Draft Manuscript for Review at the Expert Workshop: Management of Transplant Patients with HLA antibodies

28 August 2021

Guidelines for Transplanting Patients with HLA Antibodies

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Introduction

Kidney transplantation offers patients with kidney failure an opportunity for a longer life and a chance of freedom from renal dialysis. Increasingly, however, highly sensitized patients are remaining on the transplant waiting list for a suitable organ. The screening and practice of transplantation of highly sensitized patients has evolved in tandem with increases in sensitivity of HLA antigen testing, helping to improve screening and matching of patients with donor organs. The articles in this series explore the current state of knowledge around this issue and how innovation, immune-system manipulation, patient prioritization schemes and 'thinking outside the box' is increasing the likelihood that highly sensitized patients might safely obtain a transplant.

This working group, composed of leading transplant healthcare professionals from around Europe, patient group representatives, and transplant coordinators, has undertaken a review of the literature in each of six key areas:

1. Definition of sensitization
2. Comparison of practices across Europe
3. How can we risk-stratify patients?
4. Desensitization strategies
5. Outcomes after HLA incompatible transplantation
6. The place of kidney sharing schemes for sensitized patients

A standard systematic search strategy was predefined, using the PICO model to formulate clinical questions. Bibliographic searches were developed for each of the clinical questions by experienced staff from the Centre for Evidence in Transplantation, University of Oxford, UK. Systematic searches were conducted in the Transplant Library (www.transplantlibrary.com), Medline and Embase and consisted of a mixture of free text and controlled vocabulary terms. Full details of the search strategies including search dates can be found in the Appendix.

A clinical member of the work group (or a team of clinical members) then assessed the search results and wrote each chapter. The full development and review process is outlined in **Figure 1.**

A series of recommendations were developed:

Abbreviations:
cPRA, the calculated percentage of actual organ donors who express one or more unacceptable antigens
cRF, calculated reaction frequency
DSA, donor-specific antibodies
ENGAGE, EuropeaN Guidelines for the mAnagement of Graft rEcipients (ENGAGE) working group
HLA, human leukocyte antigen
SAB, single antigen bead

Standardization

- A parameter, which is based on the HLA frequencies of the actual organ donor population, such as cPRA or cRF, should be used to estimate the chance that a sensitized patient can be transplanted with a compatible donor without the need for any special treatment (Chapter 1)
- Further standardisation of solid phase assays is recommended (Chapter 1)
- When defining unacceptable mismatches in highly sensitized patients on the basis of (weak) antibody reactivities in SAB assays only, one should consider the not well-defined risk of antibody-mediated rejection in the light of a prolonged waiting time and associated mortality and morbidity (Chapter 1)
- To define the humoral risk in kidney transplantation, the use of the ENGAGE 5 strata system[1] is recommended (Chapter 3)

Organ allocation

- We recommend all countries have an active policy of prioritising highly sensitised patients for organ transplantation (Chapter 4)
- Increase access to the donor pool, through greater use of:
  - Sliding scale priority score schemes based on cPRA values (Chapters 1 and 3)
  - Prioritisation policies should be linked across countries for equity of access (Chapter 3)
  - Increased access to and harmonisation of Kidney Exchange Programmes, with greater and standardised sharing of outcomes (Chapters 2 and 6)
  - If a particular country does allow unspecified kidney donations, consider including these in kidney sharing schemes (Chapters 2 and 6)
  - Kidney paired donations should have an option to include compatible pairs and deceased donor organs (Chapter 6)
- Expand the Eurotransplant Acceptable Mismatch programme to other European countries to improve donor/recipient matching (Chapter 3)
- Kidney Paired Donation is the preferred initial option over desensitization given the better transplant outcomes and cost-effectiveness, in both ABO and HLA incompatible pairs, unless there is a need for desensitization, there is clinical urgency or a low chance of a transplant (Chapter 6)
- All kidney sharing schemes should develop calculators to help assess the probability of an organ match (Chapter 3)
- Therapeutic options should be reconsidered if there are no organ offers for a patient in a kidney sharing scheme (Chapter 3)

Desensitization

- The most efficacious desensitization strategy is to start with rounds of plasma exchanges/immunoadsorption together with B-cell immunomodulation with IVIG or B-cell depletion with anti-CD20 monoclonal antibodies to minimize post-transplantation DSA rebound

- As yet to be defined protocols including proteosome inhibitors and other anti-plasmocyte antibodies with co-stimulation blockade, B-cell immunomodulation

targeting IL-6 as well as cleavage of IgG donor-specific antibodies with imlifidase are highly promising new strategies that deserve further investigation

Areas for further research

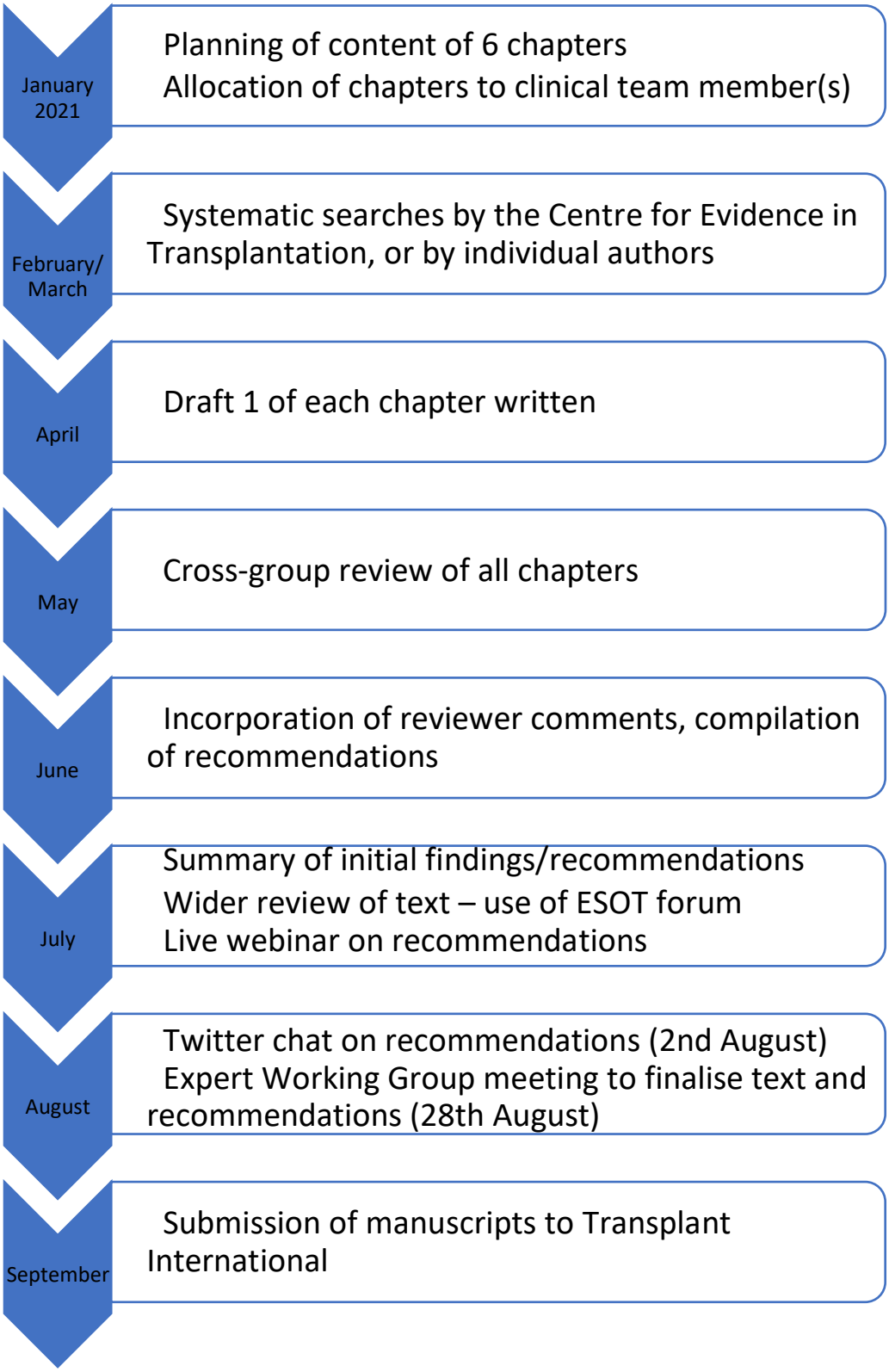
We recommend that data be collected prospectively for sensitised patients, in order to compare the effect of an HLA incompatible transplant with remaining on the waiting list. This data should include:

  - Mortality
  - Morbidity
  - Quality of Life (Level 1C) (Chapters 2 and 5)

- Continue to work to develop schemes to help patients with very high cPRA who may not be transplanted in kidney paired donations or under deceased donor priority schemes (Chapter 3)
- A further need for evidence-based information is in the role of induction immunosuppression in relation to sensitization and its role in long-term graft and patient outcomes (Chapter 3)
- Better risk stratification, thorough immunological evaluation and avoidance of HLA-DSA should be used to improve outcomes after kidney transplantation (Chapter 3)
- Better HLA matching and a restricted transfusion policy will probably diminish the number of highly sensitized patients, but more data are needed in this area (Chapter 1).

[1]Bestard O, Couzi L, Crespo M, Kessaris N, Thaunat O. Stratifying the humoral risk of candidates to a solid organ transplantation: a proposal of the ENGAGE working group. Transpl Int. 2021 Jun;34(6):1005-1018. doi: 10.1111/tri.13874. Epub 2021 Apr 22. PMID: 33786891.

Figure 1: Schematic of preparation of chapters and recommendations



Chapter 1: Definition of sensitization

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Running Title: Definition of sensitization

Keywords*:* Sensitization, Donor Specific Antibodies (DSA), Renal transplantation, Rejection, Risk assessment.

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Summary:

In the past, the degree of sensitization of renal transplant candidates was defined on the basis of antibody reactivity of their serum in complement dependent cytotoxicity (CDC) and the percentage of positive panel donors in the antibody screening assay (%PRA), which was not a proper reflection of the chance that the crossmatch with a potential organ donor would be positive.

Nowadays, more accurate parameters are available such as the virtual PRA (vPRA), calculated PRA (cPRA) or calculated reaction frequency (cRF). The parameters are based on the HLA antigens recognized by the antibodies present in the serum of the patient in relation to the HLA phenotypes of the actual organ donor population.

In contrast, the introduction of more sensitive solid phase assays has complicated the definition of a (highly) sensitized patient. The presence of specific antibodies in CDC was always considered a contra-indication for transplantation as these were associated with the occurrence of hyper-acute rejection. HLA antibodies detectable in solid phase assays only are rather considered a risk factor and not necessarily as a contra-indication.
The challenge is to define which HLA antigens recognized by the antibodies in the serum of a patient should be considered as an unacceptable mismatch for a potential organ donor. Different strategies are developed to enhance transplantation of highly sensitized patients but the best option for the patient would be prevention. Novel molecular HLA matching strategies are likely to decrease the number of highly sensitized retransplant candidates.

Introduction

In the early days of renal transplantation, it became evident that the presence of donor-specific HLA antibodies (DSA) before transplantation was associated with a high incidence of hyper-acute rejection [1,2]. Such HLA antibodies can be induced by previous blood transfusions, pregnancies or transplants [3–5]. The incidence of hyper-acute rejection was significantly diminished by the introduction of serological crossmatching and the exclusion of donors towards which the potential recipient had circulating HLA antibodies [2]. However, this strategy had an enormous impact on the transplantation rate of highly sensitized patients. Due to their broad sensitization, these patients had positive crossmatches with virtually all potential donors and accumulated on transplant waiting lists. Without special strategies, many of these patients would be unlikely to ever receive a suitable transplant, and would have a high chance of dying while on the waiting list.
The introduction of sensitive laboratory techniques that detect the presence and specificity of HLA antibodies, and their impact on clinical outcome has led to much discussion on the definition of a sensitized and a highly sensitized patient, which is the topic of this chapter.

