of AMR. In this study, patients with DSA received induction therapy with ATG with or without intravenous immune globulin (IVIG). Studies in lung recipients have shown that crossing low to intermediate levels of pre-transplant DSA (MFI <6000) without planned augmented immunosuppression is not associated with decreased chronic lung allograft dysfunction (CLAD)-free survival ($P = 0.28$) or increased Grade 3 primary graft dysfunction (PGD) ($P = 0.75$) (Courtwright et al., 2021). However, a significant association with 1-year post-transplant survival has been reported when crossing DSA with MFI>5000 compared to those with MFI<2000 (Smith et al 2014). In a retrospective study of 425 lung transplant patients, DSA MFI > 5000 had 1-year survival of 33.3% compared with 71.4% for MFI between 2000 and 5000 and 1-year survival of 62.5% for MFI less than 2000 ($p = 0.0046$) (Smith et al., 2014)).

Other factors such as the HLA Class of the antibody may influence the result of the cell-based assays and the impact of a DSA on graft outcome. In a study by Raess et al (2013), detection of Class I DSA by Luminex™ was associated with decreased 1- and 5-year survival when compared to a non-DSA group (62% and 50% versus 87% and 73%, respectively) (P = 0.038). ROC curve analysis showed a sensitivity of 76% and specificity of 73% at a cut-off value of MFI 2000 (Raess et al., 2013). In contrast, Class II DSA had no predictive value (P = 0.330). Liu et al (2012) demonstrated that Class I DSA resulted in positive CDC crossmatch at a lower MFI value than Class II DSA, and within Class II, there was difference between DR and DQ loci (Liu et al., 2012). HLA class II DSA are thought to be more significant than class I). Of all HLA class II loci, antibodies directed against HLA-DQ may be more significant (Bedford et al., 2022)

It has been shown that transplantation across DSA solely detected by Luminex™ assays has acceptable graft outcomes. However, it is not clear at what point the MFI levels translate to positive cell-based crossmatches and/or result in poor clinical outcomes. An arbitrary cut-off value of MFI 3000 has been used by many laboratories as a value to separate low titre antibodies from those considered clinically relevant.

The difficulties in correlating MFI level of DSA with FCXM, CDC XM or with clinical outcomes may be in part due to the differential expression of HLA on cells. A recent review article by Carey et al describe the factors that influence cellular expression of HLA and the implications this has for transplantation (Carey et al., 2019). In this they describe evidence of large locus-specific variations in HLA class I expression, with HLA-A and HLA-B being expressed at similar levels, but HLA-C at about 15 times lower (Apps et al., 2015). Additionally in healthy renal microvascular endothelial cells, HLA-DR expression is found at much higher levels than HLA-DQ or HLA-DP (Cross et al., 2016). They draw on evidence from the haematopoietic stem cell transplantation field where there is a direct correlation between the expression level of mismatched HLA-C, and HLA-DP antigen with both GVHD and mortality (Petersdorf et al., 2014, 2015) and suggest this lower expression of cognate antigen could represent a reduced risk of graft rejection in the cardiothoracic setting (Carey et al., 2019).

30 | Post-Transplant HLA antibody monitoring recommendations

30.1.1 Centres should have a locally agreed policy with regards to performing retrospective cell based (CDCXM or FCXM) crossmatches. There is no requirement to perform a retrospective crossmatch in a recipient who is negative for HLA specific antibodies, for other recipients, omission of a retrospective crossmatch can occur if supported by local audit evidence. [1B]

30.1.2 Each centre should have a procedure for informing the transplant unit in the event of a positive retrospective cell based crossmatch that is attributable to HLA donor specific antibodies. [1B]

30.1.3 Following transplantation, patients above standard risk should be tested for HLA-specific antibodies at 7 and 28 days; 3, 6, 9 and 12 months; and then as required. More frequent testing should be agreed as part of a local policy according to level of immunological risk, other risk factors and suspicion of rejection. [1B]

30.1.4 HLA antibody testing should be undertaken when antibody mediated rejection is suspected and when patients present with episodes of rejection associated with haemodynamic compromise. Further testing will depend on the course of the rejection episode. [1B]

Rationale

Immunosuppression regimens aim to prevent rejection of the donor organ, however rejection can still develop despite immunosuppression. Rejection can be mediated through cellular or antibody driven mechanisms, which may often be seen in combination (Kfoury & Miller, 2019). When assessing cardiac AMR, endomyocardial biopsies (EMB) are currently the key tool in classification and diagnosis, with DSA and clinical dysfunction not used (Berry et al., 2013). Recommendations from The International Society for Heart and Lung Transplantation (ISHLT) for EMB surveillance prescribe regular immunostaining for up to a year posttransplant (Berry et al., 2013). Likewise, DSA monitoring follows a similar pattern for patients who have received a higher immunological risk transplant. Both testing protocols are guided by whether the patient experiences clinical features of rejection, and so knowledge of pre-clinical manifestations of rejection is limited. The existence of asymptomatic AMR is well documented (Kfoury & Miller, 2019), but this is not reflected in monitoring protocols. Moreover, DSA testing is generally only regularly performed in patients who are above standard risk at time of transplant, thus the course of AMR development in patients who develop *de novo* (dn) DSA outside of this scenario is even less well understood. This is a particular issue as dnDSA, and in particular persistent dnDSA, is associated with worse outcomes posttransplant (Moayedi et al., 2018). While DSA are no longer considered during classification of AMR, as they are not considered to be sensitive enough for diagnosis, their presence may carry prognostic value as the presence of class II DSA may predict patients at risk of future AMR, morbidity, and mortality (Clerkin et al., 2017). Complement fixing DSA, as identified by the C1q assay, have also been seen to increase risk of AMR (Farrero Torres et al., 2017). The use of DSA identification for prognostication is useful in that it is a preferential test for the patient as opposed to a biopsy, which inherently carries a higher risk and is more unpleasant for the patient. Acute AMR, early in the post-transplant course is associated with better outcomes for patients than late developing chronic AMR (Barten & Zuckermann, 2019). In a small study with predominantly non-sensitised patients by Hodges et al. (2012), development of dnDSA was followed by late AMR at a median of 4.5 years post-transplant, and 60% died within 2 years of diagnosis (Hodges et al.,

2012), which may reflect the lack of consensus on how and when to treat AMR in these patients (Berry et al., 2013), as well as the lack of knowledge about the exact role of dnDSA in AMR. However, increased levels of cytotoxic T-cells have been seen in patients with DSA (Frank et al., 2015), although this was only identified in single biopsies at one timepoint post-transplant, with biopsies not necessarily performed on the same day as DSA measurement. Nevertheless, this indicates an area for further study in larger patient groups to identify the inflammatory cells recruited during these periods, which may help further elucidate the precise role of dnDSA in AMR, and better identify where it will be of the most use in post-transplant monitoring.

Standard immunosuppression in cardiothoracic transplant recipients involves a combination of calcineurin inhibitors, such as cyclosporine or tacrolimus, corticosteroids, antimetabolites, such as mycophenolate mofetil, and mammalian target of rapamycin inhibitors, such as sirolimus (Costanzo et al., 2010; Njue & Chih, 2019). Attempts to wean patients deemed to be of lower risk from immunosuppression, or changes to the type of drug used are often made to reduce toxicity. Adjustments are also made in response to rejection, but it is critical to ensure patients maintain adherence to immunosuppressive regimens, which has been identified as a key factor in DSA development and rejection (Barten & Zuckermann, 2019; Costanzo et al., 2010). It is clearly a fine line to stay within therapeutic range, as one study has indicated that DSA development is associated with an effective immune response, as those patients who develop DSA have been seen to be less likely to succumb to infection (Farrero Torres et al., 2017). While this study also links DSA to poor allograft survival, the question of how to treat patients with DSA, particularly those who are stable, is a pertinent one, which is as yet unclear as there have not been any trials to investigate the impact of differing treatment regimens (Njue & Chih, 2019; Tait et al., 2013). In the attempt to avoid or reduce DSA, over suppression of the immune response may have an impact on non-rejection associated deaths, as well as affecting patient quality of life through potential toxicities, and thus avoiding this where possible would be beneficial. Moreover, AMR may stop responding to treatment, as has been seen in some lung transplant cases (Levine et al., 2016), although one recent cardiac transplant case has successfully used the monoclonal antibody daratumumab, which is normally used in multiple myeloma to target malignant plasma cells, to treat refractory AMR (Aguilera Agudo et al., 2021). With the lack of clear therapy for AMR, utilisation of treatments with success in other diseases could represent a new field for study.

31 | Re-transplantation in the cardiothoracic setting

31.1.1 There is a lack of consensus opinion from H&I laboratories in the United Kingdom and a paucity of published evidence in the literature to suggest a change in approach of either timing of HLA specific antibody screening nor categorisation of level of immunological risk in patients awaiting retransplantation. We suggest that the transplant unit and H&I laboratory have an agreed policy for this scenario.[1B]

Rationale

Re-transplantation in the cardiothoracic setting is a rare event both within the United Kingdom and worldwide. Data collected by the Collaborative Transplant Study (CTS) show that of 48,318 heart transplants performed between 1990 and 2019, 1,178 (1.7%) were in recipients undergoing re-transplantation. Within this re-transplantation group 1,143 underwent a second and 35 a third heart transplant (Collaborative Transplant Study Slide H11101-0821, n.d.). When specifically considering European heart transplantation, the number of re-transplants for the same time period was 672 of 31,382 (2.1%) (Collaborative Transplant Study Slide H11101E-0821, n.d.). In the United Kingdom the latest NHSBT annual report on cardiothoracic transplantation covering the time period 2010 to 2020 shows that in adult heart transplants, 17 of 1,420 (1.2%) and in lung transplant 21 of 1,737 (2.1%) were re-transplants. Data is similar for the paediatric setting with re-transplantation accounting for 1.6% and 3% of heart and lung transplants respectively (NHSBT ODT annual report, 2020).

Overall survival following re-transplantation is in general worse than after primary transplantation with survival at 5 years approximately 60% for second and third heart transplantation versus 75% for those recipients undergoing primary transplantation (Collaborative Transplant Study Slide H11101-0821, n.d.; Collaborative Transplant Study Slide H11101E-0821, n.d.)

Information from an analysis of the Spanish heart transplant registry between 1984 and 2018 showed that re-transplantation accounted for 2.3% of total transplants and the indication for re-transplantation was primarily cardiac allograft vasculopathy (CAV) accounting for 42.2% of cases, with 80% of the re-transplantations occurring 5 years after the primary transplant. Re-transplantation was associated with higher mortality especially in the presence of acute rejection but beyond 5 years mortality rates were similar to primary transplantation (Salterain-González et al., 2022). Zhu *et al* retrospectively analysed data from their single centre and 5.8% were re-transplantations (both adult and paediatric) with 80% requiring re-transplantation due to CAV and the overall matched median survival was lower in the retransplant cohort (4.6 years vs 6.5 years) (Zhu et al., 2022).

More promisingly, Barghash and Pinney report that heart re-transplantation in the most recent era of 2002 to 2012 has been associated with improved outcomes. Where patients undergo re-transplantation for CAV more than one year following their first transplantation and patients are not in critical condition, they can have outcomes approaching those seen in primary heart transplant. This paper suggests there are other modifiable factors and patient management strategies which warrant further investigation (Barghash & Pinney, 2020).

Considering lung transplantation, an analysis of UNOS data of transplants performed between 2006 and 2017 showed re-transplantation accounted for 4% of transplants and overall one year survival was lower (76.7 vs 84.8), however there was improvement in survival with era of transplant. Patients retransplanted between 2014 -2017 had 80% survival vs 72.1% survival in the 2006 – 2009 era. Additionally the UNOS analysis showed that where centre volume was higher

survival was improved with the authors proposing that re-transplantation might be performed in fewer centres to improve outcome (Randhawa et al., 2022).

Data from Sweden in a cohort off 635 transplants performed between1991-2017, 49 were re-transplants. This group compared retransplants to primary transplants and considered timing of re-transplantation. In 8 patients (16%) re-transplantation was performed within the first year after primary transplantation. The 1-year survival for this group was 50% compared with 81% for patients who underwent re-transplantation after 1 year. They conclude that re-transplantation is a reasonable option for a selected group of patients with a number of well-established risk factors avoided and ideally performed more than 1 year after primary transplant (Wallinder et al., 2019).

With regards to strategies for HLA specific antibody screening it is not clear from the studies published in the literature whether different approaches were taken for patients awaiting re-transplantation. Within the UK an attempt was made to collate the current approaches the H&I laboratories in the UK take regarding the management of patients awaiting re-transplantation, however no clear consensus emerged.

32 | cfDNA

32.1.1 The use of dd-cfDNA assays to support the diagnosis of acute rejection in cardiothoracic transplantation is in its infancy therefore no clinical recommendation can be made.

Rationale

Cardiac allograft vasculopathy (CAV) and bronchiolitis obliterans syndrome (BOS) are leading causes of graft failure and mortality following cardiothoracic transplantation. EMB remains the gold standard test for detecting acute rejection post heart transplant. In addition, flexible bronchoscopy can be helpful in lung transplant recipients when rejection is a possibility (Du Rand et al., 2013). However, these methods are both invasive and expensive. Developments in assays of non-invasive biomarkers have the potential to become a clinical useful tool to highlight patients at risk of experiencing rejection episodes.

One of the biomarkers that has seen increasing interest in recent years is the detection of donor-derived cell-free DNA (dd-cfDNA). Cell-free DNA (cfDNA) is released into circulation as a result of cell turnover and can be detected at higher levels when tissue injury is occurring. Single Nucleotide Polymorphisms (SNP) can be used to distinguish recipient from donor DNA in genetic assays. In the allograft setting, most of the cfDNA is recipient-derived with only a small proportion derived from the donor organ. Quantification of dd-cfDNA may be used to highlight if acute rejection is occurring as damage to the transplanted organ can lead to an increase in dd-cfDNA. Studies have been performed in both heart and lung transplants investigating the correlation of dd-cfDNA to acute rejection.

In heart transplant recipients, one prospective study found that a dd-cfDNA threshold of 0.25% had a negative predictive value for acute rejection of 99% and would have eliminated 81% of biopsies (Agbor-Enoh et al., 2021) though dd-cfDNA levels appeared to be more relevant to antibody-mediated rejection, rather than cell-mediated. However, another publication reported that with a threshold of 0.2%, dd-cfDNA had 44% sensitivity to detect rejection and 97% negative prediction value (Khush et al., 2019). These findings suggest that the assay may be more useful for eliminating the presence of rejection, rather than confirming its occurrence. An earlier study reported that dd-cfDNA levels were significantly higher in recipients who were experiencing acute rejection and correlated with severity of the rejection as determined by biopsy (De Vlaminck et al., 2014). Feingold and colleagues found that circulating donor-specific antibodies were more common in patients with elevated dd-cfDNA, indicating that perhaps a combined approach to non-invasive diagnostic assays may support a case for reduced biopsies other than when rejection is suspected (Feingold et al., 2022). Although generally the assay is not recommended in the immediate period after an organ is implanted due to elevated cfDNA levels resulting from surgical tissue injury, one study serially testing paediatric heart transplant recipients within 10 days of transplant reported that of the 4 patients who died during the first year post-transplant, all had a rise or blunted decline in donor fraction from days 4 – 8 and/or persistently elevated total cfDNA levels at one week post-transplant (Zangwill et al., 2019). This indicates that the assay may be clinically useful to identify patients at increased risk of mortality who may require more intensive monitoring.

In lung transplantation, there is currently no reliable predictor for patients experiencing BOS and alterations in dd-cfDNA levels are detectable prior to clinical diagnosis or pathological features of rejection, suggesting this assay may be useful as an early, non-invasive rejection marker (Bansal et al., 2019). A multicentre, prospective study serially testing lung transplant recipients during the first 3 months post-transplant found that calculated average dd-cfDNA levels were highly variable but those patients with an average dd-cfDNA in the upper tertile (average 3.6%) had a 6.6 fold higher risk of developing allograft failure. This study calculated average dd-cfDNA levels separately for single and double lung transplants and found that median percentage dd-cfDNA values for single lung transplants were half that of double lung transplants (Agbor-Enoh et al., 2021). This was confirmed by Keller *et al* who reported that doubling dd-cfDNA levels in single lung transplant recipients to account for differences in lung mass eliminated the difference in dd-cfDNA levels. They reported an optimal threshold for the detection of acute rejection of 0.54% in single versus 1.1% in double lung transplants and state that accounting for the difference in lung mass is critical to accurate interpretation of dd-cfDNA assay results in lung transplantation (Keller & Agbor-Enoh, 2022).

33 | Non-HLA

33.1.1 The use of assays detecting non-HLA antibodies to support the diagnosis of acute rejection in cardiothoracic transplantation

is in its infancy therefore no clinical recommendation can be made.[1B]

Rationale

Biopsy proven rejection in the absence of HLA antibodies is estimated to occur in approximately 40% of heart patients (X. Zhang & Reinsmoen, 2017) and demonstrated in 47% of lung recipients (Hachem et al., 2010) raising the question of the clinical relevance of non-HLA antibodies.

Several non-HLA antibodies (Major Histocompatibility Antigen Class I Chain-related gene A (MIC-A) and autoantigens to angiotensin II type 1 receptor (AT1R), Endothelin-1 type A receptor (ETAR), Vimentin, Collagen V, K-A-1-Tubulin) have been identified and studied in single centre studies for heart and lung patients (X. Zhang & Reinsmoen, 2017).

Until recently there has been no standardised multiplex methodology available to monitor non-HLA antibodies, so studies demonstrating the population wide distribution of these antibodies and their clinical relevance are limited. However, two Luminex™ based assays have become commercially available. Limited single centre studies using these kits have been published with early findings showing heart patient groups with AMR had a greater percentage of patients with elevated reactivity to autoantigens compared to non-AMR groups although their specific role in mediating allograft injury is not yet understood (See et al., 2020; Villa et al., 2020; X. Zhang et al., 2020).

In the case of cardiothoracic transplantation any non-HLA incompatibility between the donor and recipient would be inferred only. Deceased donor typing does not include typing for these antigens and routine non-HLA antibody screening is not performed. Therefore, transplanting across non-HLA antibodies occurs at an unknown frequency. At present not enough information about the clinical relevance, the timing of appearance or ability to detect incompatibilities is available. Further work would be required before non-HLA antibodies could be considered in a virtual crossmatch and used as part of a transplant immunological risk assessment in cardiothoracic transplant.

34 | Strategies to Transplant the Highly Sensitized Patient

34.1.1 That offers for highly sensitised patients that are not defined as high immunological risk are given the appropriate level of consideration taking into account the likelihood of receiving another suitable offer for that patient. [1B]

Rationale

Highly sensitised cardiothoracic patients (cRF>85%) present a challenge in the identification of suitable crossmatch negative donors and consequently spend longer on the transplant waiting list (John et al., 2003). A retrospective audit of transplants from 2016 – 2022 undertaken in the UK showed that approximately 90% of all transplants performed were defined as standard risk (i.e., in the absence of any DSA). Evidence described in the literature previously presented shows that in some scenarios transplantation across DSA that are deemed to be of low immunological risk may well result in comparable outcomes to those transplanted as standard risk but more published evidence in the UK is needed to corroborate this and help drive clinical practice.

Studies in the literature, predominately from centres in the USA and Canada, show it is possible to establish protocols of desensitisation to HLA with varying success rates. Toronto has the largest lung transplant programme in the world, and their transplant programme institutes a perioperative antibody treatment protocol for patients who had a positive virtual crossmatch. The protocol uses plasmapheresis, intravenous immunoglobulin (IvIg) mycophenolic acid and thymoglobulin. The resulting transplants had equivalent allograft survival rates and chronic lung allograft dysfunction (CLAD) outcomes in the DSA positive cohort compared to unsensitised patients who received a lung transplant (Tinckam et al., 2015). Other protocols include use of IvIg alone (Tyan et al., 1994); IvIg and plasmapheresis (Pisani et al., 1999); the use of intra-operative plasma exchange (Issitt et al., 2022) and Belatacept (Alishetti et al., 2020). As well as clinical interventions there are also approaches to facilitate transplantation in highly sensitised cardiothoracic patients in organ allocation schemes. For example the Canadian allocation schemes prioritise highly sensitised patients (Aleksova & Ross, 2019) and a comparison of heart allocation schemes across Europe demonstrated a benefit for international exchange of organs as well as incorporating patient clinical urgency into the allocation scheme (van den Hout et al., 2003).

Supplementing the standard SAB with the use of Luminex™ assays that have been shown to detect a sub-set of antibodies capable of fixing complement and which are predictive of AMR early after transplant (Baudry et al., 2022; Chin et al., 2011b; Smith et al., 2007) is promising but comes with resource implications.

More recently, Imlifidase has emerged as a potential desensitisation agent in the kidney transplant setting. Imlifidase cleaves IgG leading to a rapid reduction in antibody levels thereby allowing transplantation to proceed (Jordan et al., 2021; Lonze, 2021). In the UK, Imlifidase has been approved for use in deceased donor kidney transplantation and its use may be extended in time to other transplant settings (NIHCE 2022 guidance TA809, 2022).

ACKNOWLEDGEMENTS

NA.

DATA AVAILABILITY STATEMENT

NA.

ORCID

Richard Battle https://orcid.org/0000-0002-4562-092X
Sarah Peacock https://orcid.org/0000-0002-4414-8432
Catherine Hastie https://orcid.org/0009-0001-3203-1368

Judith Worthington https://orcid.org/0000-0002-1241-3975
Claire Collins https://orcid.org/0000-0002-5348-1819
Natalia Diaz-Burlinson https://orcid.org/0000-0003-0001-7534
Carla Rosser https://orcid.org/0000-0001-8518-0861
Luke Foster https://orcid.org/0000-0002-1496-8851
Olivia Shaw https://orcid.org/0000-0003-4560-0619
David Briggs https://orcid.org/0000-0002-6796-7086
David Turner https://orcid.org/0000-0003-4007-6248
Arthi Anand https://orcid.org/0000-0002-7233-622X

REFERENCES

Abrol, N., Jadlowiec, C. C., & Taner, T. (2019). Revisiting the liver's role in transplant alloimmunity. *World Journal of Gastroenterology*, *25*(25), 3123–3135. https://doi.org/10.3748/wjg.v25.i25.3123

Abu-Elmagd, K. M., Costa, G., Bond, G. J., Soltys, K., Sindhi, R., Wu, T., Koritsky, D. A., Schuster, B., Martin, L., Cruz, R. J., Murase, N., Zeevi, A., Irish, W., Ayyash, M. O., Matarese, L., Humar, A., & Mazariegos, G. (2009). Five hundred intestinal and multivisceral transplantations at a single center: Major advances with new challenges. *Annals of Surgery*, *250*(4), 567–581. https://doi.org/10.1097/SLA.0b013e3181b67725

Abu-Elmagd, K. M., Wu, G., Costa, G., Lunz, J., Martin, L., Koritsky, D. A., Murase, N., Irish, W., & Zeevi, A. (2012). Preformed and de novo donor specific antibodies in visceral transplantation: Long-term outcome with special reference to the liver. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *12*(11), 3047–3060. https://doi.org/10.1111/j.1600-6143.2012.04237.x

Abu-Elmagd, K., Reyes, J., Todo, S., Rao, A., Lee, R., Irish, W., Furukawa, H., Bueno, J., McMichael, J., Fawzy, A. T., Murase, N., Demetris, J., Rakela, J., Fung, J. J., & Starzl, T. E. (1998). Clinical intestinal transplantation: New perspectives and immunologic considerations. *Journal of the American College of Surgeons*, *186*(5), 512–527. discussion 525–527. https://doi.org/10.1016/s1072-7515(98)00083-0

Adebiyi, O. O., Gralla, J., Klem, P., Freed, B., Davis, S., Wiseman, A. C., & Cooper, J. E. (2016). Clinical Significance of Pretransplant Donor-Specific Antibodies in the Setting of Negative Cell-Based Flow Cytometry Crossmatching in Kidney Transplant Recipients. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *16*(12), 3458–3467. https://doi.org/10.1111/ajt.13848

Agbor-Enoh, S., Shah, P., Tunc, I., Hsu, S., Russell, S., Feller, E., Shah, K., Rodrigo, M. E., Najjar, S. S., Kong, H., Pirooznia, M., Fideli, U., Bikineyeva, A., Marishta, A., Bhatti, K., Yang, Y., Mutebi, C., Yu, K., Kyoo Jang, M., … For the GRAfT Investigators. (2021). Cell-Free DNA to Detect Heart Allograft Acute Rejection. *Circulation*, *143*(12), 1184–1197. https://doi.org/10.1161/CIRCULATIONAHA.120.049098

Aguilera Agudo, C., Gómez Bueno, M., & Krsnik Castello, I. (2021). Daratumumab for Antibody-mediated Rejection in Heart Transplant—A Novel Therapy: Successful Treatment of Antibody-mediated Rejection. *Transplantation*, *105*(3), e30–e31. https://doi.org/10.1097/TP.0000000000003505

Aleksova, N., & Ross, H. J. (2019). Does the Canadian allocation system for highly sensitized patients work? *Current Opinion in Organ Transplantation*, *24*(3), 239–244. https://doi.org/10.1097/MOT.0000000000000635

Alishetti, S., Farr, M., Jennings, D., Serban, G., Uriel, N., Sayer, G., Vasilescu, R., Restaino, S., Chong, A. S., & Habal, M. V. (2020). Desensitizing highly sensitized heart transplant candidates with the combination of belatacept and proteasome inhibition. *American Journal of Transplantation*, *20*(12), 3620–3630. https://doi.org/10.1111/ajt.16113

Amaral, S., Sayed, B. A., Kutner, N., & Patzer, R. E. (2016). Preemptive kidney transplantation is associated with survival benefits among pediatric patients with end-stage renal disease. *Kidney International*, *90*(5), 1100–1108. https://doi.org/10.1016/j.kint.2016.07.028

Andreas, M., Freystaetter, K., Bernardi, M. H., & Zuckermann, A. (2018). Accelerated acute severe antibody-mediated graft failure related to a Ross procedure 17 years earlier†. *European Journal of Cardio-Thoracic Surgery*, *54*(2), 402–403. https://doi.org/10.1093/ejcts/ezy052

Anteby, R., Lucander, A., Bachul, P. J., Pyda, J., Grybowski, D., Basto, L., Generette, G. S., Perea, L., Golab, K., Wang, L. J., Tibudan, M., Thomas, C., Fung, J., & Witkowski, P. (2021a). Evaluating the prognostic value of islet autoantibody monitoring in islet transplant recipients with long-standing type 1 diabetes mellitus. *Journal of Clinical Medicine*, *10*(12), 2–11. https://doi.org/10.3390/jcm10122708

Anteby, R., Lucander, A., Bachul, P. J., Pyda, J., Grybowski, D., Basto, L., Generette, G. S., Perea, L., Golab, K., Wang, L.-J., Tibudan, M., Thomas, C., Fung, J., & Witkowski, P. (2021b). Evaluating the Prognostic Value of Islet Autoantibody Monitoring in Islet Transplant Recipients with Long-Standing Type 1 Diabetes Mellitus. *Journal of Clinical Medicine*, *10*(12), 2708. https://doi.org/10.3390/jcm10122708

Apps, R., Meng, Z., Del Prete, G. Q., Lifson, J. D., Zhou, M., & Carrington, M. (2015). Relative Expression Levels of the HLA Class-I Proteins in Normal and HIV-Infected Cells. *The Journal of Immunology*, *194*(8), 3594–3600. https://doi.org/10.4049/jimmunol.1403234

Aubert, O., Kamar, N., Vernerey, D., Viglietti, D., Martinez, F., Duong-Van-Huyen, J.-P., Eladari, D., Empana, J.-P., Rabant, M., Verine, J., Rostaing, L., Congy, N., Guilbeau-Frugier, C., Mourad, G., Garrigue, V., Morelon, E., Giral, M., Kessler, M., Ladrière, M., … Loupy, A. (2015). Long term outcomes of transplantation using kidneys from expanded criteria donors: Prospective, population based cohort study. *BMJ (Clinical Research Ed.)*, *351*, h3557. https://doi.org/10.1136/bmj.h3557

Aziz, F., Mandelbrot, D., Parajuli, S., Al-Qaoud, T., Redfield, R., Kaufman, D., & Odorico, J. S. (2020a). Alloimmunity in pancreas transplantation. *Current Opinion in Organ Transplantation*, *25*(4), 322–328. https://doi.org/10.1097/MOT.0000000000000776

Aziz, F., Mandelbrot, D., Parajuli, S., Al-Qaoud, T., Redfield, R., Kaufman, D., & Odorico, J. S. (2020b). Alloimmunity in pancreas transplantation. *Current Opinion in Organ Transplantation*, *25*(4), 322–328. https://doi.org/10.1097/MOT.0000000000000776

Aziz, S., Hassantash, S. A., Nelson, K., Levy, W., Kruse, A., Reichenbach, D., Himes, V., Fishbein, D., & Allen, M. D. (1998). The clinical significance of flow cytometry crossmatching in heart transplantation. *The Journal of Heart and Lung Transplantation: The Official Publication of the International Society for Heart Transplantation*, *17*(7), 686–692.

Bachelet, T., Martinez, C., Del Bello, A., Couzi, L., Kejji, S., Guidicelli, G., Lepreux, S., Visentin, J., Congy-Jolivet, N., Rostaing, L., Taupin, J.-L., Kamar, N., & Merville, P. (2016). Deleterious Impact of Donor-Specific Anti-HLA Antibodies Toward HLA-Cw and HLA-DP in Kidney Transplantation. *Transplantation*, *100*(1), 159–166. https://doi.org/10.1097/TP.0000000000000821

Badawy, A., Kaido, T., Yoshizawa, A., Yagi, S., Fukumitsu, K., Okajima, H., & Uemoto, S. (2018). Human leukocyte antigen compatibility and lymphocyte cross-matching play no significant role in the current adult-to-adult living donor liver transplantation. *Clinical Transplantation*, *32*(4), e13234. https://doi.org/10.1111/ctr.13234

Banasik, M., Boratyńska, M., Kościelska-Kasprzak, K., Kamińska, D., Zmonarski, S., Mazanowska, O., Krajewska, M., Bartoszek, D., Zabińska, M., Myszka, M., Kamińska, M., Hałoń, A., Dawiskiba, T., Szyber, P., Sas, A., & Klinger, M. (2014). Non-HLA antibodies: Angiotensin II type 1 receptor (anti-AT1R) and endothelin-1 type A receptor (anti-ETAR) are associated with renal allograft injury and graft loss. *Transplantation Proceedings*, *46*(8), 2618–2621. https://doi.org/10.1016/j.transproceed.2014.09.029

Banner, N. R., Thomas, H. L., Curnow, E., Hussey, J. C., Rogers, C. A., & Bonser, R. S. (2008). The Importance of Cold and Warm Cardiac Ischemia for Survival After Heart Transplantation. *Transplantation*, *86*(4), 542–547. https://doi.org/10.1097/TP.0b013e31818149b9

Bansal, S., Fleming, T., & Mohanakumar, T. (2019). The detection of donor-derived cell-free DNA may serve as a biomarker for the early detection of

chronic lung allograft dysfunction. *EBioMedicine*, *40*, 13–14. https://doi.org/10.1016/j.ebiom.2019.01.044

Barghash, M. H., & Pinney, S. P. (2020). Heart Retransplantation: Candidacy, Outcomes, and Management. *Current Transplantation Reports*, *7*(1), 12–17. https://doi.org/10.1007/s40472-019-00257-y

Barten, M. J., & Zuckermann, A. (2019). The meaning of donor-specific antibodies after heart transplant. *Current Opinion in Organ Transplantation*, *24*(3), 252–258. https://doi.org/10.1097/MOT.0000000000000641

Bathgate, A. J., McColl, M., Garden, O. J., Forsythe, J. L., Madhavan, K. K., & Hayes, P. C. (1998). The effect of a positive T-lymphocytotoxic crossmatch on hepatic allograft survival and rejection. *Liver Transplantation and Surgery: Official Publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society*, *4*(4), 280–284. https://doi.org/10.1002/lt.500040411

Battle, R. K., Henderson, L., Phelan, P. J., Latham, K., & Turner, D. M. (2020). A case report—Two manufacturers SAB testing kits can reveal different HLA antibody profiles—Identifying prozone and denatured antigen. *HLA*, *96*(1), 76–82. https://doi.org/10.1111/tan.13913

Battle, R. K., Rennie, T. J. W., Phelan, P. J., Abel, A. A., McConnell, S., & Turner, D. M. (2022). Highly sensitised patients awaiting deceased donor renal transplants are disadvantaged by the presence of denatured HLA antibody detected in routine HLA antibody testing. *HLA*, *100*(1), 24–36. https://doi.org/10.1111/tan.14578

Baudry, G., Pozzi, M., Aubry, M., Hugon-Vallet, E., Mocan, R., Chalabreysse, L., Portran, P., Obadia, J.-F., Thaunat, O., Girerd, N., Dubois, V., & Sebbag, L. (2022). De Novo Complement-Binding Anti-HLA Antibodies in Heart Transplanted Patients Is Associated with Severe Cardiac Allograft Vasculopathy and Poor Long-Term Survival. *Journal of Clinical Medicine*, *11*(13), 3731. https://doi.org/10.3390/jcm11133731

Bedford, A., Jervis, S., Worthington, J., Lowe, M., & Poulton, K. (2022). Human leukocyte antigen epitope mismatch loads and the development of de novo donor-specific antibodies in cardiothoracic organ transplantation. *International Journal of Immunogenetics*, *49*(1), 30–38. https://doi.org/10.1111/iji.12563

Bentall, A., Cornell, L. D., Gloor, J. M., Park, W. D., Gandhi, M. J., Winters, J. L., Chedid, M. F., Dean, P. G., & Stegall, M. D. (2013). Five-year outcomes in living donor kidney transplants with a positive crossmatch. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *13*(1), 76–85. https://doi.org/10.1111/j.1600-6143.2012.04291.x

Berger, M., Zeevi, A., Farmer, D. G., & Abu-Elmagd, K. M. (2012). Immunologic challenges in small bowel transplantation. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *12*(Suppl 4), S2–S8. https://doi.org/10.1111/j.1600-6143.2012.04332.x

Berry, G. J., Burke, M. M., Andersen, C., Bruneval, P., Fedrigo, M., Fishbein, M. C., Goddard, M., Hammond, E. H., Leone, O., Marboe, C., Miller, D., Neil, D., Rassl, D., Revelo, M. P., Rice, A., Rene Rodriguez, E., Stewart, S., Tan, C. D., Winters, G. L., … Angelini, A. (2013). The 2013 International Society for Heart and Lung Transplantation Working Formulation for the standardization of nomenclature in the pathologic diagnosis of antibody-mediated rejection in heart transplantation. *The Journal of Heart and Lung Transplantation*, *32*(12), 1147–1162. https://doi.org/10.1016/j.healun.2013.08.011