Historical definitions

Historically, complement dependent cytotoxicity (CDC) was the gold standard and the only assay available for the detection of HLA antibodies. Patients' sera were screened regularly against a panel of HLA typed blood donors and the degree of sensitization was expressed as

a percentage of panel reactive antibodies (%PRA). This %PRA was defined by the percentage of panel donors reactive with the patient serum in CDC. As the composition of the panels varied enormously between laboratories, the same held true for the %PRA reported. Furthermore, the %PRA was based on the composition of a panel consisting of voluntary blood donors, which does not necessarily reflect the frequency of the HLA antigens in the actual organ donor population. The %PRA was often reported irrespective of the specificity of the antibodies, which made this parameter an inaccurate predictor of the chance that the patient would be confronted with a positive crossmatch as other antibodies, as well as HLA antibodies, are able to cause a positive CDC reaction.

The definition of a highly sensitized patient also varied, but often a PRA>85% was considered the threshold for a highly sensitized patient [6].

Apart from the %PRA, an extensive analysis of the reaction patterns of the potential transplant patient to the HLA types of the panel donors could lead to the identification of specific antibodies, provided that the sensitization was not too broad. When an antibody reactive with a specific HLA antigen was identified i.e. anti-HLA-A2, this antigen was considered to be unacceptable and all HLA-A2-positive organ donors were excluded for this patient.

CDC crossmatch only detects those HLA antibodies that are able to activate complement i.e. IgM and the IgG subtypes IgG1 and IgG3. In order to also be able to detect the non-complement fixing IgG subclasses IgG2 and IgG4, Flow Cytometric crossmatch (FCM) was introduced in several laboratories [7,8]. Donor-specific antibodies (DSA) detectable in FCM, but not in CDC, appeared to be clinically relevant and were associated with graft rejection and graft loss in a proportion of recipients [9]. In contrast to CDC reactive DSAs, antibodies detected in FCM were considered more as a risk factor than a contra-indication for transplantation.

Both CDC and FCM are cell-based assays and a positive reaction in these assays does not necessarily mean that the target of the antibody is an HLA antigen. Clinically irrelevant antibodies reactive with other structures on lymphocytes can interfere in the outcome of both a CDC and an FCM crossmatch [10,11] leading to false positive crossmatches. These irrelevant antibodies also include auto-antibodies, which react with the patients' own lymphocytes. In addition, the endothelial cells in the kidney can express alloantigens, which

are not present on lymphocytes [12] and reliable assays to detect antibodies reactive with endothelial cell-specific antigens are currently lacking.

Impact of the introduction of solid phase assays

Solid phase assays were introduced to prevent non-HLA antibodies interfering in the establishment of HLA sensitization [13]. Targets for antibody detection in these assays are isolated HLA molecules fixed to a solid surface. Any antibody reactivity detected in this assay is by definition directed against an HLA antigen. The introduction of single antigen beads (SAB) has facilitated the detection and, especially, the identification of specific HLA antibodies (although the results are not always straightforward [14,15]). Patient serum is tested against a mix of about one hundred (and recently more) different beads, each individual bead covered with HLA molecules of the same specificity. The degree of antibody binding to a specific bead is expressed as mean fluorescence intensity (MFI). This assay appears to be far more sensitive than CDC and FCM for detecting HLA antibodies and DSA. As a consequence, the proportion of sensitized patients has significantly increased after the introduction of solid phase assays [16].

The clinical relevance of antibodies detectable in SAB assays is still a matter of debate [17]. Individual centers have tried to make correlations between the already established clinical relevance of CDC and FCM and the MFI values obtained in SAB (i.e.[18]).

Although no absolute thresholds can be defined, it is generally accepted that the highest MFI values predict a positive CDC crossmatch, although exceptions exist as some high MFIs are associated with a negative CDC. Moderate and high values are thought to be associated with a positive FCM. The risks associated with the presence of DSA with these MFIs are presumed to be similar to the ones already established for a positive CDC or Flow crossmatch (Figure 1)[19]. As the SAB assay is very sensitive, positive reactions are obtained, usually with a lower MFI, which do not correlate with a positive FCM or CDC crossmatch. The clinical value of such antibodies has been extensively studied with some conflicting results [20,21]. Overall, there seems to be a suggestion of increased risk of early antibody-mediated rejection in DSA-positive transplantation, which may be related to the MFI. The impact of these increased rejection rates on graft function and survival are less certain (Supplemental Data 1).

There are several technical issues related to SAB assays. For MFI, the parameter used to indicate the strength of the antibody reactivity is just a semi-quantitative marker [22,23] and for that reason it is virtually impossible to define an exact positive or negative reaction. Most centres use a cut-off of 1000–1500 [19] but there is no general agreement on this value. Also, the fact that two vendors provide kits with different sensitivities makes a general definition of a positive reaction impossible [24]. Amongst other things, the MFI is affected by the affinity and avidity of the antibodies but also by the number of different beads reactive with the antibody. HLA antibodies are directed against specific epitopes expressed on the target HLA antigen, but individual epitopes can be shared by (many) different HLA alleles [25]. If an antibody is directed against an epitope only expressed on one allele, its MFI will be higher than that of an antibody with exactly the same characteristic but reactive with 30 different HLA alleles as these will compete for antibody binding. Another complication is the fact that not all antibodies reactive in SAB are directed against intact HLA molecules. Studies in non-immunized males showed that their sera contained antibodies reactive with denatured HLA antigens attached to the beads leading to a positive reaction [26]. Patients with DSA directed against denatured HLA appeared to have a similar rejection incidence and graft survival as non-immunized patients [27]. Therefore, it is important to link positive reactions in SAB to known sensitizing events such as pregnancies, blood transfusions, previous transplants before considering an antibody clinically relevant and a target antigen unacceptable on a future organ donor.

cPRA, vPRA, cRF, novel parameters to define the degree of sensitization

The historical parameter for indicating the degree of sensitization was the %PRA, but this parameter was not very accurate as the specificity of the antibodies causing the positive reactions was often unknown and clinically irrelevant antibodies could contribute to the %PRA.

The introduction of solid phase assays has improved the possibility of determining the HLA specificities of the antigens recognized by the antibodies present in the patient's sera. The specificities recognized in solid phase assays are also instrumental for clarifying the specificity of the antibody patterns observed in CDC and FCM. This has led to a solid basis for the introduction of more reliable parameters for the definition of the degree of sensitization based on the antibody specificities present in the patient and the HLA

phenotypes of the actual organ donor population. Different names are now circulating for this novel parameter: vPRA (virtual PRA) [28], cPRA (calculated PRA) [29] and cRF (calculated reaction frequency) [30] but they all reflect the chance that a patient has HLA antibodies reactive with a donor derived from the actual organ donor population.

The definition of highly sensitized patients and making them eligible for prioritization in organ allocation is variable as became clear from a recent inventory by ESOT (Table 1). Several recent studies have shown that, especially in patients with a vPRA, cPRA or cRF >98%, there are difficulties in finding a suitable donor without the help of a special program or treatment [31–33].

Risk estimation in sensitized patients

As mentioned, serological crossmatching and HLA antibody screening have been introduced to prevent the occurrence of hyper-acute rejection. However, the definition of HLA antibodies and/or unacceptable mismatches has a broader application and is mainly aimed at immune risk assessment [17,19,34]. Patients with DSA detectable in CDC, Flow crossmatch and SAB (high MFI) are still at risk for hyper-acute rejection. Patients with DSA in Flow and SAB (medium MFI) but not in CDC are at risk for early antibody-mediated rejection. Patients with DSA only in SAB (lower MFI) are at a lower risk for antibody-mediated rejection and it remains to be established whether further fine-tuning SAB antibody detection will contribute to a better risk assessment. Assays have been developed to measure the complement binding capacity [35,36], or to identify the IgG subclass of the antibodies reactive in SAB [37] but it is not clear whether these modified assays really contribute to further risk assessment when performed before transplantation [34,38].

The actual challenge, in the case of SAB reactive antibodies with a low MFI, is to define whether their target antigen should be considered an unacceptable mismatch or not. A link with a specific sensitization event may help but, especially for highly sensitized patients, one should consider the low risk for a rejection event in the light of the risk of mortality or morbidity due to the fact that the patient will not be transplanted and will remain on dialysis [39,40].

Conclusions

The definition of a sensitized patient has changed enormously since the introduction of solid phase assays. Rather than the reactivity of the patient's serum against a panel of blood donors as the basis for the %PRA, the specificity of the HLA antibodies now plays a pivotal role.

Calculating the chance that specific patient antibodies react with the HLA antigens of the actual organ donor population, has created a more reliable parameter (vPR, cPRA, cRF) for the degree of sensitization. However, the sensitivity of the solid phase assays has also led to complications including the assessment of which antibodies should be considered as clinically relevant and a contra-indication for transplantation. Not every HLA antigen reactive in a solid phase assay should be considered as an unacceptable mismatch.

What has not changed is the fact that highly sensitized patients have a very low chance of being transplanted with a compatible donor organ and that special strategies are required to enable successful transplantation of highly sensitized patients.

One approach is to try to prevent patients from becoming (highly) sensitized. A recent analysis of the background of highly sensitized patients transplanted via the Eurotransplant acceptable mismatch program showed that more than 70% had been immunized by a previous transplant [41]. Better HLA matching of the first transplant and avoiding blood transfusions prevents the induction of these HLA antibodies [42]. Although classical HLA antigen or allele matching might help to some extent, novel match strategies, which take advantage of the fact that the amino acid sequence of the different HLA antigens is known, show very promising results. This has led to the identification of those amino acids responsible for the induction and reactivity of HLA antibodies called eplets or epitopes. Every HLA antigen consists of a unique set of epitopes but the individual epitopes can be expressed on different HLA antigens [25]. A patient will not make antibodies to epitopes expressed on their own HLA antigens even when these self-epitopes are expressed on a mismatched HLA antigen. As a consequence, the number of foreign epitopes on a single HLA mismatch varies and depends on the patient's HLA type. Many recent studies show a clear beneficial effect of molecular HLA matching of donors and recipients [43–47], (based on epitopes, eplets, amino acids or electrostatic properties [44]), on the induction of de novo DSA (Supplemental Data 2). Inclusion of these novel matching strategies in the allocation of donor kidneys will certainly decrease the number of (highly) sensitized retransplant candidates on the waiting list.

Figures and tables.

Figure 1: The association between the MFI of DSA detected in SAB and the outcome of CDC and Flow crossmatches is reflected in the risk estimation.