Besarani, D., Cerundolo, L., Smith, J. D., Procter, J., Barnardo, M. C. N., Roberts, I. S. D., Friend, P. J., Rose, M. L., & Fuggle, S. V. (2014). Role of anti-vimentin antibodies in renal transplantation. *Transplantation*, *98*(1), 72–78. https://doi.org/10.1097/01.TP.0000443224.66960.37

Bestard, O., Couzi, L., Crespo, M., Kessaris, N., & Thaunat, O. (2021). Stratifying the humoral risk of candidates to a solid organ transplantation: A proposal of the ENGAGE working group. *Transplant International*, *34*(6), 1005–1018. https://doi.org/10.1111/tri.13874

Bestard, O., Thaunat, O., Bellini, M. I., Böhmig, G. A., Budde, K., Claas, F., Couzi, L., Furian, L., Heemann, U., Mamode, N., Oberbauer, R., Pengel, L., Schneeberger, S., & Naesens, M. (2022). Alloimmune Risk Stratification for Kidney Transplant Rejection. *Transplant International*, *35*, 10138. https://doi.org/10.3389/ti.2022.10138

Beyzaei, Z., Geramizadeh, B., Bagheri, Z., Karimzadeh, S., & Shojazadeh, A. (2020). De Novo Donor Specific Antibody and Long-Term Outcome After Liver Transplantation: A Systematic Review and Meta-Analysis. *Frontiers in Immunology*, *11*, 613128. https://doi.org/10.3389/fimmu.2020.613128

Billen, E. V. A., Christiaans, M. H. L., Lee, J., & van den Berg-Loonen, E. M. (2009). Donor-directed HLA antibodies before and after transplantectomy detected by the luminex single antigen assay. *Transplantation*, *87*(4), 563–569. https://doi.org/10.1097/TP.0b013e3181949e37

Boggi, U., Vistoli, F., Andres, A., Arbogast, H. P., Badet, L., Baronti, W., Bartlett, S. T., Benedetti, E., Branchereau, J., Burke, G. W., Buron, F., Caldara, R., Cardillo, M., Casanova, D., Cipriani, F., Cooper, M., Cupisti, A., Davide, J., Drachenberg, C., … Berney, T. (2021). First World Consensus Conference on pancreas transplantation: Part II – recommendations. *American Journal of Transplantation*, *21*, 17–59. https://doi.org/10.1111/ajt.16750

Bond, G., Reyes, J., Mazariegos, G., Wu, T., Schaefer, N., Demetris, J., Fung, J. J., Starzl, T. E., & Abu-Elmagd, K. (2000). The impact of positive T-cell lymphocytotoxic crossmatch on intestinal allograft rejection and survival. *Transplantation Proceedings*, *32*(6), 1197–1198. https://doi.org/10.1016/s0041-1345(00)01181-7

Bouatou, Y., Seyde, O., Moll, S., Martin, P.-Y., Villard, J., Ferrari-Lacraz, S., & Hadaya, K. (2018). Clinical and histological evolution after de novo donor-specific anti-human leukocyte antigen antibodies: A single centre retrospective study. *BMC Nephrology*, *19*(1), 86. https://doi.org/10.1186/s12882-018-0886-5

Brooks, A. M. S., Carter, V., Liew, A., Marshall, H., Aldibbiat, A., Sheerin, N. S., Manas, D. M., White, S. A., & Shaw, J. A. M. (2015). De Novo Donor-Specific HLA Antibodies Are Associated With Rapid Loss of Graft Function Following Islet Transplantation in Type 1 Diabetes. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *15*(12), 3239–3246. https://doi.org/10.1111/ajt.13407

BTS Guidelines on Antibody Incompatible Transplantation Version 3. https://bts.org.uk/wp-content/uploads/2016/09/02_BTS_Antibody_Guidelines-1.pdf

Buron, F., Reffet, S., Badet, L., Morelon, E., & Thaunat, O. (2021). Immunological Monitoring in Beta Cell Replacement: Towards a Pathophysiology-Guided Implementation of Biomarkers. *Current Diabetes Reports*, *21*(6), 19. https://doi.org/10.1007/s11892-021-01386-4

Cai, J., Terasaki, P. I., Anderson, N., Lachmann, N., & Schönemann, C. (2009). Intact HLA not beta2m-free heavy chain-specific HLA class I antibodies are predictive of graft failure. *Transplantation*, *88*(2), 226–230. https://doi.org/10.1097/TP.0b013e3181ac6198

Campbell, P. M., Salam, A., Ryan, E. A., Senior, P., Paty, B. W., Bigam, D., McCready, T., Halpin, A., Imes, S., Al Saif, F., Lakey, J. R. T., & Shapiro, A. M. J. (2007). Pretransplant HLA Antibodies Are Associated with Reduced Graft Survival After Clinical Islet Transplantation. *American Journal of Transplantation*, *7*(5), 1242–1248. https://doi.org/10.1111/j.1600-6143.2007.01777.x

Cano, P., & Fernández-Viña, M. (2009). Two sequence dimorphisms of DPB1 define the immunodominant serologic epitopes of HLA-DP. *Human Immunology*, *70*(10), 836–843. https://doi.org/10.1016/j.humimm.2009.07.011

Carapito, R., Aouadi, I., Verniquet, M., Untrau, M., Pichot, A., Beaudrey, T., Bassand, X., Meyer, S., Faucher, L., Posson, J., Morlon, A., Kotova, I., Delbos, F., Walencik, A., Aarnink, A., Kennel, A., Suberbielle, C., Taupin, J.-L., Matern, B. M., … Bahram, S. (2022). The MHC class I MICA gene is a histocompatibility antigen in kidney transplantation. *Nature Medicine*, *28*(5), 989–998. https://doi.org/10.1038/s41591-022-01725-2

Cardinal, H., Dieudé, M., Brassard, N., Qi, S., Patey, N., Soulez, M., Beillevaire, D., Echeverry, F., Daniel, C., Durocher, Y., Madore, F., & Hébert, M. J. (2013). Antiperlecan antibodies are novel accelerators of

immune-mediated vascular injury. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *13*(4), 861–874. https://doi.org/10.1111/ajt.12168

Cardinal, H., Dieudé, M., & Hébert, M.-J. (2017). The Emerging Importance of Non-HLA Autoantibodies in Kidney Transplant Complications. *Journal of the American Society of Nephrology: JASN*, *28*(2), 400–406. https://doi.org/10.1681/ASN.2016070756

Carey, B. S., Poulton, K. V., & Poles, A. (2019). Factors affecting HLA expression: A review. *International Journal of Immunogenetics*, *46*(5), 307–320. https://doi.org/10.1111/iji.12443

Caro-Oleas, J. L., González-Escribano, M. F., González-Roncero, F. M., Acevedo-Calado, M. J., Cabello-Chaves, V., Gentil-Govantes, M. Á., & Núñez-Roldán, A. (2012). Clinical relevance of HLA donor-specific antibodies detected by single antigen assay in kidney transplantation. *Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplant Association - European Renal Association*, *27*(3), 1231–1238. https://doi.org/10.1093/ndt/gfr429

Chaigne, B., Geneugelijk, K., Bédat, B., Ahmed, M. A., Hönger, G., De Seigneux, S., Demuylder-Mischler, S., Berney, T., Spierings, E., Ferrari-Lacraz, S., & Villard, J. (2016). Immunogenicity of Anti-HLA Antibodies in Pancreas and Islet Transplantation. *Cell Transplantation*, *25*(11), 2041–2050. https://doi.org/10.3727/096368916X691673

Chen, C.-C., Pouliquen, E., Broisat, A., Andreata, F., Racapé, M., Bruneval, P., Kessler, L., Ahmadi, M., Bacot, S., Saison-Delaplace, C., Marcaud, M., Van Huyen, J.-P. D., Loupy, A., Villard, J., Demuylder-Mischler, S., Berney, T., Morelon, E., Tsai, M.-K., Kolopp-Sarda, M.-N., … Thaunat, O. (2018). Endothelial chimerism and vascular sequestration protect pancreatic islet grafts from antibody-mediated rejection. *The Journal of Clinical Investigation*, *128*(1), 219–232. https://doi.org/10.1172/JCI93542

Cheng, E. Y., Everly, M. J., Kaneku, H., Banuelos, N., Wozniak, L. J., Venick, R. S., Marcus, E. A., McDiarmid, S. V., Busuttil, R. W., Terasaki, P. I., & Farmer, D. G. (2017). Prevalence and Clinical Impact of Donor-Specific Alloantibody Among Intestinal Transplant Recipients. *Transplantation*, *101*(4), 873–882. https://doi.org/10.1097/TP.0000000000001391

Chin, C., Chen, G., Sequeria, F., Berry, G., Siehr, S., Bernstein, D., Rosenthal, D., Reinhartz, O., & Tyan, D. (2011a). Clinical usefulness of a novel C1q assay to detect immunoglobulin G antibodies capable of fixing complement in sensitized pediatric heart transplant patients. *The Journal of Heart and Lung Transplantation*, *30*(2), 158–163. https://doi.org/10.1016/j.healun.2010.08.020

Chin, C., Chen, G., Sequeria, F., Berry, G., Siehr, S., Bernstein, D., Rosenthal, D., Reinhartz, O., & Tyan, D. (2011b). Clinical usefulness of a novel C1q assay to detect immunoglobulin G antibodies capable of fixing complement in sensitized pediatric heart transplant patients. *The Journal of Heart and Lung Transplantation*, *30*(2), 158–163. https://doi.org/10.1016/j.healun.2010.08.020

Chowdhry, M., Makroo, R. N., Singh, M., Kumar, M., Thakur, Y., & Sharma, V. (2018). Role of Anti-MICA Antibodies in Graft Survival of Renal Transplant Recipients of India. *Journal of Immunology Research*, *2018*, 1. https://doi.org/10.1155/2018/3434050

Chowdhry, M., Patel, M., Thakur, Y., & Sharma, V. (2019). Role of de novo DQ donor-specific antibody in antibody-mediated rejection in renal transplant recipient: A case study. *Asian Journal of Transfusion Science*, *13*(2), 136–139. https://doi.org/10.4103/ajts.AJTS_1_18

Ciszek, M., Mucha, K., Foroncewicz, B., Żochowska, D., Kosieradzki, M., Grochowiecki, T., Durlik, M., Górski, A., & Pączek, L. (2017). Immune biomarkers and long-term graft survival: A prospective follow-up of 457 kidney transplant recipients. *Polish Archives of Internal Medicine*, *127*(3), 178–183. https://doi.org/10.20452/pamw.3937

Clarysse, M., Canovai, E., Vanuytsel, T., & Pirenne, J. (2020). Current state of adult intestinal transplantation in Europe. *Current Opinion in Organ Transplantation*, *25*(2), 176–182. https://doi.org/10.1097/MOT.0000000000000731

Clerkin, K. J., Farr, M. A., Restaino, S. W., Zorn, E., Latif, F., Vasilescu, E. R., Marboe, C. C., Colombo, P. C., & Mancini, D. M. (2017). Donor-specific anti-HLA antibodies with antibody-mediated rejection and long-term outcomes following heart transplantation. *The Journal of Heart and Lung Transplantation*, *36*(5), 540–545. https://doi.org/10.1016/j.healun.2016.10.016

Collaborative Transplant Study Slide H11101-0821. (n.d.). Accessed 2021. https://ctstransplant.org/

Collaborative Transplant Study Slide H11101E-0821. (n.d.). https://ctstransplant.org/

Colvin, M. M., Cook, J. L., Chang, P. P., Hsu, D. T., Kiernan, M. S., Kobashigawa, J. A., Lindenfeld, J., Masri, S. C., Miller, D. V., Rodriguez, E. R., Tyan, D. B., Zeevi, A., & On behalf of the American Heart Association Heart Failure and Transplantation Committee of the Council on Clinical Cardiology; Council on Cardiovascular Disease in the Young; Council on Cardiovascular and Stroke Nursing; and Council on Cardiovascular Surgery and Anesthesia. (2019). Sensitization in Heart Transplantation: Emerging Knowledge: A Scientific Statement From the American Heart Association. *Circulation*, *139*(12), e553–e578. https://doi.org/10.1161/CIR.0000000000000598

Connolly, J. K., Dyer, P. A., Martin, S., Parrott, N. R., Pearson, R. C., & Johnson, R. W. G. (1996). Importance of minimizing HLA-DR mismatch and cold preservation time in cadaveric renal transplantation. *Transplantation*, *61*(5), 709–714. https://doi.org/10.1097/00007890-199603150-00007

Costanzo, M. R., Costanzo, M. R., Dipchand, A., Starling, R., Anderson, A., Chan, M., Desai, S., Fedson, S., Fisher, P., Gonzales-Stawinski, G., Martinelli, L., McGiffin, D., Parisi, F., Smith, J., Taylor, D., Meiser, B., Webber, S., Baran, D., Carboni, M., … Vanhaecke, J. (2010). The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. *The Journal of Heart and Lung Transplantation*, *29*(8), 914–956. https://doi.org/10.1016/j.healun.2010.05.034

Courtwright, A. M., Kamoun, M., Diamond, J. M., Kearns, J., Ahya, V. N., Christie, J. D., Clausen, E., Hadjiliadis, D., Patel, N., Salgado, J. C., Cevasco, M., Cantu, E. E., Crespo, M. M., & Bermudez, C. A. (2021). Lung transplantation outcomes after crossing low-level donor specific antibodies without planned augmented immunosuppression. *Clinical Transplantation*, *35*(11), e14447. https://doi.org/10.1111/ctr.14447

Couzi, L., Araujo, C., Guidicelli, G., Bachelet, T., Moreau, K., Morel, D., Robert, G., Wallerand, H., Moreau, J.-F., Taupin, J.-L., & Merville, P. (2011). Interpretation of positive flow cytometric crossmatch in the era of the single-antigen bead assay. *Transplantation*, *91*(5), 527–535. https://doi.org/10.1097/TP.0b013e31820794bb

Crismale, J. F., Mahmoud, D., Moon, J., Fiel, M. I., Iyer, K., & Schiano, T. D. (2021). The role of endoscopy in the small intestinal transplant recipient: A review. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *21*(5), 1705–1712. https://doi.org/10.1111/ajt.16354

Cross, A. R., Lion, J., Loiseau, P., Charron, D., Taupin, J.-L., Glotz, D., & Mooney, N. (2016). Donor Specific Antibodies are not only directed against HLA-DR: Minding your Ps and Qs. *Human Immunology*, *77*(11), 1092–1100. https://doi.org/10.1016/j.humimm.2016.04.003

Daga, S., & Briggs, D. (2023). Defining the lower and upper limits of immunological risk of HLA antibody incompatible kidney transplantation: Current state of the art and limitations. *Transplant Immunology*, *76*, 101775. https://doi.org/10.1016/j.trim.2022.101775

Das, A., Taner, T., Kim, J., & Emamaullee, J. (2021). Crossmatch, Donor-specific Antibody Testing, and Immunosuppression in Simultaneous Liver and Kidney Transplantation: A Review. *Transplantation*, *105*(12), e285–e291. https://doi.org/10.1097/TP.0000000000003694

Day, S. L. G. (2014). Possible role of HNA3a antibodies in graft rejection following kidney. *International Journal of Immunogenetics*, 413–436. https://doi.org/10.1111/iji.12142

De Kort, H., Roufosse, C., Bajema, I. M., & Drachenberg, C. B. (2013). Pancreas transplantation, antibodies and rejection: Where do we stand? *Current Opinion in Organ Transplantation*, *18*(3), 337–344. https://doi.org/10.1097/MOT.0b013e3283614a5c

de Serre, N. P.-M., Canioni, D., Lacaille, F., Talbotec, C., Dion, D., Brousse, N., & Goulet, O. (2008). Evaluation of c4d deposition and circulating antibody in small bowel transplantation. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *8*(6), 1290–1296. https://doi.org/10.1111/j.1600-6143.2008.02221.x

De Vlaminck, I., Valantine, H. A., Snyder, T. M., Strehl, C., Cohen, G., Luikart, H., Neff, N. F., Okamoto, J., Bernstein, D., Weisshaar, D., Quake, S. R., & Khush, K. K. (2014). Circulating Cell-Free DNA Enables Noninvasive Diagnosis of Heart Transplant Rejection. *Science Translational Medicine*, *6*(241), 241ra77. https://doi.org/10.1126/scitranslmed.3007803

Del Bello, A., Thaunat, O., Le Quintrec, M., Bestard, O., Durrbach, A., Perrin, P., Gatault, P., Jambon, F., Pageaux, G.-P., Llado, L., Besch, C., Barbier, L., Neau-Cransac, M., Dumortier, J., & Kamar, N. (2020). Combined Liver-Kidney Transplantation With Preformed Anti-human Leukocyte Antigen Donor-Specific Antibodies. *Kidney International Reports*, *5*(12), 2202–2211. https://doi.org/10.1016/j.ekir.2020.09.018

Deltombe, C., Gillaizeau, F., Anglicheau, D., Morelon, E., Trébern-Launay, K., Le Borgne, F., Rimbert, M., Guérif, P., Malard-Castagnet, S., Foucher, Y., & Giral, M. (2017). Is pre-transplant sensitization against angiotensin II type 1 receptor still a risk factor of graft and patient outcome in kidney transplantation in the anti-HLA Luminex era? A retrospective study. *Transplant International: Official Journal of the European Society for Organ Transplantation*, *30*(11), 1150–1160. https://doi.org/10.1111/tri.13009

Demetris, A. J., Bellamy, C., Hübscher, S. G., O'Leary, J., Randhawa, P. S., Feng, S., Neil, D., Colvin, R. B., McCaughan, G., Fung, J. J., Del Bello, A., Reinholt, F. P., Haga, H., Adeyi, O., Czaja, A. J., Schiano, T., Fiel, M. I., Smith, M. L., Sebagh, M., … Zen, Y. (2016). 2016 Comprehensive Update of the Banff Working Group on Liver Allograft Pathology: Introduction of Antibody-Mediated Rejection. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *16*(10), 2816–2835. https://doi.org/10.1111/ajt.13909

DeVos, J. M., Gaber, A. O., Knight, R. J., Land, G. A., Suki, W. N., Gaber, L. W., & Patel, S. J. (2012). Donor-specific HLA-DQ antibodies may contribute to poor graft outcome after renal transplantation. *Kidney International*, *82*(5), 598–604. https://doi.org/10.1038/ki.2012.190

Dinavahi, R., George, A., Tretin, A., Akalin, E., Ames, S., Bromberg, J. S., Deboccardo, G., Dipaola, N., Lerner, S. M., Mehrotra, A., Murphy, B. T., Nadasdy, T., Paz-Artal, E., Salomon, D. R., Schröppel, B., Sehgal, V., Sachidanandam, R., & Heeger, P. S. (2011). Antibodies reactive to non-HLA antigens in transplant glomerulopathy. *Journal of the American Society of Nephrology: JASN*, *22*(6), 1168–1178. https://doi.org/10.1681/ASN.2010111183

Drachenberg, C. B., Torrealba, J. R., Nankivell, B. J., Rangel, E. B., Bajema, I. M., Kim, D. U., Arend, L., Bracamonte, E. R., Bromberg, J. S., Bruijn, J. A., Cantarovich, D., Chapman, J. R., Farris, A. B., Gaber, L., Goldberg, J. C., Haririan, A., Honsová, E., Iskandar, S. S., Klassen, D. K., … Bartlett, S. T. (2011). Guidelines for the diagnosis of antibody-mediated rejection in pancreas allografts-updated Banff grading schema. *American Journal of Transplantation*, *11*(9), 1792–1802. https://doi.org/10.1111/j.1600-6143.2011.03670.x

Du Rand, I. A., Blaikley, J., Booton, R., Chaudhuri, N., Gupta, V., Khalid, S., Mandal, S., Martin, J., Mills, J., Navani, N., Rahman, N. M., Wrightson, J. M., Munavvar, M., & on behalf of the British Thoracic Society Bronchoscopy Guideline Group. (2013). British Thoracic Society guideline for diagnostic flexible bronchoscopy in adults: Accredited by NICE. *Thorax*, *68*(Suppl 1), i1–i44. https://doi.org/10.1136/thoraxjnl-2013-203618

Duquesnoy, R. J. (2008). Clinical usefulness of HLAMatchmaker in HLA epitope matching for organ transplantation. *Current Opinion in Immunology*, *20*(5), 594–601. https://doi.org/10.1016/j.coi.2008.06.010

Duquesnoy, R. J. (2011). Humoral alloimmunity in transplantation: Relevance of HLA epitope antigenicity and immunogenicity. *Frontiers in Immunology*, *2*, 59. https://doi.org/10.3389/fimmu.2011.00059

Duquesnoy, R. J., Marrari, M., Mulder, A., da Mata Sousa, L. C. D., Silva, A. S., & do Monte, S. J. H. (2014). First report on the antibody verification of HLA-ABC epitopes recorded in the website-based HLA Epitope Registry. *Tissue Antigens*, *83*(6), 391–400. https://doi.org/10.1111/tan.12341

Duquesnoy, R. J., Marrari, M., Tambur, A. R., Mulder, A., da Mata Sousa, L. C. D., da Silva, A. S., & do Monte, S. J. H. (2014). First report on the antibody verification of HLA-DR, HLA-DQ and HLA-DP epitopes recorded in the HLA Epitope Registry. *Human Immunology*, *75*(11), 1097–1103. https://doi.org/10.1016/j.humimm.2014.09.012

Eby, B. C., Redfield, R. R., Ellis, T. M., Leverson, G. E., Schenian, A. R., & Odorico, J. S. (2016). Virtual HLA Crossmatching as a Means to Safely Expedite Transplantation of Imported Pancreata. *Transplantation*, *100*(5), 1103–1110. https://doi.org/10.1097/TP.0000000000001125

El-Awar, N., Terasaki, P. I., Nguyen, A., Sasaki, N., Morales-Buenrostro, L. E., Saji, H., Maruya, E., & Poli, F. (2009). Epitopes of human leukocyte antigen class I antibodies found in sera of normal healthy males and cord blood. *Human Immunology*, *70*(10), 844–853. https://doi.org/10.1016/j.humimm.2009.06.020

Eng, H. S., Bennett, G., Tsiopelas, E., Lake, M., Humphreys, I., Chang, S. H., Coates, P. T. H., & Russ, G. R. (2008). Anti-HLA donor-specific antibodies detected in positive B-cell crossmatches by Luminex predict late graft loss. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *8*(11), 2335–2342. https://doi.org/10.1111/j.1600-6143.2008.02387.x

Farmer, D. G., McDiarmid, S. V., Yersiz, H., Cortina, G., Amersi, F., Vargas, J., Gershman, G., Ament, M., & Busuttil, R. W. (2001). Outcome after intestinal transplantation: Results from one center's 9-year experience; discussion 1031–2. *Archives of Surgery (Chicago, Ill.: 1960)*, *136*(9), 1027–1031. https://doi.org/10.1001/archsurg.136.9.1027

Farmer, D. G., Venick, R. S., Colangelo, J., Esmailian, Y., Yersiz, H., Duffy, J. P., Cortina, G. R., Artavia, K., Ngo, K., McDiarmid, S. V., & Busuttil, R. W. (2010). Pretransplant predictors of survival after intestinal transplantation: Analysis of a single-center experience of more than 100 transplants. *Transplantation*, *90*(12), 1574–1580. https://doi.org/10.1097/TP.0b013e31820000a1

Farrero Torres, M., Pando, M. J., Luo, C., Luikart, H., Valantine, H., & Khush, K. (2017). The role of complement-fixing donor-specific antibodies identified by a C1q assay after heart transplantation. *Clinical Transplantation*, *31*(11), e13121. https://doi.org/10.1111/ctr.13121

Feingold, B., Rose-Felker, K., West, S. C., Zinn, M. D., Berman, P., Moninger, A., Huston, A., Stinner, B., Xu, Q., Zeevi, A., & Miller, S. A. (2022). Early findings after integration of donor-derived cell-free DNA into clinical care following pediatric heart transplantation. *Pediatric Transplantation*, *26*(1), e14124. https://doi.org/10.1111/petr.14124

Fhied, C., Kanangat, S., & Borgia, J. A. (2014). Development of a bead-based immunoassay to routinely measure vimentin autoantibodies in the clinical setting. *Journal of Immunological Methods*, *407*, 9–14. https://doi.org/10.1016/j.jim.2014.03.011

Frank, R., Dean, S. A., Molina, M. R., Kamoun, M., & Lal, P. (2015). Correlations of lymphocyte subset infiltrates with donor-specific antibodies and acute antibody-mediated rejection in endomyocardial biopsies. *Cardiovascular Pathology*, *24*(3), 168–172. https://doi.org/10.1016/j.carpath.2014.11.001

Gerlach, U. A., Lachmann, N., Sawitzki, B., Arsenic, R., Neuhaus, P., Schoenemann, C., & Pascher, A. (2014). Clinical relevance of the de novo production of anti-HLA antibodies following intestinal and multivisceral transplantation. *Transplant International: Official Journal of the European Society for Organ Transplantation*, *27*(3), 280–289. https://doi.org/10.1111/tri.12250

Giral, M., Foucher, Y., Dufay, A., Duong Van Huyen, J. P., Renaudin, K., Moreau, A., Philippe, A., Hegner, B., Dechend, R., Heidecke, H., Brouard, S., Cesbron, A., Castagnet, S., Devys, A., Soulillou, J. P., & Dragun, D.

(2013). Pretransplant sensitization against angiotensin II type 1 receptor is a risk factor for acute rejection and graft loss. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *13*(10), 2567–2576. https://doi.org/10.1111/ajt.12397

Gordon, R. D., Fung, J. J., Markus, B., Fox, I., Iwatsuki, S., Esquivel, C. O., Tzakis, A., Todo, S., & Starzl, T. E. (1986). The antibody crossmatch in liver transplantation. *Surgery*, *100*(4), 705–715.

Gralla, J., Tong, S., & Wiseman, A. C. (2013). The Impact of Human Leukocyte Antigen Mismatching on Sensitization Rates and Subsequent Retransplantation After First Graft Failure in Pediatric Renal Transplant Recipients. *Transplantation*, *95*(10), 1218–1224. https://doi.org/10.1097/TP.0b013e318288ca14

Grant, D., Abu-Elmagd, K., Mazariegos, G., Vianna, R., Langnas, A., Mangus, R., Farmer, D. G., Lacaille, F., Iyer, K., Fishbein, T., & Intestinal Transplant Association. (2015). Intestinal transplant registry report: Global activity and trends. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *15*(1), 210–219. https://doi.org/10.1111/ajt.12979

Grant, D., Wall, W., Mimeault, R., Zhong, R., Ghent, C., Garcia, B., Stiller, C., & Duff, J. (1990). Successful small-bowel/liver transplantation. *Lancet (London, England)*, *335*(8683), 181–184. https://doi.org/10.1016/0140-6736(90)90275-a

Haas, M., Loupy, A., Lefaucheur, C., Roufosse, C., Glotz, D., Seron, D., Nankivell, B. J., Halloran, P. F., Colvin, R. B., Akalin, E., Alachkar, N., Bagnasco, S., Bouatou, Y., Becker, J. U., Cornell, L. D., Duong van Huyen, J. P., Gibson, I. W., Kraus, E. S., Mannon, R. B., … Mengel, M. (2018). The Banff 2017 Kidney Meeting Report: Revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *18*(2), 293–307. https://doi.org/10.1111/ajt.14625

Hachem, R. R., & Reinsmoen, N. L. (2015). What is the Definition of a Clinically Relevant Donor HLA-Specific Antibody (DSA)? *American Journal of Transplantation*, *15*(2), 299–300. https://doi.org/10.1111/ajt.13079

Hachem, R. R., Yusen, R. D., Meyers, B. F., Aloush, A. A., Mohanakumar, T., Patterson, G. A., & Trulock, E. P. (2010). Anti-human leukocyte antigen antibodies and preemptive antibody-directed therapy after lung transplantation. *The Journal of Heart and Lung Transplantation*, *29*(9), 973–980. https://doi.org/10.1016/j.healun.2010.05.006

Halpin, A. M., Nahirniak, S., Campbell, P. M., Urschel, S., Kim, D. H., West, L. J., Pidorochynski, T., Buchholz, H., & Conway, J. (2019). HLA Alloimmunization Following Ventricular Assist Device Support Across the Age Spectrum. *Transplantation*, *103*(12), 2715–2724. https://doi.org/10.1097/TP.0000000000002798

Hawksworth, J. S., & Matsumoto, C. S. (2019). Donor-specific antibody management in intestine transplantation: Hope for improving the long-term durability of the intestine allograft? *Current Opinion in Organ Transplantation*, *24*(2), 212–218. https://doi.org/10.1097/MOT.0000000000000619

He, J., Li, C., Yuan, X., Zhang, J., Li, Y., Wei, X., & Hou, J. (2013). Anti-human leukocyte antigens and anti-major histocompatibility complex class I-related chain A antibody expression in kidney transplantation during a four-year follow-up. *Chinese Medical Journal*, *126*(15), 2815–2820.

Heidt, S., Haasnoot, G. W., & Claas, F. H. J. (2018). How the definition of acceptable antigens and epitope analysis can facilitate transplantation of highly sensitized patients with excellent long-term graft survival. *Current Opinion in Organ Transplantation*, *23*(4), 493–499. https://doi.org/10.1097/MOT.0000000000000545

Higgins, R., Lowe, D., Daga, S., Hathaway, M., Williams, C., Lam, F. T., Kashi, H., Tan, L. C., Imray, C., Fletcher, S., Krishnan, N., Hart, P., Zehnder, D., & Briggs, D. (2015). Pregnancy-induced HLA antibodies respond more vigorously after renal transplantation than antibodies induced by prior transplantation. *Human Immunology*, *76*(8), 546–552. https://doi.org/10.1016/j.humimm.2015.06.013

Hilbrands, R., Gillard, P., Van der Torren, C. R., Ling, Z., Verheyden, S., Jacobs-Tulleneers-Thevissen, D., Roep, B. O., Claas, F. H. J., Demanet, C., Gorus, F. K., Pipeleers, D., & Keymeulen, B. (2013). Predictive factors of allosensitization after immunosuppressant withdrawal in recipients of long-term cultured islet cell grafts. *Transplantation*, *96*(2), 162–169. https://doi.org/10.1097/TP.0b013e3182977afc

Hodges, A. M., Lyster, H., McDermott, A., Rice, A. J., Smith, J. D., Rose, M. L., & Banner, N. R. (2012). Late Antibody-Mediated Rejection After Heart Transplantation Following the Development of De Novo Donor-Specific Human Leukocyte Antigen Antibody. *Transplantation*, *93*(6), 650–656. https://doi.org/10.1097/TP.0b013e318244f7b8

Issitt, R., Shetty, P., Crook, R., Cross, N., Henwood, S., Broadhead, M., Spencer, H., Aurora, P., Gupta, A., Kallon, D., Fenton, M., & Muthialu, N. (2022). Lung transplantation in an 18-month-old with donor specific antibodies – The use of intraoperative, targeted plasma exchange. *Perfusion*, 026765912211149. https://doi.org/10.1177/02676591221114958

Jackson, A. M., Wiebe, C., & Hickey, M. J. (2020). The role of non-HLA antibodies in solid organ transplantation: A complex deliberation. *Current Opinion in Organ Transplantation*, *25*(6), 536–542. https://doi.org/10.1097/MOT.0000000000000811

Jacob, E. K., De Goey, S. R., & Gandhi, M. J. (2011). Positive virtual crossmatch with negative flow crossmatch results in two cases. *Transplant Immunology*, *25*(1), 77–81. https://doi.org/10.1016/j.trim.2011.05.007

John, R., Lietz, K., Schuster, M., Naka, Y., Rao, V., Mancini, D. M., Rose, E. A., Smith, C. R., Oz, M. C., Edwards, N. M., & Itescu, S. (2003). Immunologic sensitization in recipients of left ventricular assist devices. *The Journal of Thoracic and Cardiovascular Surgery*, *125*(3), 578–591. https://doi.org/10.1067/mtc.2003.30

Joosten, S. A., Sijpkens, Y. W. J., van Ham, V., Trouw, L. A., van der Vlag, J., van den Heuvel, B., van Kooten, C., & Paul, L. C. (2005). Antibody response against the glomerular basement membrane protein agrin in patients with transplant glomerulopathy. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *5*(2), 383–393. https://doi.org/10.1111/j.1600-6143.2005.00690.x

Jordan, S. C., Legendre, C., Desai, N. M., Lorant, T., Bengtsson, M., Lonze, B. E., Vo, A. A., Runström, A., Laxmyr, L., Sjöholm, K., Schiött, Å., Sonesson, E., Wood, K., Winstedt, L., Kjellman, C., & Montgomery, R. A. (2021). Imlifidase Desensitization in Crossmatch-positive, Highly Sensitized Kidney Transplant Recipients: Results of an International Phase 2 Trial (Highdes). *Transplantation*, *105*(8), 1808–1817. https://doi.org/10.1097/TP.0000000000003496

Jucaud, V., Ravindranath, M. H., & Terasaki, P. I. (2017). Conformational Variants of the Individual HLA-I Antigens on Luminex Single Antigen Beads Used in Monitoring HLA Antibodies: Problems and Solutions. *Transplantation*, *101*(4), 764–777. https://doi.org/10.1097/TP.0000000000001420

Kang, Z.-Y., Liu, C., Liu, W., & Li, D.-H. (2022). Effect of C1q-binding donor-specific anti-HLA antibodies on the clinical outcomes of patients after renal transplantation: A systematic review and meta-analysis. *Transplant Immunology*, *72*, 101566. https://doi.org/10.1016/j.trim.2022.101566

Kannabhiran, D., Lee, J., Schwartz, J. E., Friedlander, R., Aull, M., Muthukumar, T., Campbell, S., Epstein, D., Seshan, S. V., Kapur, S., Sharma, V. K., Suthanthiran, M., & Dadhania, D. (2015). Characteristics of Circulating Donor Human Leukocyte Antigen-specific Immunoglobulin G Antibodies Predictive of Acute Antibody-mediated Rejection and Kidney Allograft Failure. *Transplantation*, *99*(6), 1156–1164. https://doi.org/10.1097/TP.0000000000000511

Karahan, G. E., de Vaal, Y., Bakker, K., Roelen, D., Claas, F. H. J., & Heidt, S. (2021). Comparison of different luminex single antigen bead kits for memory B cell-derived HLA antibody detection. *HLA*, *98*(3), 200–206. https://doi.org/10.1111/tan.14356

Karahan, G. E., de Vaal, Y. J. H., Krop, J., Wehmeier, C., Roelen, D. L., Claas, F. H. J., & Heidt, S. (2017). A Memory B Cell Crossmatch Assay for Quantification of Donor-Specific Memory B Cells in the Peripheral Blood of HLA-Immunized Individuals. *American Journal of Transplantation*, *17*(10), 2617–2626. https://doi.org/10.1111/ajt.14293

Keller, M., & Agbor-Enoh, S. (2022). Cell-free DNA in lung transplantation: Research tool or clinical workhorse? *Current Opinion in Organ Transplantation*, *27*(3), 177–183. https://doi.org/10.1097/MOT.0000000000000979

Kessler, L., Parissiadis, A., Bayle, F., Moreau, F., Pinget, M., Froelich, N., Cazenave, J.-P., Berney, T., Benhamou, P. Y., Hanau, D., & GRAGIL Study Group. (2009). Evidence for humoral rejection of a pancreatic islet graft and rescue with rituximab and IV immunoglobulin therapy. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *9*(8), 1961–1966. https://doi.org/10.1111/j.1600-6143.2009.02711.x

Key, T., Carter, V., & Day, S. (2019). Human Neutrophic antibodies associated with early and chronic antibody mediated rejection in kidney transplant recipients. *Journal of Nephrology and Renal Transplantation Science*, *2*, 81–84.