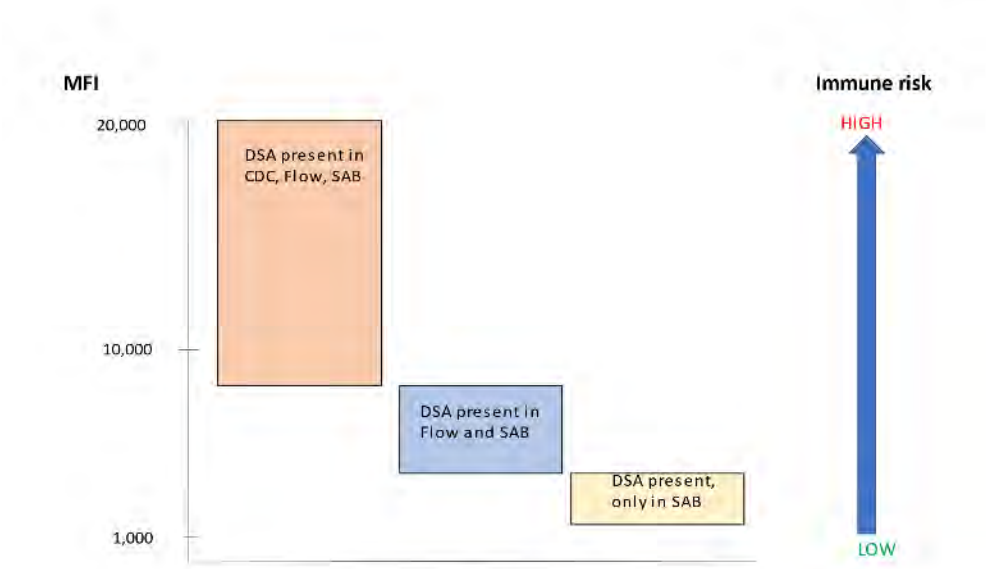
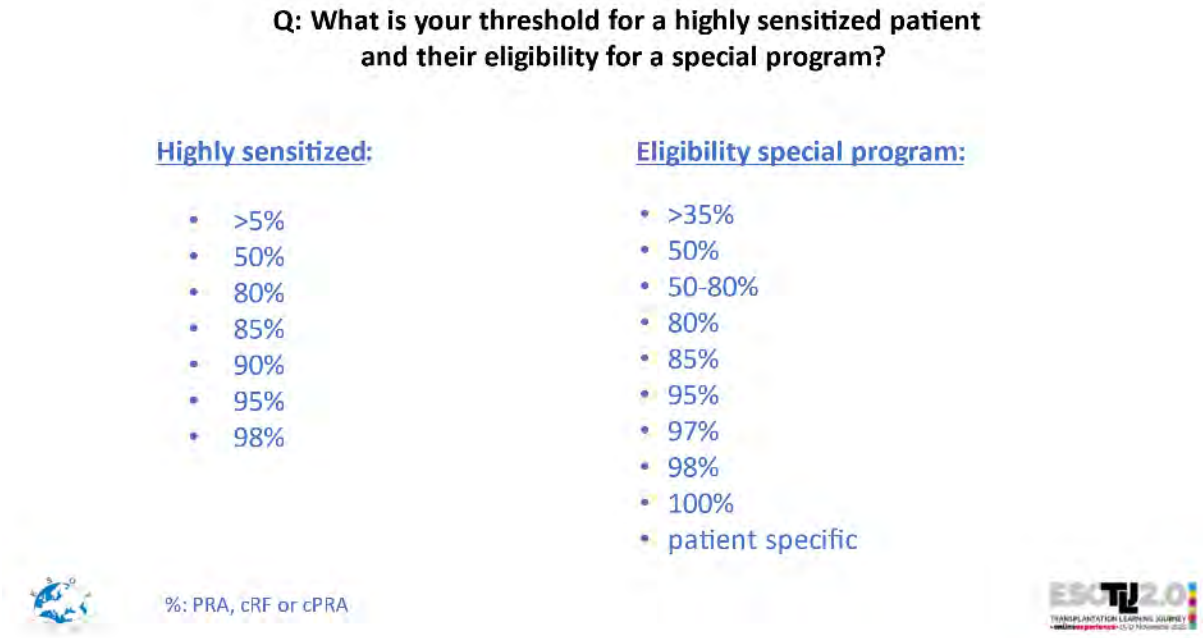


Table 1: Outcome of an inventory made by ESOT on the definition of a highly sensitized patient and eligibility for a special program. The results are based on the reactions of 45 centers.



Possible Supplemental Data 1: Evidence report on the clinical relevance of DSA detectable in SAB only.

Possible Supplemental Data 2: Evidence report on the beneficial effect of eplet matching on the induction of DSA.

Recommendations

Standardization

- A parameter, which is based on the HLA frequencies of the actual organ donor population, such as cPRA or cRF, should be used to estimate the chance that a sensitized patient can be transplanted with a compatible donor without the need for any special treatment
- Further standardisation of solid phase assays is recommended
- When defining unacceptable mismatches in highly sensitized patients on the basis of (weak) antibody reactivities in SAB assays only, one should consider the not well-defined risk of antibody-mediated rejection in the light of a prolonged waiting time and associated mortality and morbidity

Organ allocation

- Increase access to the donor pool, through greater use of:

- Sliding scale priority score schemes based on cPRA values (Chapters 1 and 3)

Areas for further research

- Better HLA matching and a restricted transfusion policy will probably diminish the number of highly sensitized patients, but more data are needed in this area

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Chapter 2: Comparison of practices across Europe for dealing with highly sensitized transplant candidates

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The management of highly sensitized recipients is a challenge for all European countries, and initiatives have been introduced to increase the opportunities for this disadvantaged group to receive a transplant from either a deceased or living donor. Although there is some variability in approach between countries, there is broad recognition that to allow equity of access novel strategies are needed. In the setting of deceased donor transplantation, this

includes prioritisation for highly sensitised patients should a compatible donor become available or the development of an acceptable mismatch programme. For living donor transplantation, Kidney Exchange Programmes have been established to allow compatible transplantation, and many individual units have undertaken antibody removal to allow HLA incompatible transplantation to proceed. Challenges remain, in particular to achieve a consensus on best practice and ensure there is the potential for all patients to receive a successful transplant.

Introduction

Organ transplantation has been one of the major medical advances of the 20th Century, providing life-saving treatment to millions. Assessment of sensitisation and the detection of HLA antibodies has become increasingly sophisticated, and potential recipients now have a detailed antibody profile compiled prior to transplantation. Around 1 in 4 potential kidney transplant candidates are highly sensitised, limiting their available donor pool and increasing their waiting time before receiving a transplant. The barrier of sensitisation is a frustrating situation for both clinicians and transplant candidates, but fortunately several options are now available to increase transplant opportunities for this group. In addition to the interventions developed in single centres, there have also been national initiatives to facilitate deceased organ donation, and a rapidly increasing number of countries have established Kidney Exchange Programmes (KEP) for those with a living donor. Living donation offers the greatest opportunity for treatment to modulate antibody levels, but this has also been applied in the context of deceased organ donation. There is considerable variability in practice across Europe, not only in terms of rates of deceased and living donation, but also in approaches to antibody removal and access to a KEP. The best outcome for an individual recipient depends both on their degree of sensitisation and risk stratification, and the availability of different treatment options in their locality.

Organisation of transplantation in Europe

Both deceased and living donations are coordinated on either a national basis (for example the United Kingdom, France, Switzerland, Italy, Spain and Portugal), or on behalf of a group of countries (http://www.accord-ja.eu/background). Eurotransplant (https://www.eurotransplant.org/) is responsible for allocation of donor organs in Austria,

Belgium, Croatia, Germany, Hungary, Luxembourg, the Netherlands and Slovenia. Scandiatransplant (http://www.scandiatransplant.org/) is the organ exchange organisation for Denmark, Finland, Iceland, Norway, Sweden and Estonia. Larger donor pools would be expected to increase the likelihood of identifying a compatible donor for those who are hard to match, either due to an unusual tissue type or a high level of sensitisation.

Deceased organ offering and allocation

Deceased donor allocation schemes that do not take HLA sensitisation into account will inevitably lead to fewer offers being made to these candidates and longer waiting times. Offering schemes can adjust for this bias, either by increasing the weighting given to those who are hard to match, as in the UK Kidney Offering Scheme (https://www.odt.nhs.uk/transplantation/kidney/kidney-offering-and-matching/) or by the development of an Acceptable Mismatch (AM) programme, first established by Eurotransplant over 30 years ago [1]. The Eurotransplant AM programme has enabled successful transplantation of Highly Sensitised Patients (HSP) with excellent outcomes [2]. Despite this success, a subgroup of patients will not receive the offer of a transplant because a suitable donor is not available in the Eurotransplant donor population.
A similar observation has been made in other transplant organisations in North America [3, 4]. The EUROSTAM project (a Europe-wide Strategy to enhance Transplantation of highly sensitised patients on the basis of Acceptable HLA Mismatches) has compared data from five European registries (Eurotransplant, UK National Health Service Blood and Transplant, Barcelona, Athens and Prague), to determine whether expanding the donor pool across different populations will result in increased rates of transplantation for those with >95% sensitisation [5]. In total, 195 (27%) of the 724 HSP who had been registered for at least 5 years at each organisation had an increased chance of a compatible kidney transplant offer in a different European pool. This makes a strong case for sharing kidneys between European countries for selected difficult to transplant patients.

Living Donor Transplantation

Kidney Exchange Programmes (KEPs)

Europe's first kidney exchange was carried out in Switzerland in 1999 [6], however, the Netherlands was the first country to establish a nationally coordinated KEP in 2004 [7]. The UK Kidney Sharing Schemes (KSS) were initiated in 2007 [8], and to date this programme has performed the greatest number of transplants [9]. The Spanish national programme began in 2009. Over the last decade, there has been a further rapid expansion in the number of programmes, which are now established in Austria, the Czech Republic, Poland, Belgium, France, Italy and Portugal, and are developing elsewhere [9].

The active KEPs are organised centrally. Approaches to living donation vary between countries [10], which has an impact on the number of donors enrolled and the chance of an HSP receiving an offer. For example, in the UK, altruistic donation is permitted, and all altruistic donors are enrolled in the KSS to initiate donor chains. The inclusion of compatible pairs is also permitted, with the most usual reasons for consideration of inclusion being a significant age difference between donor and recipient and poor HLA match. In addition to short chains, the UK scheme also allows three-way exchanges. Altruistic donation is not possible in France, Poland, Greece and Switzerland. In France, only two-way exchanges are possible, and in France and Portugal only incompatible pairs can participate [9].

The Austrian and Czech programmes both commenced in 2011, and merged in 2015, including the option for altruistic donor-initiated chains [11]. This transnational merger has demonstrated the feasibility of increasing the size of the donor pool, although whilst matching rates in Austria doubled, those in the Czech programme actually fell, partly due to the introduction of more stringent threshold criteria for HLA antibodies. Further collaborations have been introduced between Italy, Portugal and Spain [12], and Sweden, Norway and Denmark, although these collaborations have mainly considered patients left unmatched in their national KEPs.

The European Network for Collaboration on Kidney Exchange Programmes (ENCKEP, https://www.eurotransplant.org/) was established in 2016 "in order to establish and foster a preferential discussion channel for the various essential themes that have to be addressed for the implementation of a collaborative KEP". The programme has contributed to aspirations for future developments, including modelling of European KEPs with the aim of future optimisation [13].

HLA incompatible transplantation

Rates of HLA incompatible (HLAi) transplantation vary considerably between centres and countries, depending on the availability of alternative approaches, likelihood of achieving a compatible transplant, the clinician's interpretation of the individual patient's risk and the acceptability of the proposed antibody removal regimen and predicted outcome to the recipient.