Key, T., Carter, V., & Goodwin, J. (2018). Human neutrophil antibodies are associated with severe early rejection in kidney transplant recipients. *Transplantation*, *102*, S214.

Kfoury, A. G., & Miller, D. V. (2019). The impact of asymptomatic antibody-mediated rejection on outcome after heart transplantation. *Current Opinion in Organ Transplantation*, *24*(3), 259–264. https://doi.org/10.1097/MOT.0000000000000640

Khan, S. M., Sumbal, R., & Schenk, A. D. (2021). Impact of Anti-HLA De Novo Donor Specific Antibody on Graft Outcomes in Pancreas Transplantation: A Meta-Analysis. *Transplantation Proceedings*, *53*(10), 3022–3029. https://doi.org/10.1016/j.transproceed.2021.08.052

Khush, K. K., Patel, J., Pinney, S., Kao, A., Alharethi, R., DePasquale, E., Ewald, G., Berman, P., Kanwar, M., Hiller, D., Yee, J. P., Woodward, R. N., Hall, S., & Kobashigawa, J. (2019). Noninvasive detection of graft injury after heart transplant using donor-derived cell-free DNA: A prospective multicenter study. *American Journal of Transplantation*, *19*(10), 2889–2899. https://doi.org/10.1111/ajt.15339

Kobashigawa, J., Colvin, M., Potena, L., Dragun, D., Crespo-Leiro, M. G., Delgado, J. F., Olymbios, M., Parameshwar, J., Patel, J., Reed, E., Reinsmoen, N., Rodriguez, E. R., Ross, H., Starling, R. C., Tyan, D., Urschel, S., & Zuckermann, A. (2018). The management of antibodies in heart transplantation: An ISHLT consensus document. *The Journal of Heart and Lung Transplantation*, *37*(5), 537–547. https://doi.org/10.1016/j.healun.2018.01.1291

Koo, J., & Wang, H. L. (2016). Small Bowel Transplant Pathology. In W. D. Wallace, & B. V. Naini (Eds.), *Practical Atlas of Transplant Pathology* (pp. 133–151). Springer International Publishing. https://doi.org/10.1007/978-3-319-23054-2_6

Kosmoliaptsis, V., Bradley, J. A., Peacock, S., Chaudhry, A. N., & Taylor, C. J. (2009). Detection of immunoglobulin G human leukocyte antigen-specific alloantibodies in renal transplant patients using single-antigen-beads is compromised by the presence of immunoglobulin M human leukocyte antigen-specific alloantibodies. *Transplantation*, *87*(6), 813–820. https://doi.org/10.1097/TP.0b013e318199c581

Kubal, C., Mangus, R., Saxena, R., Lobashevsky, A., Higgins, N., Fridell, J., & Tector, A. J. (2015). Prospective Monitoring of Donor-specific Anti-HLA Antibodies After Intestine/Multivisceral Transplantation: Significance of De Novo Antibodies. *Transplantation*, *99*(8), e49–e56. https://doi.org/10.1097/TP.0000000000000614

Kueht, M., Jindra, P., Stevenson, H. L., Galvan, T. N., Murthy, B., Goss, J., Anton, J., Abbas, R., & Cusick, M. F. (2021). Intra-operative kinetics of anti-HLA antibody in simultaneous liver-kidney transplantation. *Molecular Genetics and Metabolism Reports*, *26*, 100705. https://doi.org/10.1016/j.ymgmr.2020.100705

Kwon, H., Kim, Y. H., Choi, J. Y., Shin, S., Jung, J. H., Park, S.-K., & Han, D. J. (2018). Impact of pretransplant donor-specific antibodies on kidney allograft recipients with negative flow cytometry cross-matches. *Clinical Transplantation*, *32*(6), e13266. https://doi.org/10.1111/ctr.13266

Lablanche, S., Borot, S., Thaunat, O., Bayle, F., Badet, L., Morelon, E., Thivolet, C., Wojtusciszyn, A., Frimat, L., Kessler, L., Penfornis, A., Brault, C., Colin, C., Bosco, D., Berney, T., Benhamou, P. Y., & GRAGIL Network. (2014). Impact of anti-insulin antibodies on islet transplantation outcome: Data from the GRAGIL Network. *Transplantation*, *98*(4), 475–482. https://doi.org/10.1097/TP.0000000000000081

Ladowski, J. M., Mullins, H., Romine, M., Kloda, D., Young, C., Hauptfeld-Dolejsek, V., Houp, J., & Locke, J. (2021). Eplet mismatch scores and de novo donor-specific antibody development in simultaneous pancreas-kidney transplantation. *Human Immunology*, *82*(3), 139–146. https://doi.org/10.1016/j.humimm.2020.12.009

Lan, J. H., & Tinckam, K. (2018). Clinical Utility of Complement Dependent Assays in Kidney Transplantation. *Transplantation*, *102*(1S), S14–S22. https://doi.org/10.1097/TP.0000000000001819

Lauro, A., Oltean, M., & Marino, I. R. (2018). Chronic Rejection After Intestinal Transplant: Where Are We in Order to Avert It? *Digestive Diseases and Sciences*, *63*(3), 551–562. https://doi.org/10.1007/s10620-018-4909-7

Leca, N., Warner, P., Bakthavatsalam, R., Nelson, K., Halldorson, J., Rayhill, S., Kendrick, E., Davis, C., & Reyes, J. (2013). Outcomes of simultaneous liver and kidney transplantation in relation to a high level of preformed donor-specific antibodies. *Transplantation*, *96*(10), 914–918. https://doi.org/10.1097/TP.0b013e3182a192f5

Lee, J., Huh, K. H., Park, Y., Park, B. G., Yang, J., Jeong, J. C., Lee, J., Park, J. B., Cho, J.-H., Lee, S., Ro, H., Han, S.-Y., Kim, M. S., Kim, Y. S., Kim, S. J., Kim, C.-D., Chung, W., Park, S.-B., Ahn, C., & KNOW-KT Study Group. (2017). The clinicopathological relevance of pretransplant anti-angiotensin II type 1 receptor antibodies in renal transplantation. *Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplant Association - European Renal Association*, *32*(7), 1244–1250. https://doi.org/10.1093/ndt/gfv375

Lee, J.-H. (2019). Letter to the editor. *Journal of Immunological Methods*, *474*, 112449. https://doi.org/10.1016/j.jim.2018.05.001

Lefaucheur, C., Loupy, A., Hill, G. S., Andrade, J., Nochy, D., Antoine, C., Gautreau, C., Charron, D., Glotz, D., & Suberbielle-Boissel, C. (2010). Preexisting donor-specific HLA antibodies predict outcome in kidney transplantation. *Journal of the American Society of Nephrology: JASN*, *21*(8), 1398–1406. https://doi.org/10.1681/ASN.2009101065

Lemy, A., Andrien, M., Lionet, A., Labalette, M., Noel, C., Hiesse, C., Delahousse, M., Suberbielle-Boissel, C., De Meyer, M., Latinne, D., Mourad, M., Delsaut, S., Racapé, J., Wissing, K. M., Toungouz, M., & Abramowicz, D. (2012). Posttransplant major histocompatibility complex class I chain-related gene A antibodies and long-term graft outcomes in a multicenter cohort of 779 kidney transplant recipients. *Transplantation*, *93*(12), 1258–1264. https://doi.org/10.1097/TP.0b013e31824fd8f1

Lemy, A., Andrien, M., Wissing, K. M., Ryhahi, K., Vandersarren, A., Racapé, J., Heylen, C., Ghisdal, L., Broeders, E., Vereerstraeten, P., Toungouz, M., & Abramowicz, D. (2010). Major histocompatibility complex class 1 chain-related antigen a antibodies: Sensitizing events and impact on renal graft outcomes. *Transplantation*, *90*(2), 168–174. https://doi.org/10.1097/TP.0b013e3181e228f7

Levine, D. J., Glanville, A. R., Aboyoun, C., Belperio, J., Benden, C., Berry, G. J., Hachem, R., Hayes, D., Neil, D., Reinsmoen, N. L., Snyder, L. D., Sweet, S., Tyan, D., Verleden, G., Westall, G., Yusen, R. D., Zamora, M., & Zeevi, A. (2016). Antibody-mediated rejection of the lung: A consensus report of the International Society for Heart and Lung Transplantation. *The Journal of Heart and Lung Transplantation: The Official Publication of the International Society for Heart Transplantation*, *35*(4), 397–406. https://doi.org/10.1016/j.healun.2016.01.1223

Levitsky, J., Kaneku, H., Jie, C., Walsh, R. C., Abecassis, M., & Tambur, A. R. (2016). Donor-Specific HLA Antibodies in Living Versus Deceased Donor Liver Transplant Recipients. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *16*(8), 2437–2444. https://doi.org/10.1111/ajt.13757

Li, L., Wadia, P., Chen, R., Kambham, N., Naesens, M., Sigdel, T. K., Miklos, D. B., Sarwal, M. M., & Butte, A. J. (2009). Identifying compartment-specific non-HLA targets after renal transplantation by integrating transcriptome and 'antibodyome' measures. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(11), 4148–4153. https://doi.org/10.1073/pnas.0900563106

Lionaki, S., Panagiotellis, K., Iniotaki, A., & Boletis, J. N. (2013). Incidence and clinical significance of de novo donor specific antibodies after kidney transplantation. *Clinical & Developmental Immunology*, *2013*, 849835. https://doi.org/10.1155/2013/849835

Liu, C., Wetter, L., Pang, S., Phelan, D. L., Mohanakumar, T., & Morris, G. P. (2012). Cutoff values and data handling for solid-phase testing for antibodies to HLA: Effects on listing unacceptable antigens for thoracic organ transplantation. *Human Immunology*, *73*(6), 597–604. https://doi.org/10.1016/j.humimm.2012.04.016

Lucisano, G., Thiruvengadam, S., Hassan, S., Gueret-Wardle, A., Brookes, P., Santos-Nunez, E., & Willicombe, M. (2020). Donor-specific antibodies detected by single antigen beads alone can help risk stratify patients undergoing retransplantation across a repeat HLA mismatch. *American Journal of Transplantation*, *20*(2), 441–450. https://doi.org/10.1111/ajt.15595

Lonze, B. E. (2021). A review of imlifidase in solid organ transplantation. *Expert Opinion on Biological Therapy*, *21*(2), 135–143. https://doi.org/10.1080/14712598.2021.1850685

Lopez-Soler, R. I., Borgia, J. A., Kanangat, S., Fhied, C. L., Conti, D. J., Constantino, D., Ata, A., Chan, R., & Wang, Z. (2016). Anti-vimentin Antibodies Present at the Time of Transplantation May Predict Early Development of Interstitial Fibrosis/Tubular Atrophy. *Transplantation Proceedings*, *48*(6), 2023–2033. https://doi.org/10.1016/j.transproceed.2016.04.009

Loupy, A., Haas, M., Roufosse, C., Naesens, M., Adam, B., Afrouzian, M., Akalin, E., Alachkar, N., Bagnasco, S., Becker, J. U., Cornell, L. D., Clahsen-van Groningen, M. C., Demetris, A. J., Dragun, D., Duong van Huyen, J.-P., Farris, A. B., Fogo, A. B., Gibson, I. W., Glotz, D., … Mengel, M. (2020). The Banff 2019 Kidney Meeting Report (I): Updates on and clarification of criteria for T cell- and antibody-mediated rejection. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *20*(9), 2318–2331. https://doi.org/10.1111/ajt.15898

Martins, L. S., Henriques, A. C., Fonseca, I. M., Rodrigues, A. S., Oliverira, J. C., Dores, J. M., Dias, L. S., Cabrita, A. M., Silva, J. D., & Noronha, I. L. (2014). Pancreatic autoantibodies after pancreas-kidney transplantation—Do they matter? *Clinical Transplantation*, *28*(4), 462–469. https://doi.org/10.1111/ctr.12337

Matko, J., Bushkin, Y., Wei, T., & Edidin, M. (1994). Clustering of class I HLA molecules on the surfaces of activated and transformed human cells. *Journal of Immunology (Baltimore, Md.: 1950)*, *152*(7), 3353–3360.

Matsumoto, C. S., & Rosen-Bronson, S. (2021). Donor-specific antibody and sensitized patients in intestinal transplantation. *Current Opinion in Organ Transplantation*, *26*(2), 245–249. https://doi.org/10.1097/MOT.0000000000000853

McCarthy, J. F., Cook, D. J., Massad, M. G., Sano, Y., O'Malley, K. J., Ratliff, N. R., Stewart, R. W., Smedira, N. G., Starling, R. C., Young, J. B., & McCarthy, P. M. (1998). Vascular rejection post heart transplantation is associated with positive flow cytometric cross-matching1. *European Journal of Cardio-Thoracic Surgery*, *14*(2), 197–200. https://doi.org/10.1016/S1010-7940(98)00159-6

McCaughan, J. A., Battle, R. K., Singh, S. K. S., Tikkanen, J. M., Moayedi, Y., Ross, H. J., Singer, L. G., Keshavjee, S., & Tinckam, K. J. (2018). Identification of risk epitope mismatches associated with de novo donor-specific HLA antibody development in cardiothoracic transplantation. *American Journal of Transplantation*, *18*(12), 2924–2933. https://doi.org/10.1111/ajt.14951

McCaughan, J. A., Robertson, V., Falconer, S. J., Cryer, C., Turner, D. M., & Oniscu, G. C. (2016). Preformed donor-specific HLA antibodies are associated with increased risk of early mortality after liver transplantation. *Clinical Transplantation*, *30*(12), 1538–1544. https://doi.org/10.1111/ctr.12851

Meneghini, M., Crespo, E., Niemann, M., Torija, A., Lloberas, N., Pernin, V., Fontova, P., Melilli, E., Favà, A., Montero, N., Manonelles, A., Cruzado, J. M., Palou, E., Martorell, J., Grinyó, J. M., & Bestard, O. (2021). Donor/Recipient HLA Molecular Mismatch Scores Predict Primary Humoral and Cellular Alloimmunity in Kidney Transplantation. *Frontiers in Immunology*, *11*, 623276. https://doi.org/10.3389/fimmu.2020.623276

Middleton, D., Jones, J., & Lowe, D. (2014). Nothing's perfect: The art of defining HLA-specific antibodies. *Transplant Immunology*, *30*(4), 115–121. https://doi.org/10.1016/j.trim.2014.02.003

Middleton, S. J., & Jamieson, N. V. (2005). The current status of small bowel transplantation in the UK and internationally. *Gut*, *54*(11), 1650–1657. https://doi.org/10.1136/gut.2004.062612

Min, J. W., Lee, H., Choi, B. S., Park, C. W., Yang, C. W., Kim, Y.-S., Choi, Y. J., Oh, E.-J., & Chung, B. H. (2018). Clinical Impact of Pre-transplant Antibodies Against Angiotensin II Type I Receptor and Major Histocompatibility Complex Class I-Related Chain A in Kidney Transplant Patients. *Annals of Laboratory Medicine*, *38*(5), 450–457. https://doi.org/10.3343/alm.2018.38.5.450

Mittal, S., Page, S. L., Friend, P. J., Sharples, E. J., & Fuggle, S. V. (2014). De novo donor-specific HLA antibodies: Biomarkers of pancreas transplant failure. *American Journal of Transplantation*, *14*(7), 1664–1671. https://doi.org/10.1111/ajt.12750

Moayedi, Y., Fan, C.-P. S., Tinckam, K. J., Ross, H. J., & McCaughan, J. A. (2018). De novo donor-specific HLA antibodies in heart transplantation: Do transient de novo DSA confer the same risk as persistent de novo DSA? *Clinical Transplantation*, *32*(11), e13416. https://doi.org/10.1111/ctr.13416

Mohan, S., Palanisamy, A., Tsapepas, D., Tanriover, B., Crew, R. J., Dube, G., Ratner, L. E., Cohen, D. J., & Radhakrishnan, J. (2012). Donor-specific antibodies adversely affect kidney allograft outcomes. *Journal of the American Society of Nephrology: JASN*, *23*(12), 2061–2071. https://doi.org/10.1681/ASN.2012070664

Mohanakumar, T., Narayanan, K., Desai, N., Ramachandran, S., Shenoy, S., Jendrisak, M., Susskind, B. M., Olack, B., Benshoff, N., Phelan, D. L., Brennan, D. C., Fernandez, L. A., Odorico, J. S., & Polonsky, K. S. (2006). A significant role for histocompatibility in human islet transplantation. *Transplantation*, *82*(2), 180–187. https://doi.org/10.1097/01.tp.0000226161.82581.b2

Morris, G. P., Phelan, D. L., Jendrisak, M. D., & Mohanakumar, T. (2010). Virtual crossmatch by identification of donor-specific anti-human leukocyte antigen antibodies by solid-phase immunoassay: A 30-month analysis in living donor kidney transplantation. *Human Immunology*, *71*(3), 268–273. https://doi.org/10.1016/j.humimm.2010.01.003

Morrison, A. H., Gupta, M., Lloyd, K., Trofe-Clark, J., Ann Lim, M., Limonte, C., Levine, M. H., Sawinski, D., Kamoun, M., & Porrett, P. M. (2019). Class and Kinetics of Weakly Reactive Pretransplant Donor-specific HLA Antibodies Predict Rejection in Kidney Transplant Recipients. *Transplantation Direct*, *5*(8), e478. https://doi.org/10.1097/TXD.0000000000000926

Motter, J. D., Jackson, K. R., Long, J. J., Waldram, M. M., Orandi, B. J., Montgomery, R. A., Stegall, M. D., Jordan, S. C., Benedetti, E., Dunn, T. B., Ratner, L. E., Kapur, S., Pelletier, R. P., Roberts, J. P., Melcher, M. L., Singh, P., Sudan, D. L., Posner, M. P., El-Amm, J. M., … Garonzik-Wang, J. M. (2021). Delayed graft function and acute rejection following HLA-incompatible living donor kidney transplantation. *American Journal of Transplantation: Official Journal of the American Society of*

Transplantation and the American Society of Transplant Surgeons, *21*(4), 1612–1621. https://doi.org/10.1111/ajt.16471

Murase, N., Ye, Q., Nalesnik, M. A., Demetris, A. J., Abu-Elmagd, K., Reyes, J., Ichikawa, N., Okuda, T., Fung, J. J., & Starzl, T. E. (2000). Immunomodulation for intestinal transplantation by allograft irradiation, adjunct donor bone marrow infusion, or both. *Transplantation*, *70*(11), 1632–1641. https://doi.org/10.1097/00007890-200012150-00016

Musat, A. I., Pigott, C. M., Ellis, T. M., Agni, R. M., Leverson, G. E., Powell, A. J., Richards, K. R., D'Alessandro, A. M., & Lucey, M. R. (2013). Pretransplant donor-specific anti-HLA antibodies as predictors of early allograft rejection in ABO-compatible liver transplantation. *Liver Transplantation: Official Publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society*, *19*(10), 1132–1141. https://doi.org/10.1002/lt.23707

Nakamura, T., & Shirouzu, T. (2021). Antibody-Mediated Rejection and Recurrent Primary Disease: Two Main Obstacles in Abdominal Kidney, Liver, and Pancreas Transplants. *Journal of Clinical Medicine*, *10*(22), 5417. https://doi.org/10.3390/jcm10225417

Newell, H., Smith, J. D., Rogers, P., Birks, E., Danskine, A. J., Fawson, R. E., & Roseb, M. L. (2006). Sensitization Following LVAD Implantation Using Leucodepleted Blood Is Not Due to HLA Antibodies. *American Journal of Transplantation*, *6*(7), 1712–1717. https://doi.org/10.1111/j.1600-6143.2006.01342.x

NHSBT. (2012). *Bowel Advisory Group—HLA specific antibodies in bowel transplantation: Standardisation of testing, reporting and crossmatchinng protocols in the UK*. https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/20032/kidney-annual-report-2019-20-final.pdf

NHSBT. (2020). *Annual report on Intestine Transplantation—Report for 2019/2020*. https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/19865/nhsbt-annual-report-on-intestine-transplantation-201920.pdf

NHSBT. (2021). *Annual report on living donor kidney transplantation April 2006–2021*. https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/25370/annual-report-on-living-donor-kidney-transplantation-2020-21.pdf

NHSBT liver transplantation report 2020. (2021). https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/27814/nhsbt-liver-transplant-report-2122-final.pdf

NHSBT ODT allocation Liver policy. (2018). https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/7893/national-liver-offering-scheme-outline.pdf

NHSBT ODT annual report. (2020). https://www.odt.nhs.uk/statistics-and-reports/annual-activity-report/

NHSBT ODT POL 228/13. (2023). https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/29680/pol228.pdf

NHSBT ODT POL 230. (2023). https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/29405/pol230.pdf

NHSBT ODT SPN1439/3. (2022). https://www.odt.nhs.uk/transplantation/pathology-services/donor-characterisation/

NIHCE 2022 guidance TA809. (2022). https://www.nice.org.uk/guidance/ta809/informationforpublic

Nikaein, A., El-Awar, N., Hunt, J., Rosenthal, E. J., Eichhorn, E., Hall, S., Boehrer, J., Magee, M., Pieniek, M., Brinkman, W., & Dewey, T. (2012). Clinically irrelevant circulating human leukocyte antigen antibodies in the presence of ventricular assist devices. *The Journal of Heart and Lung Transplantation*, *31*(5), 443–447. https://doi.org/10.1016/j.healun.2011.10.006

Njue, F., & Chih, S. (2019). When to intervene for donor-specific antibody after heart transplantation. *Current Opinion in Organ Transplantation*, *24*(3), 271–278. https://doi.org/10.1097/MOT.0000000000000634

Oaks, M., Michel, K., Sulemanjee, N. Z., Thohan, V., & Downey, F. X. (2014). Practical value of identifying antibodies to cryptic HLA epitopes in cardiac transplantation. *The Journal of Heart and Lung Transplantation*, *33*(7), 713–720. https://doi.org/10.1016/j.healun.2014.02.013

Ohe, H., Uchida, Y., Yoshizawa, A., Hirao, H., Taniguchi, M., Maruya, E., Yurugi, K., Hishida, R., Maekawa, T., Uemoto, S., & Terasaki, P. I. (2014). Association of anti-human leukocyte antigen and anti-angiotensin II type 1 receptor antibodies with liver allograft fibrosis after immunosuppression withdrawal. *Transplantation*, *98*(10), 1105–1111. https://doi.org/10.1097/TP.0000000000000185

O'Leary, J. G., Gebel, H. M., Ruiz, R., Bray, R. A., Marr, J. D., Zhou, X. J., Shiller, S. M., Susskind, B. M., Kirk, A. D., & Klintmalm, G. B. (2013). Class II alloantibody and mortality in simultaneous liver-kidney transplantation. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *13*(4), 954–960. https://doi.org/10.1111/ajt.12147

O'Leary, J. G., Kaneku, H., Banuelos, N., Jennings, L. W., Klintmalm, G. B., & Terasaki, P. I. (2015). Impact of IgG3 subclass and C1q-fixing donor-specific HLA alloantibodies on rejection and survival in liver transplantation. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *15*(4), 1003–1013. https://doi.org/10.1111/ajt.13153

O'Leary, J. G., Kaneku, H., Jennings, L. W., Bañuelos, N., Susskind, B. M., Terasaki, P. I., & Klintmalm, G. B. (2013). Preformed class II donor-specific antibodies are associated with an increased risk of early rejection after liver transplantation. *Liver Transplantation: Official Publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society*, *19*(9), 973–980. https://doi.org/10.1002/lt.23687

Olymbios, M. (2017). Heart Transplant across Low or Moderate Level Donor-Specific Antibodies Have Acceptable Outcome (Abstract B35). *American Journal of Transplantation*, *17*(Supplement 3). https://doi.org/10.1111/ajt.14306

Ong, S. C., White, J., Hauptfeld-Dolejsek, V., & Kumar, V. (2016). Outcomes in Simultaneous Liver Kidney Transplants in the Setting of a Positive Crossmatch: A Single Center Experience. *Clinical Transplants*, *32*, 119–125.

Orandi, B. J., Garonzik-Wang, J. M., Massie, A. B., Zachary, A. A., Montgomery, J. R., Van Arendonk, K. J., Stegall, M. D., Jordan, S. C., Oberholzer, J., Dunn, T. B., Ratner, L. E., Kapur, S., Pelletier, R. P., Roberts, J. P., Melcher, M. L., Singh, P., Sudan, D. L., Posner, M. P., El-Amm, J. M., … Segev, D. L. (2014). Quantifying the risk of incompatible kidney transplantation: A multicenter study. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *14*(7), 1573–1580. https://doi.org/10.1111/ajt.12786

Otten, H. G., Verhaar, M. C., Borst, H. P. E., van Eck, M., van Ginkel, W. G. J., Hené, R. J., & van Zuilen, A. D. (2013). The significance of pretransplant donor-specific antibodies reactive with intact or denatured human leucocyte antigen in kidney transplantation. *Clinical and Experimental Immunology*, *173*(3), 536–543. https://doi.org/10.1111/cei.12127

Pandey, P., Pande, A., Kumar Devra, A., Kumar Sinha, V., & Prasad Bhatt, A. (2021). Comparative analysis of complement-dependent lymphocytotoxicity crossmatch and flow cytometry crossmatch results versus Luminex single-antigen bead-based donor-specific IgG class I antibody MFI values in live related renal transplant cases; a retrospective observation in 102 cases. *Journal of Immunoassay and Immunochemistry*, *42*(3), 300–313. https://doi.org/10.1080/15321819.2020.1862865

Panigrahi, A., Gupta, N., Siddiqui, J. A., Margoob, A., Bhowmik, D., Guleria, S., & Mehra, N. K. (2007). Post transplant development of MICA and anti-HLA antibodies is associated with acute rejection episodes and renal allograft loss. *Human Immunology*, *68*(5), 362–367. https://doi.org/10.1016/j.humimm.2007.01.006

Parajuli, S., Arunachalam, A., Swanson, K. J., Aziz, F., Garg, N., Bath, N., Redfield, R. R., Kaufman, D., Djamali, A., Odorico, J., & Mandelbrot, D. A. (2019). Pancreas Retransplant after Pancreas Graft Failure in Simultaneous Pancreas-kidney Transplants Is Associated with Better Kidney Graft Survival. *Transplantation Direct*, *5*(8), 1–8. https://doi.org/10.1097/TXD.0000000000000919

Parajuli, S., Aziz, F., Blazel, J., Muth, B. L., Garg, N., Mohamed, M., Rice, J., Mezrich, J. D., Hidalgo, L. G., & Mandelbrot, D. (2021a). The Utility of Donor-specific Antibody Monitoring and the Role of Kidney Biopsy in Simultaneous Liver and Kidney Recipients With De Novo Donor-specific Antibodies. *Transplantation*, *105*(7), 1548–1555. https://doi.org/10.1097/TP.0000000000003399

Parajuli, S., Aziz, F., Blazel, J., Muth, B. L., Garg, N., Mohamed, M., Rice, J., Mezrich, J. D., Hidalgo, L. G., & Mandelbrot, D. (2021b). The Utility of Donor-specific Antibody Monitoring and the Role of Kidney Biopsy in Simultaneous Liver and Kidney Recipients With De Novo Donor-specific Antibodies. *Transplantation*, *105*(7), 1548–1555. https://doi.org/10.1097/TP.0000000000003399

Parajuli, S., Aziz, F., Blazel, J., Muth, B. L., Garg, N., Mohamed, M., Rice, J., Mezrich, J. D., Hidalgo, L. G., & Mandelbrot, D. (2021c). The Utility of Donor-specific Antibody Monitoring and the Role of Kidney Biopsy in Simultaneous Liver and Kidney Recipients With De Novo Donor-specific Antibodies. *Transplantation*, *105*(7), 1548–1555. https://doi.org/10.1097/TP.0000000000003399

Parajuli, S., Bath, N. M., Hidalgo, L., Leverson, G., Garg, N., Redfield, R., & Mandelbrot, D. A. (2021). Impact of low-level pretransplant donor-specific antibodies on outcomes after kidney transplantation. *Immunity, Inflammation and Disease*, *9*(4), 1508–1519. https://doi.org/10.1002/iid3.504

Patel, R., & Terasaki, P. I. (1969). Significance of the positive crossmatch test in kidney transplantation. *The New England Journal of Medicine*, *280*(14), 735–739. https://doi.org/10.1056/NEJM196904032801401

Peacock, S., Briggs, D., Barnardo, M., Battle, R., Brookes, P., Callaghan, C., Clark, B., Collins, C., Day, S., Diaz Burlinson, N., Dunn, P., Fernando, R., Fuggle, S., Harmer, A., Kallon, D., Keegan, D., Key, T., Lawson, E., Lloyd, S., … Worthington, J. (2022). BSHI/BTS guidance on crossmatching before deceased donor kidney transplantation. *International Journal of Immunogenetics*, *49*(1), 22–29. https://doi.org/10.1111/iji.12558

Petersdorf, E. W., Gooley, T. A., Malkki, M., Bacigalupo, A. P., Cesbron, A., Du Toit, E., Ehninger, G., Egeland, T., Fischer, G. F., Gervais, T., Haagenson, M. D., Horowitz, M. M., Hsu, K., Jindra, P., Madrigal, A., Oudshoorn, M., Ringdén, O., Schroeder, M. L., Spellman, S. R., … International Histocompatibility Working Group in Hematopoietic Cell Transplantation. (2014). HLA-C expression levels define permissible mismatches in hematopoietic cell transplantation. *Blood*, *124*(26), 3996–4003. https://doi.org/10.1182/blood-2014-09-599969

Petersdorf, E. W., Malkki, M., O'hUigin, C., Carrington, M., Gooley, T., Haagenson, M. D., Horowitz, M. M., Spellman, S. R., Wang, T., & Stevenson, P. (2015). High HLA-DP Expression and Graft-versus-Host Disease. *New England Journal of Medicine*, *373*(7), 599–609. https://doi.org/10.1056/NEJMoa1500140

Philogene, M. C., Zhou, S., Lonze, B. E., Bagnasco, S., Alasfar, S., Montgomery, R. A., Kraus, E., Jackson, A. M., Leffell, M. S., & Zachary, A. A. (2018). Pretransplant Screening for Non-HLA Antibodies: Who should be Tested? *Human Immunology*, *79*(4), 195–202. https://doi.org/10.1016/j.humimm.2018.02.001

Piemonti, L., Everly, M. J., Maffi, P., Scavini, M., Poli, F., Nano, R., Cardillo, M., Melzi, R., Mercalli, A., Sordi, V., Lampasona, V., Espadas de Arias, A., Scalamogna, M., Bosi, E., Bonifacio, E., Secchi, A., & Terasaki, P. I. (2013). Alloantibody and autoantibody monitoring predicts islet transplantation outcome in human type 1 diabetes. *Diabetes*, *62*(5), 1656–1664. https://doi.org/10.2337/db12-1258

Piñeiro, G. J., Rovira, J., Montagud-Marrahí, E., Torregrosa, J. V., Ríos, J., Cucchiari, D., Ugalde-Altamirano, J., Ventura-Aguiar, P., Gelpi, R., Palou, E., Colmenero, J., Navasa, M., Diekmann, F., & Esforzado, N. (2020). Kidney Graft Outcomes in High Immunological Risk Simultaneous Liver-Kidney Transplants. *Liver Transplantation: Official Publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society*, *26*(4), 517–527. https://doi.org/10.1002/lt.25726

Pisani, B. A., Mullen, G. M., Malinowska, K., Lawless, C. E., Mendez, J., Silver, M. A., Radvany, R., & Robinson, J. A. (1999). Plasmapheresis with intravenous immunoglobulin G is effective in patients with elevated panel reactive antibody prior to cardiac transplantation. *The Journal of Heart and Lung Transplantation*, *18*(7), 701–706. https://doi.org/10.1016/S1053-2498(99)00022-4

Pouliquen, E., Baltzinger, P., Lemle, A., Chen, C.-C., Parissiadis, A., Borot, S., Frimat, L., Girerd, S., Berney, T., Lablanche, S., Benhamou, P. Y., Morelon, E., Badet, L., Dubois, V., Kessler, L., Thaunat, O., & GRAGIL Network. (2017). Anti-Donor HLA Antibody Response After Pancreatic Islet Grafting: Characteristics, Risk Factors, and Impact on Graft Function. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *17*(2), 462–473. https://doi.org/10.1111/ajt.13936

Rabant, M., Racapé, M., Petit, L.-M., Taupin, J. L., Aubert, O., Bruneau, J., Barbet, P., Goulet, O., Chardot, C., Suberbielle, C., Lacaille, F., Canioni, D., & Duong Van Huyen, J.-P. (2018). Antibody-mediated rejection in pediatric small bowel transplantation: Capillaritis is a major determinant of C4d positivity in intestinal transplant biopsies. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *18*(9), 2250–2260. https://doi.org/10.1111/ajt.14685

Raess, M., Fröhlich, G., Roos, M., Rüsi, B., Wilhelm, M. J., Noll, G., Ruschitzka, F., Fehr, T., & Enseleit, F. (2013). Donor-specific anti-HLA antibodies detected by Luminex: Predictive for short-term but not long-term survival after heart transplantation. *Transplant International*, *26*(11), 1097–1107. https://doi.org/10.1111/tri.12170

Randhawa, S. K., Yang, Z., Morkan, D. B., Yan, Y., Chang, S.-H., Hachem, R. R., Witt, C. A., Byers, D. E., Kulkarni, H. S., Guillamet, R. V., Kozower, B. D., Nava, R. G., Meyers, B. F., Patterson, G. A., Kreisel, D., & Puri, V. (2022). One-Year Survival Worse for Lung Retransplants Relative to Primary Lung Transplants. *The Annals of Thoracic Surgery*, *113*(4), 1265–1273. https://doi.org/10.1016/j.athoracsur.2021.03.112

Reed, E. F., Rao, P., Zhang, Z., Gebel, H., Bray, R. A., Guleria, I., Lunz, J., Mohanakumar, T., Nickerson, P., Tambur, A. R., Zeevi, A., Heeger, P. S., & Gjertson, D. (2013). Comprehensive assessment and standardization of solid phase multiplex-bead arrays for the detection of antibodies to HLA. *American Journal of Transplantation*, *13*(7), 1859–1870. https://doi.org/10.1111/ajt.12287