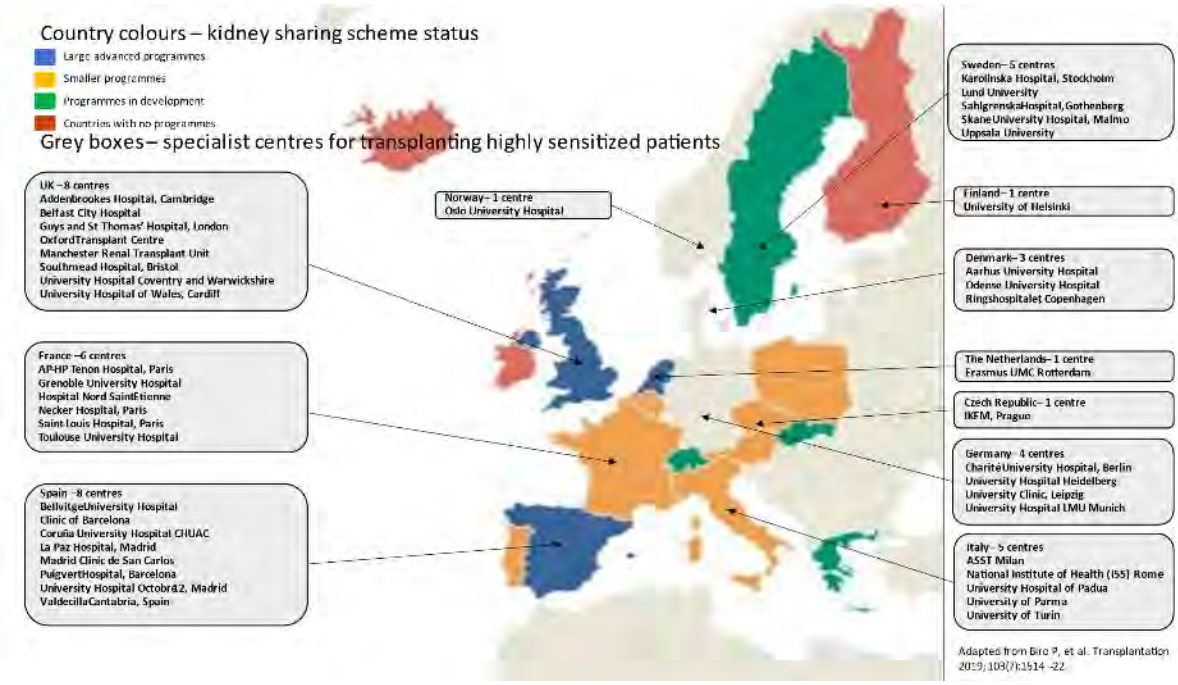
No country has a published national consensus on their optimal recommended management pathway for HSP. Several European centres have published their protocols and outcomes following HLAi transplantation [14–17], but a more general overview of how widely this option is offered is not available. A recent survey of European transplant centres demonstrated substantial variability in the definition of sensitisation, approaches to improve opportunities for deceased and living transplantation and perceived success of HLAi transplantation. This was an informal survey carried out by ESOT, which queried European transplantation professionals regarding approaches to patients with HLA antibodies. There were 47 responses from 25 European countries (21 complete responses). The majority of respondents (>80%) agreed that new strategies were needed to more effectively manage highly sensitised transplant candidates.

In the UK, the recognition that results following HLAi transplantation may be inferior to compatible [18], albeit with similar recipient survival, has led to increased reliance on the KSS. Although the success of the KSS has been to the benefit of many, there are a subgroup of patients who have little chance of receiving a compatible transplant and at present may not be offered the opportunity and potential benefit of an HLAi transplant [19].

Conclusion

The barrier of sensitisation remains a significant hurdle for many transplant candidates. In the future, on-going research will improve the accuracy of risk stratification for HLAi transplantation, and prospective data collection of patient outcomes from the time of initiation of dialysis will contribute to more informed decision making by transplant candidates and their clinicians.

Figure 1: Countries with kidney sharing schemes and specialist centres for tansplnating highly sensitized patients



Recommendations

Organ allocation

- Increased access to and harmonisation of Kidney Exchange Programmes, with greater and standardised sharing of outcomes (Chapters 2 and 6)
- If a particular country does allow unspecified kidney donations, consider including these in kidney sharing schemes (Chapters 2 and 6)

Areas for further research

We recommend that data be collected prospectively for sensitised patients, in order to compare the effect of an HLA incompatible transplant with remaining on the waiting list. This data should include:

- Mortality
- Morbidity
- Quality of Life (Level 1C) (Chapters 2 and 5)

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Chapter 3: Strategies for access to HLA compatible kidney transplantation in highly sensitized patients

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Immunologic risk stratification in kidney transplantation

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Introduction

A main barrier to successful transplantation is the Human Leukocyte Antigen (HLA) molecular disparity between donors and recipients, which triggers a robust recall alloimmune response, ultimately leading to allograft rejection and graft loss [1]. Notably, previous HLA antigen encounters through blood transfusions, pregnancies or previous transplants may lead to the development of a long-lasting allogeneic immune memory, mostly characterized by the presence of serum IgG antibodies directed to distinct HLA antigens [2]. With currently available immunological tools, detection of circulating anti-HLA antibodies can be accurately assessed and thus, transplant candidates may be stratified by their immunological risk of humoral rejection of a transplant organ. While the outstanding sensitivity and specificity of these assays in detecting serum antibodies have allowed a clear reduction of severe, hyperacute antibody-mediated rejection (ABMR), a progressively increasing proportion of kidney transplant candidates worldwide may be considered as highly sensitized to HLA antigens. These patients have a significantly lower chance of finding an HLA compatible kidney organ donor and remain for longer periods of time on the waiting list for transplantation. Importantly, a precise understanding of the different biological features of circulating anti-HLA antibodies according to different immunological tests, will determine their clinical relevance in predicting the precise risks of post-transplant allograft rejection and survival.

Desensitization therapies have shown poorer mid-/long-term transplant outcomes than seen in those undergoing HLA compatible transplants. In light of this, in recent years, transplant physicians have developed a number of strategies, both for deceased and living-donor transplantation, aimed at facilitating access to HLA compatible transplantation for these highly sensitized patients before they undergo desensitization therapies.

Among the most successful transplant policies are i) sliding scales - local, regional or national priority points programmes; ii) establishing an allocation system based on acceptable mismatch (AM) HLA antigens rather than in the avoidance of unacceptable ones to improve donor/recipient matching; iii) favoring different living donor kidney transplantation modalities to achieve HLA compatibility, such as overcoming ABO incompatibility or kidney paired donation exchange programmes.

In this chapter we discuss the different approaches to establish a definition of the immunological risk of a transplant candidate, as well as different major straightforward strategies to increase transplant rates in highly immunized transplant candidates.

Stratification of the immunological risk of kidney transplant candidates

The molecular basis of the HLA system relies on a highly polymorphic system that allows for strong adaptive immune responses driven both by alloreactive T and B lymphocytes. However, while alloreactive T cells are key players promoting and facilitating allograft rejection, there is a lack of sensitive and validated immune tools that can be implemented in clinical transplantation to mitigate these effects [3,4]. Conversely, current immune-risk stratification in kidney transplant candidates is solely focused on the humoral effector pathway of adaptive immunity through the detection of serum anti-HLA antibodies directed against donor antigens using a plethora of highly sensitive *in vitro* immune assays (please see **chapter 1**).

Since a high number of unacceptable alloantigens diminishes the likelihood of an organ offer, precise identification of circulating anti-HLA antibodies is highly warranted. In addition, the risk of undergoing kidney transplantation should be balanced with the risk of post-transplant rejection, allograft survival, as well as life expectancy and quality of life while remaining on the transplant waiting list for an extended period of time.

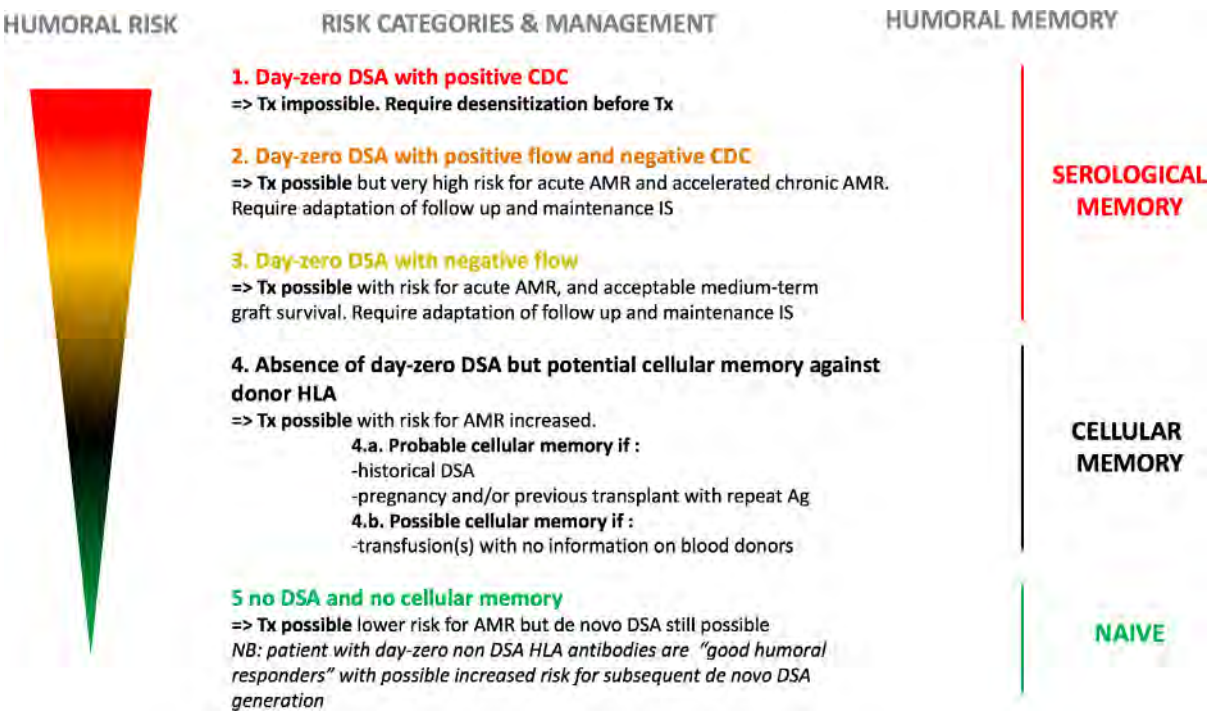
It is widely accepted that patients with a positive complement-dependent cytotoxicity (CDC) crossmatch test, targeting complement-fixing class I and/or II donor-specific antibodies (DSA), have a very high risk of hyperacute rejection and graft failure [5]. With the implementation of flow-cytometry cross-matching (FCXM), serum DSA against either class I or II donor antigens may be more accurately detected than by CDC, reducing the high risk of

early post-transplant acute ABMR. However, a positive FCXM in the absence of detectable DSA by single antigen bead (SAB) assays may not necessarily be predictive of rejection [6,7], most likely because these antibodies may recognize antigens not present at the endothelial cell surface. Notably, with the development of highly sensitive SAB assays, the identification of unacceptable antigens has become easier, but entails a high degree of interpretation and expertise in each laboratory. SAB assays detect amounts of antibodies present in the serum (and quantified as Mean Fluorescence Intensity [MFI]), and can identify purified class I and II antigens adhered to plastic beads with fluorescent-labelled antibodies to IgG, thus providing a reliable virtual crossmatch, which does not require donor cells. Several modifications of SAB assays have been developed, including an assay to evaluate complement-binding DSA (both C1q or C3d), although their absence does not rule out the negative impact of DSA [8-10]. IgG subclasses can also be delineated and have also been associated with a diverse range of severity of graft damage due to their complement binding potential [11], as well as their Fcγ receptors, which trigger innate immune responses [12]. The impact of preformed DSA has classically been linked to the degree of MFI, although there is no general consensus regarding MFI cut-off levels (as discussed in Chapter 1). Notably, it is important to bear in mind that a number of distinct factors may impact the interpretation of SAB data, such as antibody titer, prozone effect, competition of shared epitopes on different beads, as well as irrelevant antibody reactivity against denatured HLA molecules [13–15]. Thus, the ability of DSA identified by SAB to bind donor cells *ex vivo* in FCXM is a good predictor of subsequent AMR lesions and graft loss (in 50% and 30% of recipients, respectively [16–18]). Importantly, by accepting every SAB signal, a high number of patients would be defined as highly sensitized, with the consequently lower chance of receiving an organ offer through regular allocation systems – likely reducing a patient's chance by up to five-fold [5]. Therefore, an individualized risk-assessment of previous sensitizing events, adding a thorough epitope analysis and most importantly, the likelihood of receiving an HLA compatible transplant in their respective region, should be taken into account.