Rennie, T. J. W., Battle, R. K., Abel, A. A., McConnell, S., McLaren, R., Phelan, P. J., Geddes, C., Padmanabhan, N., Clancy, M. J., Little, A., & Turner, D. M. (2022). Comparison of kidney transplant outcomes in HLA compatible and incompatible transplantation: A national cohort study. *Nephrology*, *27*(12), 962–972. https://doi.org/10.1111/nep.14102

Rios, P., Baidal, D., Lemos, J., Camhi, S. S., Infante, M., Padilla, N., Alvarez Gil, A. M., Fuenmayor, V., Ambut, J., Qasmi, F. A., Mantero, A. M., Cayetano, S. M., Ruiz, P., Ricordi, C., & Alejandro, R. (2021). Long-term Persistence of Allosensitization After Islet Allograft Failure. *Transplantation*, *105*(11), 2490–2498. https://doi.org/10.1097/TP.0000000000003635

Roelen, D. L., Doxiadis, I. I. N., & Claas, F. H. J. (2012). Detection and clinical relevance of donor specific HLA antibodies: A matter of debate. *Transplant International: Official Journal of the European Society for Organ Transplantation*, *25*(6), 604–610. https://doi.org/10.1111/j.1432-2277.2012.01491.x

Rohan, V. S., Pilch, N., Moussa, O., Nadig, S. N., Dubay, D., Baliga, P. K., & Taber, D. J. (2020). Virtual Crossmatching in Kidney Transplantation: The Wait Is Over. *Journal of the American College of Surgeons*, *230*(4), 373–379. https://doi.org/10.1016/j.jamcollsurg.2019.12.031

Rose, M. L. (2013). Role of anti-vimentin antibodies in allograft rejection. *Human Immunology*, *74*(11), 1459–1462. https://doi.org/10.1016/j.humimm.2013.06.006

Ruiz, P., Bagni, A., Brown, R., Cortina, G., Harpaz, N., Magid, M. S., & Reyes, J. (2004). Histological criteria for the identification of acute cellular rejection in human small bowel allografts: Results of the pathology

workshop at the VIII International Small Bowel Transplant Symposium. *Transplantation Proceedings*, *36*(2), 335–337. https://doi.org/10.1016/j.transproceed.2004.01.079

Russo, A., Oliveira, G., Berglund, S., Greco, R., Gambacorta, V., Cieri, N., Toffalori, C., Zito, L., Lorentino, F., Piemontese, S., Morelli, M., Giglio, F., Assanelli, A., Stanghellini, M. T. L., Bonini, C., Peccatori, J., Ciceri, F., Luznik, L., & Vago, L. (2018). NK cell recovery after haploidentical HSCT with posttransplant cyclophosphamide: Dynamics and clinical implications. *Blood*, *131*(2), 247–262. https://doi.org/10.1182/blood-2017-05-780668

Russo, M. J., Chen, J. M., Sorabella, R. A., Martens, T. P., Garrido, M., Davies, R. R., George, I., Cheema, F. H., Mosca, R. S., Mital, S., Ascheim, D. D., Argenziano, M., Stewart, A. S., Oz, M. C., & Naka, Y. (2007). The effect of ischemic time on survival after heart transplantation varies by donor age: An analysis of the United Network for Organ Sharing database. *The Journal of Thoracic and Cardiovascular Surgery*, *133*(2), 554–559. https://doi.org/10.1016/j.jtcvs.2006.09.019

Rutter, C. S., Amin, I., Russell, N. K., Sharkey, L. M., Butler, A. J., & Middleton, S. J. (2016). Adult Intestinal and Multivisceral Transplantation: Experience From a Single Center in the United Kingdom. *Transplantation Proceedings*, *48*(2), 468–472. https://doi.org/10.1016/j.transproceed.2015.10.079

Salterain-González, N., Rábago Juan-Aracil, G., Gómez-Bueno, M., Almenar-Bonet, L., Crespo-Leiro, M. G., Arizón Del Prado, J. M., García-Cosío, M. D., Martínez-Sellés, M., Mirabet-Pérez, S., Sobrino-Márquez, J. M., González-Costello, J., Pérez-Villa, F., Díaz-Molina, B., De La Fuente-Galán, L., Blasco-Peiró, T., Garrido-Bravo, I. P., García-Guereta Silva, L., Gil-Villanueva, N., Gran, F., & González-Vilchez, F. (2022). Results of heart retransplantation: Subanalysis of the Spanish Heart Transplant Registry. *Revista Española de Cardiología (English Edition)*, *75*(1), 60–66. https://doi.org/10.1016/j.rec.2021.06.009

Salvadé, I., Aubert, V., Venetz, J.-P., Golshayan, D., Saouli, A.-C., Matter, M., Rotman, S., Pantaleo, G., & Pascual, M. (2016). Clinically-relevant threshold of preformed donor-specific anti-HLA antibodies in kidney transplantation. *Human Immunology*, *77*(6), 483–489. https://doi.org/10.1016/j.humimm.2016.04.010

Sánchez-Zapardiel, E., Castro-Panete, M. J., Castillo-Rama, M., Morales, P., Lora-Pablos, D., Valero-Hervás, D., Ruiz-García, R., Apaza, J., Talayero, P., Andrés, A., Morales, J. M., & Paz-Artal, E. (2013). Harmful effect of preformed anti-MICA antibodies on renal allograft evolution in early posttransplantation period. *Transplantation*, *96*(1), 70–78. https://doi.org/10.1097/TP.0b013e3182943506

Schinstock, C. A., Smith, B. H., Montgomery, R. A., Jordan, S. C., Bentall, A. J., Mai, M., Khamash, H. A., & Stegall, M. D. (2019). Managing highly sensitized renal transplant candidates in the era of kidney paired donation and the new kidney allocation system: Is there still a role for desensitization? *Clinical Transplantation*, *33*(12), e13751. https://doi.org/10.1111/ctr.13751

Schnaidt, M., Weinstock, C., Jurisic, M., Schmid-Horch, B., Ender, A., & Wernet, D. (2011). HLA antibody specification using single-antigen beads—A technical solution for the prozone effect. *Transplantation*, *92*(5), 510–515. https://doi.org/10.1097/TP.0b013e31822872dd

Schwaiger, E., Wahrmann, M., Bond, G., Eskandary, F., & Böhmig, G. A. (2014). Complement component C3 activation: The leading cause of the prozone phenomenon affecting HLA antibody detection on single-antigen beads. *Transplantation*, *97*(12), 1279–1285. https://doi.org/10.1097/01.TP.0000441091.47464.c6

See, S. B., Mantell, B. S., Clerkin, K. J., Ray, B., Vasilescu, E. R., Marboe, C. C., Naka, Y., Restaino, S., Colombo, P. C., Addonizio, L. J., Farr, M. A., & Zorn, E. (2020). Profiling non-HLA antibody responses in antibody-mediated rejection following heart transplantation. *American Journal of Transplantation*, *20*(9), 2571–2580. https://doi.org/10.1111/ajt.15871

Seitz, A., Mounsey, K., Hughes, P., Cullen, K., Welberry Smith, M., Daga, S., Carter, C., Clark, B., & Baker, R. (2022). Isolated Pre-existing HLA-DP Donor-Specific Antibodies are Associated With Poorer Outcomes in Renal Transplantation. *Kidney International Reports*, *7*(10), 2251–2263. https://doi.org/10.1016/j.ekir.2022.07.014

Selvaggi, G., Gaynor, J. J., Moon, J., Kato, T., Thompson, J., Nishida, S., Levi, D., Ruiz, P., Cantwell, P., & Tzakis, A. G. (2007). Analysis of acute cellular rejection episodes in recipients of primary intestinal transplantation: A single center, 11-year experience. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *7*(5), 1249–1257. https://doi.org/10.1111/j.1600-6143.2007.01755.x

Senev, A., Coemans, M., Lerut, E., Van Sandt, V., Kerkhofs, J., Daniëls, L., Driessche, M. V., Compernolle, V., Sprangers, B., Van Loon, E., Callemeyn, J., Claas, F., Tambur, A. R., Verbeke, G., Kuypers, D., Emonds, M.-P., & Naesens, M. (2020). Eplet Mismatch Load and De Novo Occurrence of Donor-Specific Anti-HLA Antibodies, Rejection, and Graft Failure after Kidney Transplantation: An Observational Cohort Study. *Journal of the American Society of Nephrology: JASN*, *31*(9), 2193–2204. https://doi.org/10.1681/ASN.2020010019

Senev, A., Lerut, E., Van Sandt, V., Coemans, M., Callemeyn, J., Sprangers, B., Kuypers, D., Emonds, M.-P., & Naesens, M. (2019). Specificity, strength, and evolution of pretransplant donor-specific HLA antibodies determine outcome after kidney transplantation. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *19*(11), 3100–3113. https://doi.org/10.1111/ajt.15414

Setia, G., Nishihara, K., Singer-Englar, T., Zhang, X., Patel, J., & Kobashigawa, J. (2021). Crossing low/moderate-level donor-specific antibodies during heart transplantation. *Clinical Transplantation*, *35*(3), e14196. https://doi.org/10.1111/ctr.14196

Shah, S., Suddle, A., Callaghan, C., Karydis, N., Shaw, O., Horsfield, C., Koffman, G., & Heaton, N. (2020). Kidney Rejection Following Simultaneous Liver-kidney Transplantation. *Transplantation Direct*, *6*(7), e569. https://doi.org/10.1097/TXD.0000000000001004

Shamsaeefar, A., Saleh, T., Kazemi, K., Nikeghbalian, S., Dehghani, M., Mansurian, M., Gholam, S., Khosravi, B., & Malek Hosseini, S. A. (2021). Retransplant of the Liver: 12-Year Experience of the Shiraz Organs Transplantation Center. *Experimental and Clinical Transplantation: Official Journal of the Middle East Society for Organ Transplantation*, *19*(1), 44–49. https://doi.org/10.6002/ect.2017.0246

Shankar, N., Daly, R., Geske, J., Kushwaha, S. K., Timmons, M., Joyce, L., Stulak, J., Gandhi, M., Kremers, W., Park, S., & Pereira, N. L. (2013). LVAD Implant as a Bridge to Heart Transplantation Is Associated with Allosensitization as Measured by Single Antigen Bead Assay. *Transplantation*, *96*(3), 324–330. https://doi.org/10.1097/TP.0b013e3182985371

Simmons, D. P., Kafetzi, M. L., Wood, I., Macaskill, P. C., Milford, E. L., & Guleria, I. (2016). Antibodies against HLA-DP recognize broadly expressed epitopes. *Human Immunology*, *77*(12), 1128–1139. https://doi.org/10.1016/j.humimm.2016.09.008

Smith, J. D., Hamour, I. M., Banner, N. R., & Rose, M. L. (2007). C4d Fixing, Luminex Binding Antibodies—A New Tool for Prediction of Graft Failure After Heart Transplantation. *American Journal of Transplantation*, *7*(12), 2809–2815. https://doi.org/10.1111/j.1600-6143.2007.01991.x

Smith, J. D., Ibrahim, M. W., Newell, H., Danskine, A. J., Soresi, S., Burke, M. M., Rose, M. L., & Carby, M. (2014). Pre-transplant donor HLA-specific antibodies: Characteristics causing detrimental effects on survival after lung transplantation. *The Journal of Heart and Lung Transplantation: The Official Publication of the International Society for Heart Transplantation*, *33*(10), 1074–1082. https://doi.org/10.1016/j.healun.2014.02.033

South, A. M., & Grimm, P. C. (2016). Transplant immuno-diagnostics: Cross-match and antigen detection. *Pediatric Nephrology (Berlin, Germany)*, *31*(6), 897–905. https://doi.org/10.1007/s00467-015-3145-z

Statistics and Clinical Research, N. B. and T. (2022). *Organ and Tissue Donation and Transplantation Activity Report 2021/22*. https://www.organdonation.nhs.uk/helping-you-to-decide/about-organ-donation/statistics-about-organ-donation/transplant-activity-report/

Su, J. A., Baxter-Lowe, L. A., Kantor, P. F., Szmuszkovicz, J. R., & Menteer, J. (2019). The clinical impact of donor-specific antibodies on antibody-mediated rejection and long-term prognosis after heart transplantation. *Current Opinion in Organ Transplantation*, *24*(3), 245–251. https://doi.org/10.1097/MOT.0000000000000636

Sullivan, H. C., Gebel, H. M., & Bray, R. A. (2017). Understanding solid-phase HLA antibody assays and the value of MFI. *Human Immunology*, *78*(7–8), 471–480. https://doi.org/10.1016/j.humimm.2017.05.007

Sultani, B., Marget, M., Briem-Richter, A., Herrmann, J., Meisner, S., Grabhorn, E. F., Ozga, A.-K., Weidemann, S., Herden, U., Fischer, L., & Sterneck, M. (2021). Presence of donor specific HLA class 2 antibodies (DSA class 2) is associated with development of graft fibrosis more than 10 years after liver transplantation-a retrospective single center study. *Clinical Transplantation*, *35*(7), e14336. https://doi.org/10.1111/ctr.14336

Summers, D. M., Johnson, R. J., Allen, J., Fuggle, S. V., Collett, D., Watson, C. J., & Bradley, J. A. (2010). Analysis of factors that affect outcome after transplantation of kidneys donated after cardiac death in the UK: A cohort study. *Lancet (London, England)*, *376*(9749), 1303–1311. https://doi.org/10.1016/S0140-6736(10)60827-6

Tait, B. D., Süsal, C., Gebel, H. M., Nickerson, P. W., Zachary, A. A., Claas, F. H. J., Reed, E. F., Bray, R. A., Campbell, P., Chapman, J. R., Coates, P. T., Colvin, R. B., Cozzi, E., Doxiadis, I. I. N., Fuggle, S. V., Gill, J., Glotz, D., Lachmann, N., Mohanakumar, T., … Opelz, G. (2013). Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. *Transplantation*, *95*(1), 19–47. https://doi.org/10.1097/TP.0b013e31827a19cc

Takaya, S., Bronsther, O., Iwaki, Y., Nakamura, K., Abu-Elmagd, K., Yagihashi, A., Demetris, A. J., Kobayashi, M., Todo, S., & Tzakis, A. G. (1992). The adverse impact on liver transplantation of using positive cytotoxic crossmatch donors. *Transplantation*, *53*(2), 400–406. https://doi.org/10.1097/00007890-199202010-00026

Tambur, A. R., Campbell, P., Chong, A. S., Feng, S., Ford, M. L., Gebel, H., Gill, R. G., Kelsoe, G., Kosmoliaptsis, V., Mannon, R. B., Mengel, M., Reed, E. F., Valenzuela, N. M., Wiebe, C., Dijke, I. E., Sullivan, H. C., & Nickerson, P. (2020). Sensitization in transplantation: Assessment of risk (STAR) 2019 Working Group Meeting Report. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *20*(10), 2652–2668. https://doi.org/10.1111/ajt.15937

Tambur, A. R., Campbell, P., Claas, F. H., Feng, S., Gebel, H. M., Jackson, A. M., Mannon, R. B., Reed, E. F., Tinckam, K., Askar, M., Chandraker, A., Chang, P. P., Colvin, M., Demetris, A.-J., Diamond, J. M., Dipchand, A. I., Fairchild, R. L., Ford, M. L., Friedewald, J., … Nickerson, P. (2018). Sensitization in Transplantation: Assessment of Risk (STAR) 2017 Working Group Meeting Report. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *18*(7), 1604–1614. https://doi.org/10.1111/ajt.14752

Tambur, A. R., Herrera, N. D., Haarberg, K. M. K., Cusick, M. F., Gordon, R. A., Leventhal, J. R., Friedewald, J. J., & Glotz, D. (2015). Assessing Antibody Strength: Comparison of MFI, C1q, and Titer Information. *American Journal of Transplantation*, *15*(9), 2421–2430. https://doi.org/10.1111/ajt.13295

Tambur, A. R., Kosmoliaptsis, V., Claas, F. H. J., Mannon, R. B., Nickerson, P., & Naesens, M. (2021). Significance of HLA-DQ in kidney transplantation: Time to reevaluate human leukocyte antigen-matching priorities to improve transplant outcomes? An expert review and recommendations. *Kidney International*, *100*(5), 1012–1022. https://doi.org/10.1016/j.kint.2021.06.026

Tambur, A. R., & Lavee, J. (2016). Incorporating human leukocyte antibody results into clinical practice. *The Journal of Heart and Lung Transplantation*, *35*(7), 851–856. https://doi.org/10.1016/j.healun.2016.05.010

Tambur, A. R., & Schinstock, C. (2022). Clinical utility of serial serum dilutions for HLA antibody interpretation. *HLA*, *100*(5), 457–468. https://doi.org/10.1111/tan.14781

Tambur, A. R., & Wiebe, C. (2018). HLA Diagnostics: Evaluating DSA Strength by Titration. *Transplantation*, *102*(Supplement 1), S23–S30. https://doi.org/10.1097/TP.0000000000001817

Tamura, K., Tohyama, T., Watanabe, J., Nakamura, T., Ueno, Y., Inoue, H., Honjo, M., Sakamoto, K., Takai, A., Ogawa, K., & Takada, Y. (2019). Preformed donor-specific antibodies are associated with 90-day mortality in living-donor liver transplantation. *Hepatology Research: The Official Journal of the Japan Society of Hepatology*, *49*(8), 929–941. https://doi.org/10.1111/hepr.13352

Taner, T. (2017). Liver transplantation: Rejection and tolerance. *Liver Transplantation: Official Publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society*, *23*(S1), S85–S88. https://doi.org/10.1002/lt.24840

Taner, T., Gandhi, M. J., Sanderson, S. O., Poterucha, C. R., De Goey, S. R., Stegall, M. D., & Heimbach, J. K. (2012). Prevalence, course and impact of HLA donor-specific antibodies in liver transplantation in the first year. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *12*(6), 1504–1510. https://doi.org/10.1111/j.1600-6143.2012.03995.x

Taner, T., Gustafson, M. P., Hansen, M. J., Park, W. D., Bornschlegl, S., Dietz, A. B., & Stegall, M. D. (2018). Donor-specific hypo-responsiveness occurs in simultaneous liver-kidney transplant recipients after the first year. *Kidney International*, *93*(6), 1465–1474. https://doi.org/10.1016/j.kint.2018.01.022

Taylor, C. J., Kosmoliaptsis, V., Sharples, L. D., Prezzi, D., Morgan, C. H., Key, T., Chaudhry, A. N., Amin, I., Clatworthy, M. R., Butler, A. J., Watson, C. J. E., & Bradley, J. A. (2010). Ten-year experience of selective omission of the pretransplant crossmatch test in deceased donor kidney transplantation. *Transplantation*, *89*(2), 185–193. https://doi.org/10.1097/TP.0b013e3181c926f2

Taylor, C. J., Smith, S. I., Morgan, C. H., Stephenson, S., Key, T., Jones, P., Watson, C., Jacques, B., Welsh, K. I., & Bradley, J. A. (2000). Selective omission of the donor cross-match before renal transplantation: Efficacy, safety and effects on cold storage time. *Transplantation*, *69*(5), 719–723. https://doi.org/10.1097/00007890-200003150-00008

Thammanichanond, D., Ingsathit, A., Mongkolsuk, T., Rattanasiri, S., Kantachuvesiri, S., Sakhonrat, C., Leenanupan, C., Worawichawongs, S., & Kitpoka, P. (2012). Pre-transplant donor specific antibody and its clinical significance in kidney transplantation. *Asian Pacific Journal of Allergy and Immunology*, *30*(1), 48–54.