Indeed, there is still no precise definition of the different strata for the humoral risk in kidney transplantation, which ultimately represents a major barrier to evolve clinical care in this area. Currently, a wide range of different patient profiles are mixed together. Aiming to move this field forward, a European working group endorsed by the European Society of

Organ Transplantation (ENGAGE), has put forward an initiative proposing an integrative consensus of the most consistent evidence to stratify kidney transplant candidates into five distinct risk categories with the aim of conferring the best chance of successful transplantation. These risk categories take into account an individual patient's past immunological clinical background, integrated with an assessment of serological alloimmune memory using CDC-XM, FC-XM and SAB assays [19] (**Figure 1**)**.** While further novel technologies assessing the impact of other immune effector pathways favoring transplant rejection are in development and validation, these different immunological humoral-risk categories should help stratify the risks of kidney transplant candidates.

Figure 1. Humoral risk stratification of kidney transplant candidates (adapted from reference 19)

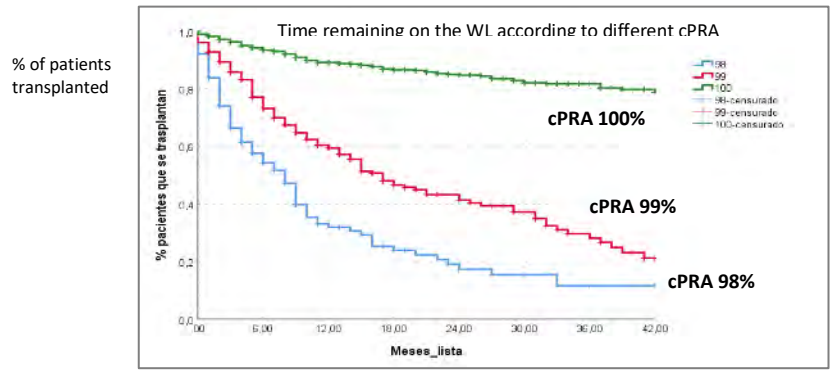


The high humoral immune-risk, together with higher mortality rates of patients remaining on the waiting list for long periods of times [20,21], have prompted a number of strategies to be put forward worldwide aiming to increase transplantation rates in highly sensitized kidney transplant candidates.

Sliding scale priority points for deceased-donor kidney transplantation

A widely established policy to enhance the kidney transplant rates of highly sensitized kidney transplant candidates is based on the implementation of a local, regional or national sliding scale priority points system for the regular allocation of deceased donor organs. These programmes aim to increase the pool of suitable donors with compatible kidneys based on a virtual crossmatch. These systems award extra points based on calculated panel-reactive antibody (cPRA) and by implementing local, regional, and national sharing for those with a high calculated cPRA, which may vary between different countries. The Kidney Allocation System (KAS) promoted in the United States, helps patients from a starting cPRA threshold of 20%. Those with a cPRA ≥98% receive a higher sliding scale priority point score, in which ABO compatible (A2/A2B to B organ) offers are also permitted due to their lower immunogenicity, and these patients are eligible for local, regional and national priority donor allocation [22–24]. Remarkably, kidney transplant rates among these patients dramatically increased from 2.5% to 13.4% during the first year after implementation, notwithstanding an important bolus effect [25]. Consequently, the median waiting time dropped from >19 years to 3.2 years [26]. The implementation of this KAS also increased sharing of high Kidney Donor Profile Index (KDPI) kidneys and decreased the hazard of graft loss without an impact on patient survival [27]. A similar scheme has been developed in Spain, with a national sliding scale priority programme using an ABO identical deceased organ donor allocation system (PATHI) [28]. However, while these programmes have significantly helped the access to transplantation for this increasingly prevalent patient population, these outcomes only hold true for those transplant candidates with a cPRA <100% [25,29,30]. For those with 100% cPRA, sliding priority points schemes do not seem to increase their chance of receiving a kidney transplant, or even an organ offer, especially when stratifying the levels of sensitization into decimals (99.95–100%) [31] **Figure 2.**

Figure 2: Percentage of patients receiving a kidney transplant relative to their cPRA value in the priority program for highly sensitized kidney transplant patients (www-ONT.es)



An illustrative example of how the interpretation of the SAB cut-offs defining unacceptable HLA antigens may directly impact on access to transplantation was clearly reported by Houp and colleagues. This group showed that including very weak MFI levels of anti-HLA antibodies as unacceptable antibodies in intermediately sensitized patients, deleteriously impacted on severely sensitized patients competing for similar priority organ donors [30]. Importantly, excellent short-term kidney graft and patient outcomes under this new priority system have been reported, with acceptable low rejection rates. Although the organs had longer cold ischemia time and subsequently a higher incidence of delayed graft function, this did not negatively impact graft outcomes [25,32]. Nevertheless, whether long-term graft survival will mimic those short-term outcomes still remains to be evaluated and certain concerns have been raised. These relate to the generally low donor/recipient HLA matching for these patients – using an unacceptable HLA antigen policy rather than an acceptable antigen mismatch program thus eventually leading to poorer long-term graft outcomes [33, 34].

In summary, while sliding scale priority points strategies have enabled highly sensitized transplant candidates to have access to kidney transplantation, showing optimal short/mid-term graft outcomes, important questions still remain. Patients with the highest sensitization status (cPRA 100%) do not seem to be positively impacted, with their chance of receiving a transplant offer remaining extremely low. Furthermore, whether HLA matching within these programmes should emphasize donor/recipient acceptable antigen matching

rather than concentrating on prohibited ones to ultimately gain longer transplant survival rates is unclear.

Living-donor KSS / ABOi but HLA compatible

For those patients with an antibody incompatible (ABO or HLA) living donor, a kidney sharing scheme (KSS) remains an option. This is discussed in detail in Chapter 6, but one of the difficulties faced by clinicians is assessing the likelihood of success through a KSS for an individual patient. This will clearly vary according to a number of factors:

1) National demographics: the incidence of blood groups and HLA types varies across different countries, and will therefore affect the chances within a KSS - for example, where blood group B is uncommon (as in most Western European countries) the chance for group B recipients will be lower, **Figure 3**

Figure 3: UK figures for the chance of a transplant by blood group (https://www.odt.nhs.uk)

Impact of Altruistic Donor Chains - Chance of transplant by blood group

NHS Blood and Transplant

2012 onwards

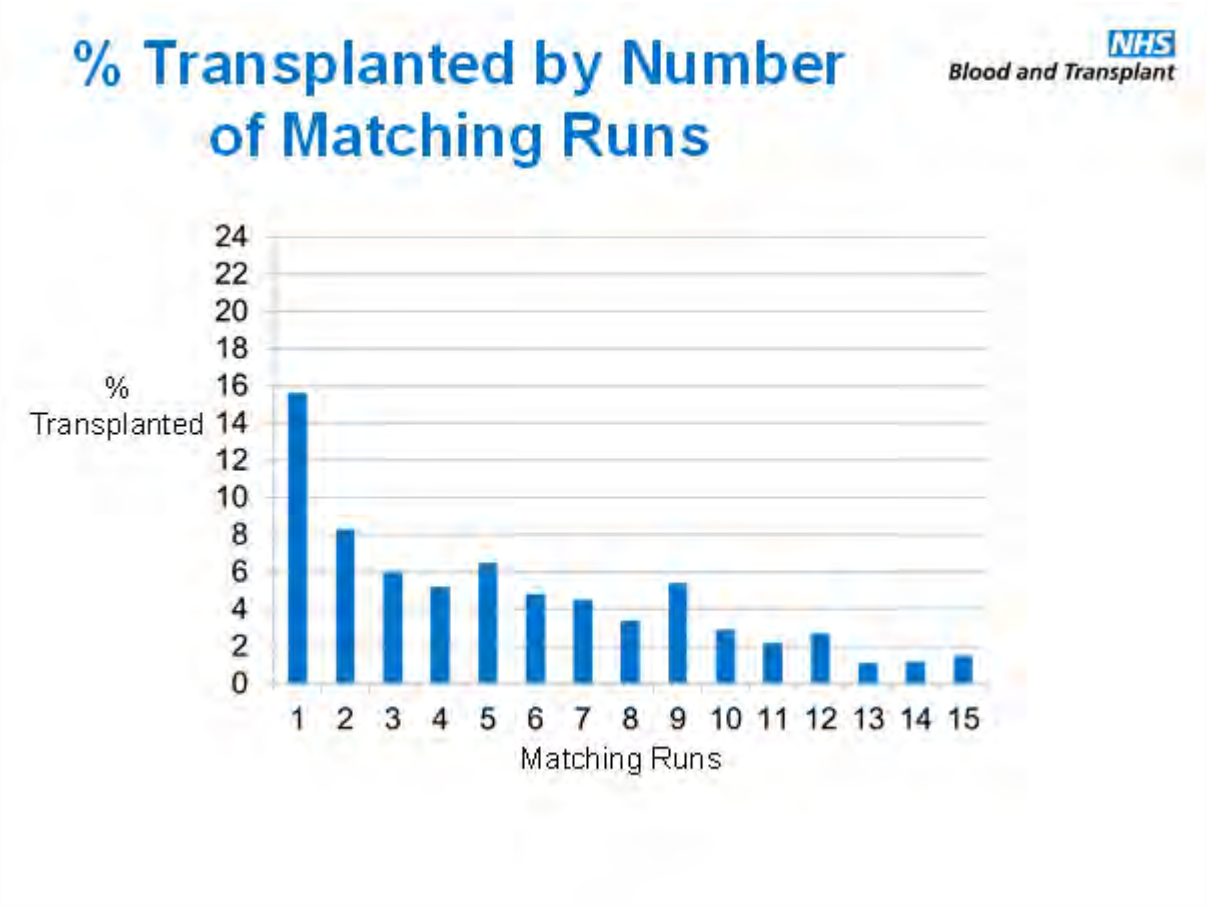
Donor ABO \ Recipient ABO	O	A	B	AB
O	95/307 (31%)	81/144 (56%)	21/43 (49%)	6/12 (50%)
A	106/429 (25%)	56/192 (29%)	27/57 (47%)	2/14 (14%)
B	32/107 (30%)	21/54 (39%)	13/44 (30%)	1/6 (17%)
AB	4/17 (24%)	8/16 (50%)	3/14 (21%)	0/5 (0%)

2) The size of the scheme: generally speaking, the larger the scheme the greater the chances of a match, although there is probably a maximum size beyond which there is no incremental advantage
3) Recipient characteristics: for example, those who are very highly sensitized (eg cPRA/CRF 100%) will have a low or even negligible chance in a KSS, for the same reasons that they will have a low chance of receiving a deceased donor transplant
4) KSS algorithm: each KSS will have its own algorithm, which will affect the chances an individual has for a match in the scheme. This should be considered when entering a patient into the scheme.

The easiest way to address these factors is to access an online calculator which incorporates the factors into a probability of a match, ideally with confidence intervals. An example from the UK scheme is given at:
https://www.odt.nhs.uk/living-donation/tools-and-resources
and at:
https://www.odt.nhs.uk/transplantation/tools-policies-and-guidance/calculators/
which addresses the likelihood of a deceased donor transplant for sensitized patients.

Finally, an important point to consider is that entry into a KSS should not be considered as a definitive solution. The figure below, from the UK KSS, shows that the incremental chance of a match after 6 or 7 'runs' is low, and thus, at this stage, if there are alternatives, such as a direct antibody incompatible transplant, these should be considered, **Figure 4**.

Figure 4: Correlation of the chance of a transplant relative to the number of matching runs (https://www.odt.nhs.uk)



Acceptable mismatch program

Although a priority point strategy is anticipated to improve access to transplantation for sensitized patients, this is not necessarily helpful for the most highly sensitized patients, who may still have difficulty securing a transplantation. While desensitization strategies (See chapter 4) could offer a solution, the Eurotransplant Acceptable Mismatch (AM) programme [33] represents a valid alternative for patients in Austria, Belgium, Croatia, Germany, Hungary, Luxembourg, Slovenia, and The Netherlands. This program fully prioritizes the allocation of compatible donor kidneys to highly sensitized patients (>85% cPRA), to increase their chances for transplantation, focusing on finding acceptable matches rather than to prohibit matches. It is an alternative approach to the sliding scale programs of the US and Spain mentioned previously. The main advantage of the AM over prioritization schemes is that it entails better matching and thus may lead to better long-term outcomes because of less susceptibility to primary alloimmune activation. Unfortunately, it does not seem to increase access to transplantation for those very highly sensitized patients (>99% cPRA).

Donor kidney offers (<65 years) are first allocated in the AM program, and only if no match is found in this program, are the kidneys allocated to the standard Eurotransplant Kidney Allocation System (ETKAS). First priority is therefore checking immunological compatibility for the most highly sensitized patients, without considering other factors. In the AM program, the HLA mismatches that most likely will not result in a positive crossmatch are defined, based on detailed evaluation of the HLA antigens to which the patient has not yet reacted, and therefore might be acceptable for the patient. This detailed evaluation also encompasses computer algorithms such as HLAMatchmaker which helps in defining acceptable HLA or epitope mismatches [35,36]. Only patients who have been included on the standard Eurotransplant (ETKAS) waiting list for at least 2 years are eligible, and their cPRA% should be ≥85%. After the antibodies detected with CDC, only antibodies identified using solid phase assays are considered for the evaluation of the cPRA%, if they can be explained by previous immunizing events, e.g., HLA mismatches with previous donor(s) or a specific sensitization of the recipient such as HLA antigens of their partner or children in women. Not only are the classic HLA loci (HLA-A, B and -DR) considered, but also HLA-C and HLA-DQ. Access to the AM program is strictly controlled by the Eurotransplant Reference Laboratory (ETRL) in Leiden, The Netherlands, which reviews all relevant patient-level data before enrolling patients. Not all patients with a cPRA >85% are registered in the AM program, for several reasons, including the strict criteria for inclusion (**Figures 5 and 6**).

By fully prioritizing patients who are very highly sensitized by well-defined HLA antibodies, the Eurotransplant AM program clearly increases the chances for transplantation for this group of patients. The number of actively waiting patients included in the AM program has remained relatively constant over the past decade, as well as the numbers of patients transplanted within this program (**Figures 7 and 8**). A considerable number of patients have already been transplanted within the AM program [33], both first and repeat transplantations. Waiting times for transplantations in the AM program (thus of very highly sensitized patients) are significantly shorter than seen in similarly sensitized patients (cPRA >85%) not included in this program [33], illustrating that the AM program fulfils its primary goal, to increase access to transplantation for the most difficult to transplant patients.

In addition to benefits in terms of access to transplantation, there are also other very important messages we gain from detailed evaluation of the AM program. Kidney transplant

failure is significantly lower in the highly sensitized patients included in the AM program, compared with highly sensitized patients not included in the AM program. Furthermore, death-censored graft survival rate is similar to the rate in non-sensitized patients, and better than seen in mildly sensitized patients [33]; and is related to a lower chance of rejection in the highly sensitized patients included in the AM program [34]. Although at first sight counterintuitive, this important finding, that the most highly sensitized patients have the lowest risk of rejection, clearly illustrates that better risk stratification, thorough immunological evaluation (as in the AM program), and avoidance of HLA-DSA is highly beneficial for outcomes after kidney transplantation. This message is important beyond the implications for highly sensitized patients and makes the case for better (molecular) matching for general kidney transplants as a means to further decrease the risk of graft failure.

Although the AM program is highly successful [33] in terms of access to transplantation and outcomes after transplantation, a subset of patients enrolled in the AM program remain on the transplant waiting list because no compatible donors are available in the Eurotransplant donor population. This is exemplified by the discrepancy between the number of patients waiting in the AM program and the number of patients effectively transplanted each year (**Figure 8**), and represents the population of cPRA 99–100% mentioned previously. The patients remaining without transplants are mainly those with a rare HLA type compared with the HLA types of the actual donor population. Part of the population, therefore, remains waiting for a transplant, even in the AM program, with very limited chance of finding a suitable (HLA-DSA negative) donor. For this, the EUROSTAM project was initiated, which intends to expand the Eurotransplant AM program to a Europe-wide acceptable mismatch program [37]. Simulations suggest that one in four of the highly sensitized patients who have been waiting a long time for a transplant (in total >700 patients identified), registered at each partner organisation, have increased chances of transplant in a different European donor pool. Although the simulation exercises make a strong case for kidney sharing between European countries for selected patients, further practical and logistical work is needed before this Europe-wide AM program is implemented clinically [37].

Figure 5: Relative numbers of patients in the Eurotransplant and Acceptable Mismatch program (image reproduced from the online Eurotransplant database: https://statistics.eurotransplant.org)

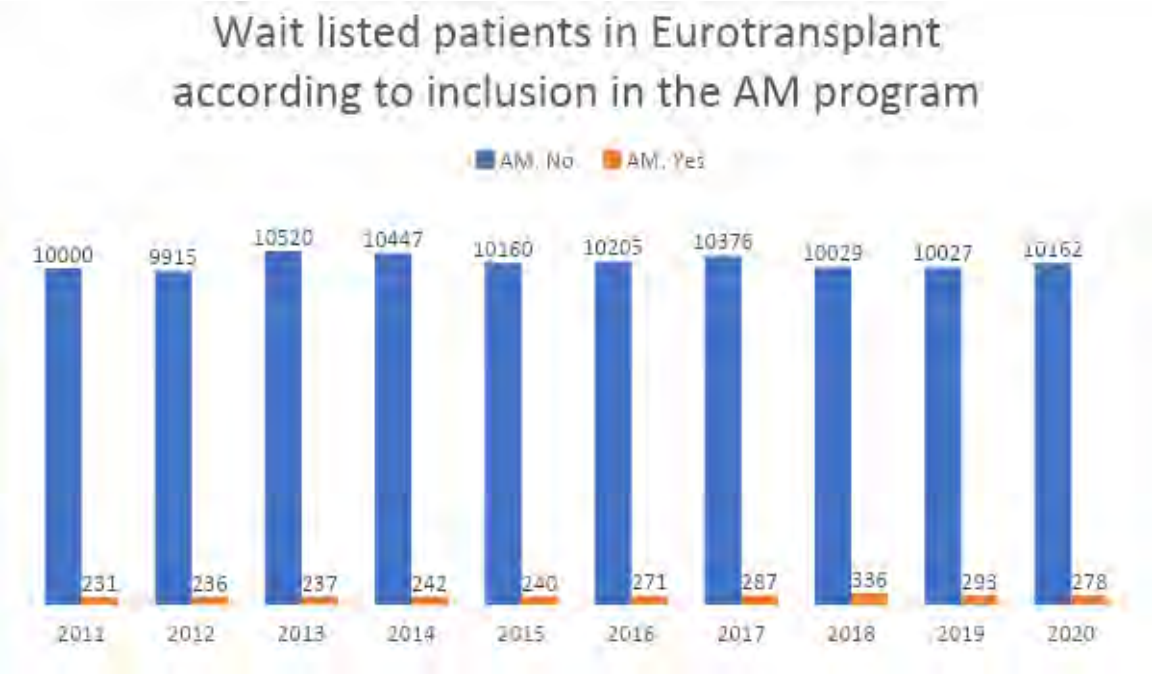


Figure 6: Relative numbers of highly sensitized patients in the Eurotransplant and Acceptable Mismatch programmes (image reproduced from the online Eurotransplant database: https://statistics.eurotransplant.org)

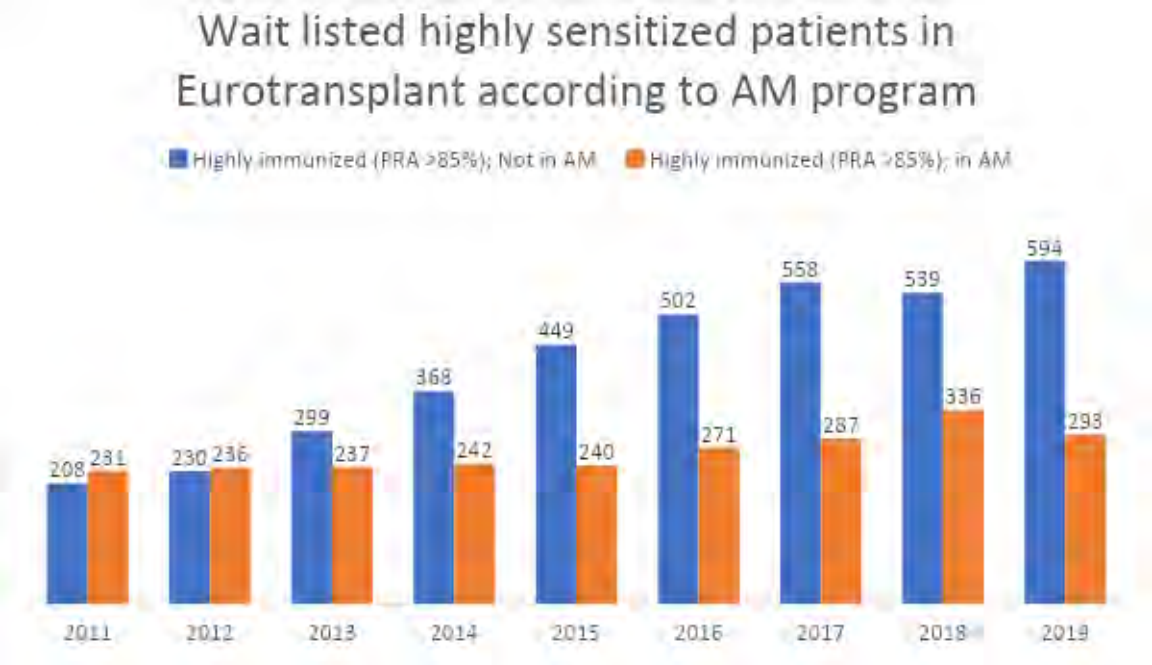


Figure 7: Relative numbers of kidney transplantations achieved by Eurotransplant and by the Acceptable Mismatch program (image reproduced from the online Eurotransplant database: https://statistics.eurotransplant.org)