Tinckam, K. J., Keshavjee, S., Chaparro, C., Barth, D., Azad, S., Binnie, M., Chow, C. W., De Perrot, M., Pierre, A. F., Waddell, T. K., Yasufuku, K., Cypel, M., & Singer, L. G. (2015). Survival in Sensitized Lung Transplant Recipients With Perioperative Desensitization. *American Journal of Transplantation*, *15*(2), 417–426. https://doi.org/10.1111/ajt.13076

Tsai, H.-L., Island, E. R., Chang, J.-W., Gonzalez-Pinto, I., Tryphonopoulos, P., Nishida, S., Selvaggi, G., Tekin, A., Moon, J., Levi, D., Woodle, E. S., Ruiz, P., Weppler, D., Lee, O. K. S., & Tzakis, A. G. (2011). Association between donor-specific antibodies and acute rejection and resolution in small bowel and multivisceral transplantation. *Transplantation*, *92*(6), 709–715. https://doi.org/10.1097/TP.0b013e318229f752

Turner, D., Battle, R., Akbarzad-Yousefi, A., & Little, A.-M. (2019). The omission of the 'wet' pre-transplant crossmatch in renal transplant centres in Scotland. *HLA*, *94*(1), 3–10. https://doi.org/10.1111/tan.13558

Tyan, D. B., Li, V. A., Czer, L., Trento, A., & Jordan, S. C. (1994). Intravenous immunoglobulin suppression of HLA alloantibody in highly sensitized transplant candidates and transplantation with a histoincompatible organ. *Transplantation*, *57*(4), 553–562.

Uva, P. D., Quevedo, A., Roses, J., Toniolo, M. F., Pilotti, R., Chuluyan, E., & Casadei, D. H. (2020). Anti-Hla donor-specific antibody monitoring in pancreas transplantation: Role of protocol biopsies. *Clinical Transplantation*, *34*(8), 0–3. https://doi.org/10.1111/ctr.13998

van den Hout, W. B., Smits, J. M. A., Deng, M. C., Hummel, M., Schoendube, F., Scheld, H. H., Persijn, G. G., Laufer, G., Comparative Outcome and Clinical Profiles in Transplantation study group, & Eurotransplant heart

transplant programs. (2003). The heart-allocation simulation model: A tool for comparison of transplantation allocation policies. *Transplantation*, *76*(10), 1492–1497. https://doi.org/10.1097/01.TP.0000092005.95047.E9

van Erp, E. A., Luytjes, W., Ferwerda, G., & van Kasteren, P. B. (2019). Fc-Mediated Antibody Effector Functions During Respiratory Syncytial Virus Infection and Disease. *Frontiers in Immunology*, *10*, 548. https://doi.org/10.3389/fimmu.2019.00548

Vandendriessche, K., Tchana-Sato, V., Ledoux, D., Degezelle, K., Rex, S., Neyrinck, A., Jochmans, I., Monbaliu, D., Vandenbriele, C., Cleemput, J. V., Meyns, B., & Rega, F. (2021). Transplantation of donor hearts after circulatory death using normothermic regional perfusion and cold storage preservation. *European Journal of Cardio-Thoracic Surgery: Official Journal of the European Association for Cardio-Thoracic Surgery*, *60*(4), 813–819. https://doi.org/10.1093/ejcts/ezab139

Vandevoorde, K., Ducreux, S., Bosch, A., Guillaud, O., Hervieu, V., Chambon-Augoyard, C., Poinsot, D., André, P., Scoazec, J.-Y., Robinson, P., Boillot, O., Dubois, V., & Dumortier, J. (2018). Prevalence, Risk Factors, and Impact of Donor-Specific Alloantibodies After Adult Liver Transplantation. *Liver Transplantation: Official Publication of the American Association for the Study of Liver Diseases and the International Liver Transplantation Society*, *24*(8), 1091–1100. https://doi.org/10.1002/lt.25177

Varni, J. W., Seid, M., & Rode, C. A. (1999). The PedsQL™: Measurement Model for the Pediatric Quality of Life Inventory. *Medical Care*, *37*(2), 126–139. https://doi.org/10.1097/00005650-199902000-00003

Vendrame, F., Hopfner, Y. Y., Diamantopoulos, S., Virdi, S. K., Allende, G., Snowhite, I. V., Reijonen, H. K., Chen, L., Ruiz, P., Ciancio, G., Hutton, J. C., Messinger, S., Burke, G. W., & Pugliese, A. (2016a). Risk factors for type 1 diabetes recurrence in immunosuppressed recipients of simultaneous pancreas-kidney transplants. *American Journal of Transplantation*, *16*(1), 235–245. https://doi.org/10.1111/ajt.13426

Vendrame, F., Hopfner, Y.-Y., Diamantopoulos, S., Virdi, S. K., Allende, G., Snowhite, I. V., Reijonen, H. K., Chen, L., Ruiz, P., Ciancio, G., Hutton, J. C., Messinger, S., Burke, G. W., & Pugliese, A. (2016b). Risk Factors for Type 1 Diabetes Recurrence in Immunosuppressed Recipients of Simultaneous Pancreas-Kidney Transplants. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *16*(1), 235–245. https://doi.org/10.1111/ajt.13426

Villa, C., Mesa, K., Cristy Smith, M., Mooney, D. M., Coletti, A., & Klohe, E. (2020). Hyperacute graft dysfunction in an orthotopic heart transplant in the presence of non-HLA antibodies. *American Journal of Transplantation*, *20*(2), 593–599. https://doi.org/10.1111/ajt.15564

Visentin, J., Guidicelli, G., Bachelet, T., Jacquelinet, C., Audry, B., Nong, T., Dubois, V., Moreau, J.-F., Lee, J.-H., Couzi, L., Merville, P., & Taupin, J.-L. (2014). Denatured class I human leukocyte antigen antibodies in sensitized kidney recipients: Prevalence, relevance, and impact on organ allocation. *Transplantation*, *98*(7), 738–744. https://doi.org/10.1097/TP.0000000000000229

Visentin, J., Marroc, M., Guidicelli, G., Bachelet, T., Nong, T., Moreau, J.-F., Lee, J.-H., Merville, P., Couzi, L., & Taupin, J.-L. (2015). Clinical impact of preformed donor-specific denatured class I HLA antibodies after kidney transplantation. *Clinical Transplantation*, *29*(5), 393–402. https://doi.org/10.1111/ctr.12529

Vistoli, F., Kauffmann, E. F., & Boggi, U. (2021). Pancreas transplantation. *Current Opinion in Organ Transplantation*, *26*(4), 381–389. https://doi.org/10.1097/MOT.0000000000000900

Wallinder, A., Danielsson, C., Magnusson, J., Riise, G. C., & Dellgren, G. (2019). Outcomes and Long-term Survival After Pulmonary Retransplantation: A Single-Center Experience. *The Annals of Thoracic Surgery*, *108*(4), 1037–1044. https://doi.org/10.1016/j.athoracsur.2019.04.028

Wehmeier, C., Amico, P., Sidler, D., Wirthmüller, U., Hadaya, K., Ferrari-Lacraz, S., Golshayan, D., Aubert, V., Schnyder, A., Sunic, K., Schachtner, T., Nilsson, J., Schaub, S., & Swiss Transplant Cohort Study. (2021). Pretransplant donor-specific HLA antibodies and risk for poor first-year renal transplant outcomes: Results from the Swiss Transplant Cohort Study. *Transplant International: Official Journal of the European Society for Organ Transplantation*, *34*(12), 2755–2768. https://doi.org/10.1111/tri.14119

Wehmeier, C., Hönger, G., & Schaub, S. (2020). Caveats of HLA antibody detection by solid-phase assays. *Transplant International: Official Journal of the European Society for Organ Transplantation*, *33*(1), 18–29. https://doi.org/10.1111/tri.13484

Wehmeier, C., Karahan, G. E., Krop, J., de Vaal, Y., Langerak-Langerak, J., Binet, I., Schaub, S., Roelen, D. L., Claas, F. H. J., Heidt, S., & Swiss Transplant Cohort Study. (2020). Donor-specific B Cell Memory in Alloimmunized Kidney Transplant Recipients: First Clinical Application of a Novel Method. *Transplantation*, *104*(5), 1026–1032. https://doi.org/10.1097/TP.0000000000002909

Weinstock, C., & Schnaidt, M. (2013). The complement-mediated prozone effect in the Luminex single-antigen bead assay and its impact on HLA antibody determination in patient sera. *International Journal of Immunogenetics*, *40*(3), 171–177. https://doi.org/10.1111/j.1744-313X.2012.01147.x

Wiebe, C., Gibson, I. W., Blydt-Hansen, T. D., Karpinski, M., Ho, J., Storsley, L. J., Goldberg, A., Birk, P. E., Rush, D. N., & Nickerson, P. W. (2012). Evolution and Clinical Pathologic Correlations of De Novo Donor-Specific HLA Antibody Post Kidney Transplant. *American Journal of Transplantation*, *12*(5), 1157–1167. https://doi.org/10.1111/j.1600-6143.2012.04013.x

Williams, R. C., West, L. J., & Opelz, G. (2018). The Risk of Failure With HLA Mismatch and Recipient Age in First Pediatric (<18 years) Kidney Transplants. *Transplantation Direct*, *4*(7), e365. https://doi.org/10.1097/TXD.0000000000000801

Willicombe, M., Brookes, P., Sergeant, R., Santos-Nunez, E., Steggar, C., Galliford, J., McLean, A., Cook, T. H., Cairns, T., Roufosse, C., & Taube, D. (2012). De novo DQ donor-specific antibodies are associated with a significant risk of antibody-mediated rejection and transplant glomerulopathy. *Transplantation*, *94*(2), 172–177. https://doi.org/10.1097/TP.0b013e3182543950

Wozniak, L. J., Naini, B. V., Hickey, M. J., Bhattacharyya, S., Reed, E. F., Busuttil, R. W., Farmer, D. G., Vargas, J. H., Venick, R. S., & McDiarmid, S. V. (2017). Acute antibody-mediated rejection in ABO-compatible pediatric liver transplant recipients: Case series and review of the literature. *Pediatric Transplantation*, *21*(1), e12791. https://doi.org/10.1111/petr.12791

Wu, G., & Cruz, R. J. (2018). Liver-inclusive intestinal transplantation results in decreased alloimmune-mediated rejection but increased infection. *Gastroenterology Report*, *6*(1), 29–37. https://doi.org/10.1093/gastro/gox043

Wu, G.-S. (2016). Updates on antibody-mediated rejection in intestinal transplantation. *World Journal of Transplantation*, *6*(3), 564–572. https://doi.org/10.5500/wjt.v6.i3.564

Wu, T., Abu-Elmagd, K., Bond, G., & Demetris, A. J. (2004). A clinicopathologic study of isolated intestinal allografts with preformed IgG lymphocytotoxic antibodies. *Human Pathology*, *35*(11), 1332–1339. https://doi.org/10.1016/j.humpath.2004.07.001

Yang, B., Dieudé, M., Hamelin, K., Hénault-Rondeau, M., Patey, N., Turgeon, J., Lan, S., Pomerleau, L., Quesnel, M., Peng, J., Tremblay, J., Shi, Y., Chan, J. S., Hébert, M. J., & Cardinal, H. (2016). Anti-LG3 Antibodies Aggravate Renal Ischemia-Reperfusion Injury and Long-Term Renal Allograft Dysfunction. *American Journal of Transplantation: Official Journal of the American Society of Transplantation and the American Society of Transplant Surgeons*, *16*(12), 3416–3429. https://doi.org/10.1111/ajt.13866

Zahr Eldeen, F., Mabrouk Mourad, M., Liossis, C., & Bramhall, S. R. (2014). Liver retransplant for primary disease recurrence. *Experimental and Clinical Transplantation: Official Journal of the Middle East Society for Organ Transplantation*, *12*(3), 175–183.

Zakaria, H., Saleh, Y., Zidan, A., Sturdevant, M., Alabbad, S., Elsheikh, Y., Al-Hamoudi, W., Albenmousa, A., Troisi, R. I., & Broering, D. (2020). Is It Justified to Use Liver Grafts From Living Donors for Retransplant? A

Single-Center Experience. *Experimental and Clinical Transplantation: Official Journal of the Middle East Society for Organ Transplantation*, *18*(2), 188–195. https://doi.org/10.6002/ect.2019.0262

Zangwill, S. D., Stamm, K. D., Hidestrand, M., Tomita-Mitchell, A., & Mitchell, M. E. (2019). Effect of endomyocardial biopsy on levels of donor-specific cell-free DNA. *The Journal of Heart and Lung Transplantation*, *38*(10), 1118–1120. https://doi.org/10.1016/j.healun.2019.06.005

Zhang, R. (2018). Donor-Specific Antibodies in Kidney Transplant Recipients. *Clinical Journal of the American Society of Nephrology: CJASN*, *13*(1), 182–192. https://doi.org/10.2215/CJN.00700117

Zhang, X., Levine, R., Patel, J. K., Kittleson, M., Czer, L., & Kobashigawa, J. A. (2020). Association of vimentin antibody and other non-HLA antibodies with treated antibody mediated rejection in heart transplant recipients. *Human Immunology*, *81*(12), 671–674. https://doi.org/10.1016/j.humimm.2020.09.003

Zhang, X., & Reinsmoen, N. L. (2017). Impact of Non-Human Leukocyte Antigen-Specific Antibodies in Kidney and Heart Transplantation. *Frontiers in Immunology*, *8*, 434. https://doi.org/10.3389/fimmu.2017.00434

Zhu, Y., Shudo, Y., Lingala, B., Baiocchi, M., Oyer, P. E., & Woo, Y. J. (2022). Outcomes after heart retransplantation: A 50-year single-center experience. *The Journal of Thoracic and Cardiovascular Surgery*, *163*(2), 712–720.e6. https://doi.org/10.1016/j.jtcvs.2020.06.121

Ziemann, M., Altermann, W., Angert, K., Arns, W., Bachmann, A., Bakchoul, T., Banas, B., von Borstel, A., Budde, K., Ditt, V., Einecke, G., Eisenberger, U., Feldkamp, T., Görg, S., Guthoff, M., Habicht, A., Hallensleben, M., Heinemann, F. M., Hessler, N., … Lachmann, N. (2019). Preformed Donor-Specific HLA Antibodies in Living and Deceased Donor Transplantation: A Multicenter Study. *Clinical Journal of the American Society of Nephrology*, *14*(7), 1056–1066. https://doi.org/10.2215/CJN.13401118

Ziemann, M., Suwelack, B., Banas, B., Budde, K., Einecke, G., Hauser, I., Heinemann, F. M., Kauke, T., Kelsch, R., Koch, M., Lachmann, N., Reuter, S., Seidl, C., Sester, U., & Zecher, D. (2022). Determination of unacceptable HLA antigen mismatches in kidney transplant recipients. *HLA*, *100*(1), 3–17. https://doi.org/10.1111/tan.14521

How to cite this article: Battle, R., Pritchard, D., Peacock, S., Hastie, C., Worthington, J., Jordan, S., McCaughlan, J. A., Barnardo, M., Cope, R., Collins, C., Diaz-Burlinson, N., Rosser, C., Foster, L., Kallon, D., Shaw, O., Briggs, D., Turner, D., Anand, A., Akbarzad-Yousefi, A., & Sage, D. (2023). BSHI and BTS UK guideline on the detection of alloantibodies in solid organ (and islet) transplantation. *International Journal of Immunogenetics*, 1–61. https://doi.org/10.1111/iji.12641