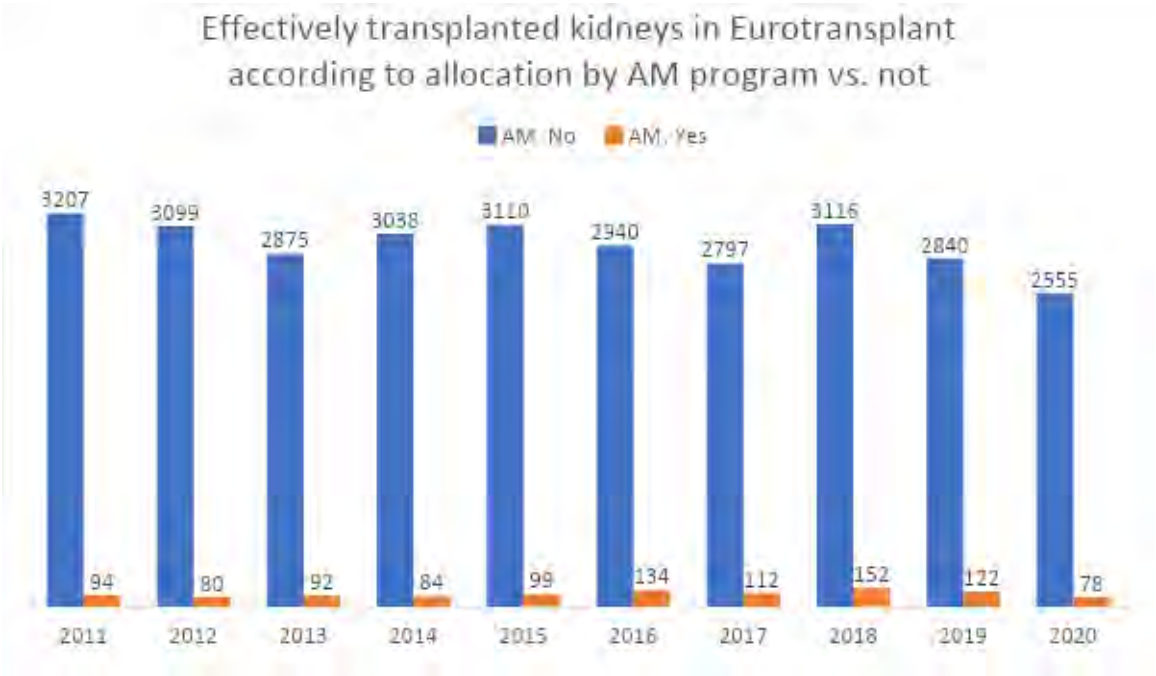
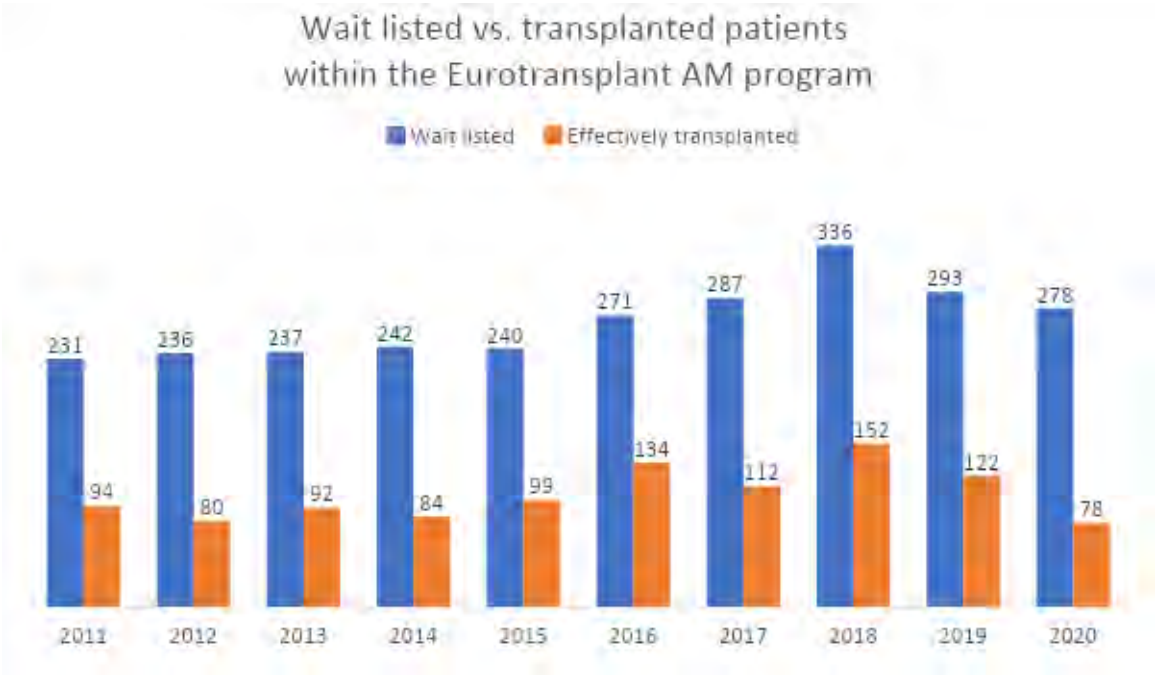


Figure 8: Relative number of kidney transplantations versus those remaining on the wait list within the Acceptable Mismatch program (image reproduced from the online Eurotransplant database: https://statistics.eurotransplant.org)



Recommendations

Standardization

- To define the humoral risk in kidney transplantation, the use of the ENGAGE 5 strata system is recommended

Organ allocation

- Increase access to the donor pool, through greater use of:
  - Sliding scale priority score schemes based on cPRA values (Chapters 1 and 3)
- Prioritisation policies should be linked across countries for equity of access
- Expand the Eurotransplant Acceptable Mismatch programme to other European countries to improve donor/recipient matching
- All kidney sharing schemes should develop calculators to help assess the probability of an organ match
- Therapeutic options should be reconsidered if there are no organ offers for a patient in a kidney sharing scheme

Desensitization

- The most efficacious desensitization strategy is to start with rounds of plasma exchanges/immunoadsorption together with B-cell immunomodulation with IVIG or B-cell depletion with anti-CD20 monoclonal antibodies to minimize post-transplantation DSA rebound

- As yet to be defined protocols including proteosome inhibitors and other anti-plasmocyte antibodies with co-stimulation blockade, B-cell immunomodulation targeting IL-6 as well as cleavage of IgG donor-specific antibodies with imlifidase are highly promising new strategies that deserve further investigation

Areas for further research

- Continue to work to develop schemes to help patients with very high cPRA who may not be transplanted in kidney paired donations or under deceased donor priority schemes
- A further need for evidence-based information is in the role of induction immunosuppression in relation to sensitization and its role in long-term graft and patient outcomes

- Better risk stratification, thorough immunological evaluation and avoidance of HLA-DSA should be used to improve outcomes after kidney transplantation

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Chapter 4: Desensitization strategies in kidney transplantation

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As the number of HLA-sensitized or even hyper-sensitized patients grows on transplant waiting lists, the need for desensitization strategies becomes ever more important. In this short review, first we describe the current treatments that are or have been used in order to

transplant these patients, and secondly, we examine the best ways to evaluate the results from using these strategies. It is currently possible to remove or block donor-specific antibodies, but the problem or rebound with a high incidence of antibody-mediated rejection is still unsolved.

Introduction

The percentage of patients registered on kidney transplant lists who are anti-HLA immunized or even hyper-immunized is currently increasing in the majority of transplant teams throughout the world [1]. For example, in our group (Necker Hospital, Paris, France), 62% of listed patients are anti-HLA immunized and 33% are hyper-immunized – both these groups of patients spend significantly more time waiting for a suitable kidney, and are therefore on dialysis until an organ is available and can be transplanted. Time on dialysis is a bad prognostic factor in the long-term, mainly because of cardiovascular complications. Therefore, there is a need to improve the transplantability of these patients – a fact especially true for patients waiting for retransplantations.

Strategies to improve patient transplantability

Strategy one is to wait for a well-matched donor kidney, but the anticipated price to pay is a long time spent on the waiting list!

Strategy two relies on acceptable mismatch programs [2], which look for a donor with or without low titers of donor-specific antibodies (DSA); the donor kidney can be coupled to kidney-paired exchange programs [3], or not. This strategy is providing excellent long-term results and increases the number of patients transplanted. However, it is not the solution for all patients.

Strategy three is desensitization and consists of interfering with DSAs either before transplantation (when a potential living donor has been identified) [4], immediately before transplantation in order to facilitate the crossmatch [5] or just after transplantation, in the case of transplantation with a kidney from a deceased donor [6]. This chapter will focus mainly on this third strategy.

Desensitization in kidney transplantation: What are the current options?

There are several ways to desensitize HLA-immunized patients, which involve utilizing specific drugs or monoclonal antibodies. Historically, polyvalent intravenous immunoglobulins (IVIgs) were used alone [7]. In a randomized trial [8], it was shown that IVIgs alone allowed more patients to be transplanted, but the overall benefit was still quite limited. IVIgs exert their effects by several complex modes of action, including modulation of antibody action, anti-complement effects and anti-cytokine effects [9].

It is relatively simple to decrease the global level of IVIgs through plasma exchange (PE) or by immune-adsorption - an equivalent method. Both methods have drawbacks including the frequent need for a central catheter (with the incumbent risk of infection this creates) and modification of coagulation factors, which increases the risk of bleeding. The number of PEs necessary to lower the IgG level is about five and the gain of increasing the number of PEs beyond that is small [4].

Rituximab, the anti-CD20 monoclonal antibody that is expressed on pre-plasmocyte precursors can be used to desensitize patients prior to transplantation. This drug aims to decrease the rebound effect linked to decreased levels of immunoglobulins in the plasma. Efficacy is monitored using the expression of CD19 on B cells.

Currently, the two methods used to desensitize patients are either a combination of anti-CD20 antibodies and high-dose IVIgs (2 g/kg over 2 to 4 days) [10], probably useful for immunized patients but less so for those highly sensitized, or a combination of 3 to 5 sessions of PE followed after each session by an infusion of low-dose IVIgs (0.1 g/kg) to avoid the rebound following a decreased level of circulating IVIgs [4]. New anti-CD20 monoclonal antibodies (such as ocrelizumab or obinutuzumab) may be more efficient, as well as anti-CD19 antibodies.

It is possible to decrease the synthesis of proteins (DSAs) using proteasome inhibitors such as the first-generation drug, bortezomib [11]. This drug was tested in a study with such a complex design (including the testing of many drugs as well as bortezomib) that it is difficult to clearly see its role in desensitization [12]! Second generation drugs such as carfilzomib or ixazomib may be more efficient. These drugs require administration of steroids at the same time, and their main problem is their neurological toxicity that might not be reversible [12].

A logical approach to desensitization is to block the activity of complement in order to decrease the effect of antibodies such as DSAs. The anti-C5 monoclonal antibody, eculizumab, was the first to be tested in this indication. In a non-randomized study using

eculizumab in addition to desensitization and historical controls, Stegall et al [13] showed a very significant decrease in the incidence of antibody-mediated rejection (ABMR) and transplant glomerulopathy (TG) on screening biopsies. Unfortunately, increasing the number of biopsies led to an equivalent incidence of TG in this study. A randomized study was designed for patients receiving a kidney from a living donor and comparing the use of eculizumab for 3 months post-transplantation with a control group who received desensitization [14]. Unfortunately, the results were rather disappointing or at least, difficult to interpret, with no significant difference found between the two groups. One explanation of these results is the difficulties in defining ABMR (with results varying depending on whether biopsies were graded by local or central pathologists) and probably more importantly, the use of anti-C5 in patients with DSAs not fixing the complement [15]. It is likely that this treatment could be efficient in certain circumstances, but it remains to be demonstrated. In contrast, in a study in patients being transplanted with an organ from a deceased donor, it was possible to get a low incidence of ABMR (around 10% during the first 3 months). However, there were no controls in this study, so the overall results are not clear-cut, but it remains a logical approach that may be used in selected groups of patients. Other complement blockers (such as a C1-inhibitor) are the subject of current clinical trials [16].

Another recent approach is the use of a cysteine protease (IG endopeptidase, Ides, Imlifidase and Idefirix®) that cleaves all IgGs, without regard to their specificity with an immediate action that lasts around 5 to 7 days; this drug cannot be re-used due to immunogenicity [17]. Cysteine protease has been used in hyper-immunized patients with good and safe results, and, apart from its impressive efficacy in facilitating crossmatches, it does not require a central IV line (**Figure 1**). On the other hand, it also cleaves rabbit IgG which complicates the choice of immunosuppressant, and leads to a rebound effect and ABMR with a frequency equivalent to other desensitization methods.

An additional desensitization strategy is the manipulation of the cytokines involved in B cells action. In this indication, tocilizumab, an anti-IL6 receptor monoclonal antibody has been giving promising results in a randomized trial, used in addition to current desensitization protocols [18]. Antibodies to anti-IL6 are also undergoing study for this indication. Belimumab, an anti-BAFF monoclonal antibody, might be a useful adjunct to standard care

immunosuppression in renal transplantation patients, as it shows no major increased risk of infection and potential beneficial effects on humoral alloimmunity [19].
Finally, it would be logical to use a monoclonal antibody against plasmocytes (such as daratumumab), which gives promising results in non-human primate models and in a few patients [20].

How to evaluate the efficacy of desensitization?

This is the most important issue, but not a simple one.
The main goal of desensitization is to allow patients who would otherwise not have been transplanted to receive a donor organ with an 'acceptable result'. In the literature, patient and graft survival have been used to show efficacy. For example, at the Johns Hopkins Hospital in Baltimore, USA, patient survival of desensitized recipients (transplanted with a living donor kidney) was statistically better than survival either in a group of non-listed patients receiving dialysis or in a group of listed patients, transplanted or not, but without desensitization [4]. This provides outcomes on the efficacy of desensitization as well as on the 'quality of dialysis', but this result may not be generalizable.
Using data from several transplant centers in the USA, the same group showed that there was a negative correlation between number of graft losses and immunological risk [21]. Graft survival at five years was highest in the reference group, then decreased in order to patients without DSAs, then to patients with DSAs but a negative flow crossmatch, to patients with DSAs but a positive flow crossmatch and finally to patients with DSAs and a positive CDC crossmatch.
More recently [22], it was shown that patients with a living donor and desensitization had a better graft outcome than patients either listed or not listed but in dialysis. These data outline very clearly that defining what is an 'acceptable transplantation' is very subjective and variable from one country to another.
The experience from the UK is very interesting [23]. Survival of sensitized patients undergoing HLA-incompatible transplantation is comparable with those on dialysis awaiting a compatible organ, many of which are unlikely to receive a transplant. Choosing a direct HLAi transplant has no detrimental effect on survival, but offers no survival benefit, which is in contrast with similar patients studied in a North American multicentre cohort [21].

In Seoul, Republic of Korea, the average waiting time for an HLA-compatible deceased donor kidney transplant (DDKT) is long, >5 years, which impacts the relative benefit of each transplant option. In a study of outcomes, significantly better patient survival was seen in those undergoing HLAi living-donor kidney transplant (LDKT) compared with those remaining on the waiting list and compared with those on the waiting list or who had received an HLA-compatible DDKT. In addition, the HLAi LDKT group survival benefit was seen at all strengths of donor-specific antibodies, suggesting that HLAi LDKT as a good option for sensitized patients with kidney failure in countries with prolonged waiting times for DDKT, such as the Republic of Korea [24].

Finally, in our group [6], patients with DSAs at the time of transplantation (mean of 9421 MFI) and desensitized not before, but after transplantation, exhibited a graft survival of 78% at 7 years, which is not very different from patients transplanted without DSAs. An increased incidence of infections was unfortunately the price to pay.

Overall, there is no doubt that transplanting patients with DSAs negatively impacts the results of transplantation and there is a necessary balance between the benefits of transplantation and complications, especially infectious ones.

The results of desensitization also rely on a careful analysis of DSAs (Luminex SA®, MFI, acceptable threshold, dilution test etc.), flow cytometry crossmatch (channel shift, positivity or negativity) and also CDC crossmatch. There is a correlation between DSA titers and histological lesions from normal biopsies to clinically active humoral rejection. In most studies about desensitization, the frequency of acute antibody-mediated rejection is still around 30 to 40% during the first year. The TG (cg in the Banff classification) lesion may also be a prognostic factor. Finally, renal function and proteinuria are also good prognostic factors; however, as there are not many randomized trials in this group of patients, there is still discussion about this.

Even though the Luminex® test to detect DSAs is only semi-quantitative, there are some correlations between MFI and clinical events, immunological risk and final graft survival. Also, the correlation between level of MFI and positivity of crossmatches, either cellular or flow-cytometric, is far from perfect (**Figure 2**).

Conclusion

Desensitization is an option that will need to be used more and more often in organ transplantation to produce 'acceptable' results. It will also have to be considered in allocation policies as they are updated, because the number of patients who are immunized and hyper-immunized is growing in most countries. Cocktails of medications will be necessary to manage desensitization efficiently with an 'acceptable' safety profile (**Figure 3**).

Figure 1: Efficacy of imlifidase cleaving IgG [17]

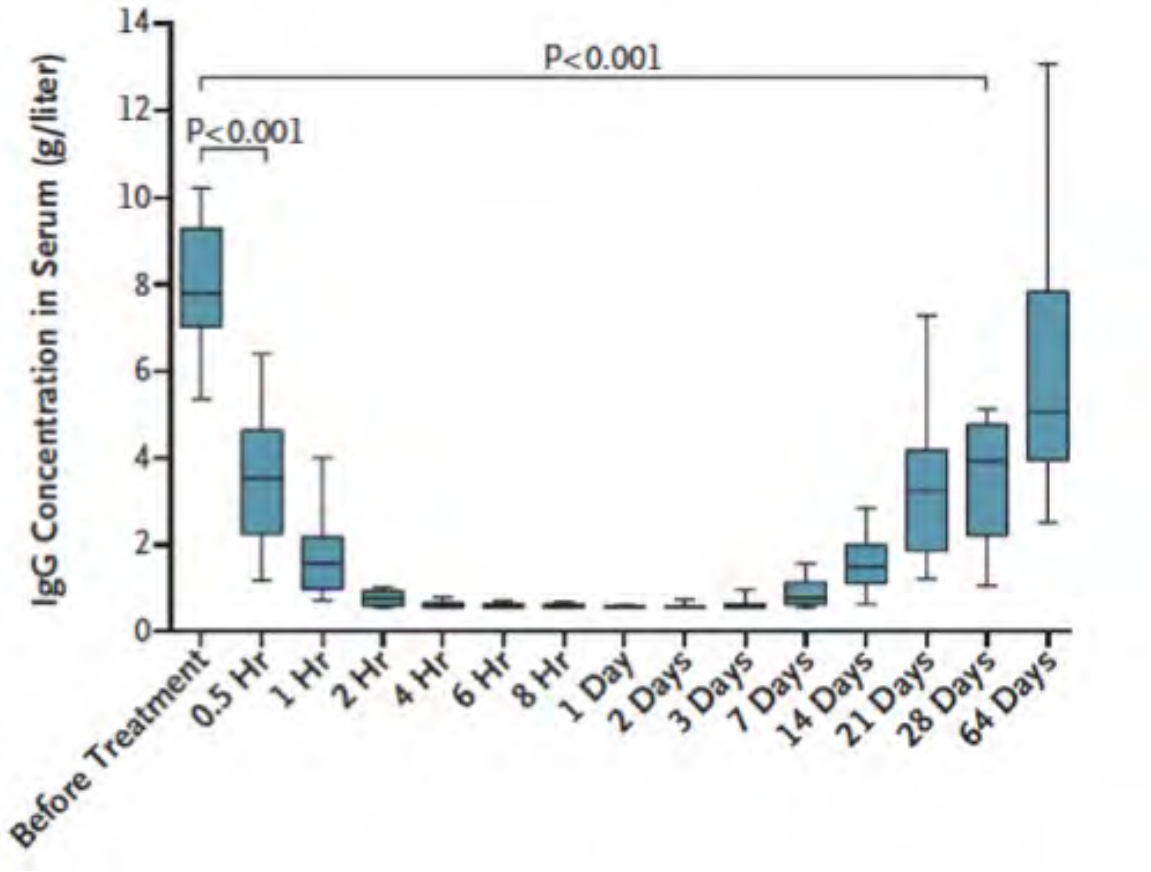


Figure 2: A schematic representation of the relationship between risk of rejection and the level of DSAs, measured by MFI. The positivity of either cytometry or CDC crossmatches does not occur at the same value of MFI, making interpretation of the immunological risk complex at the very least

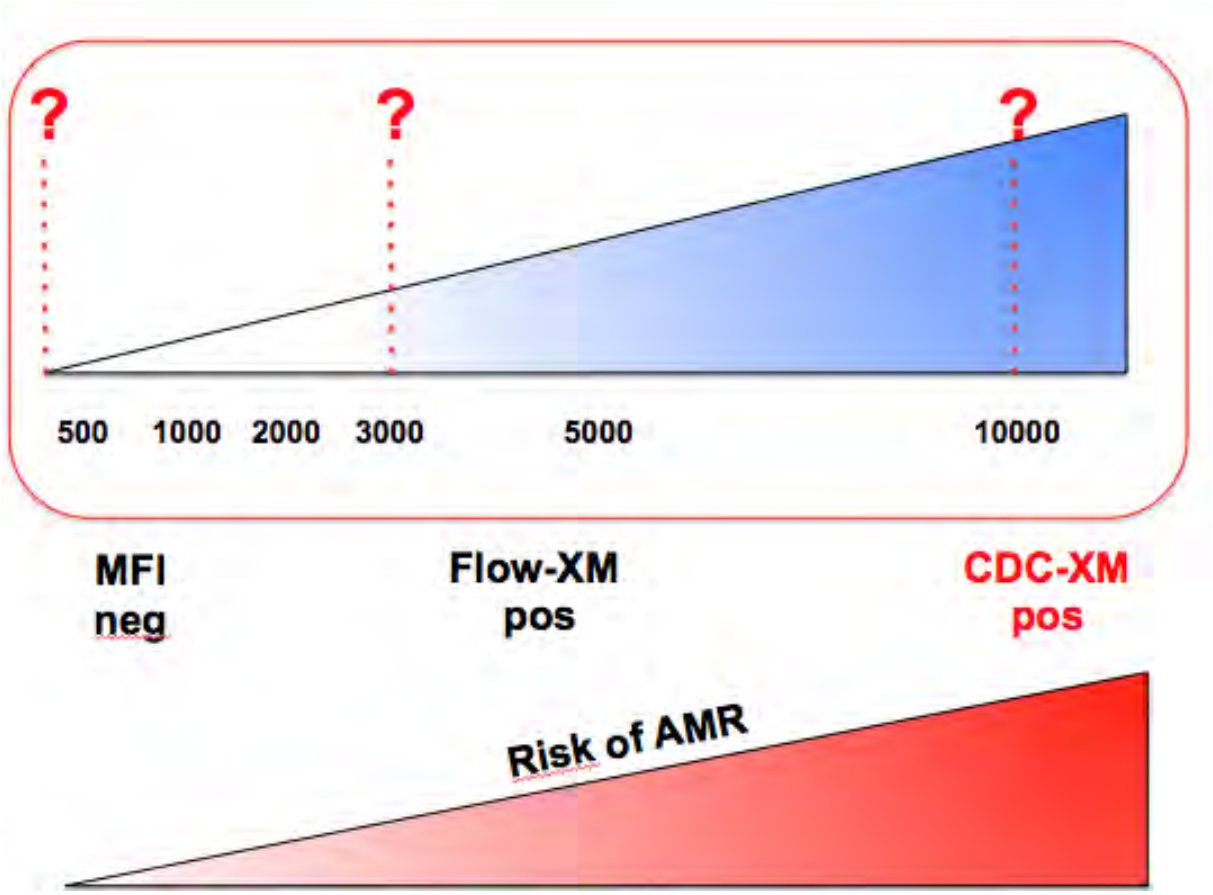
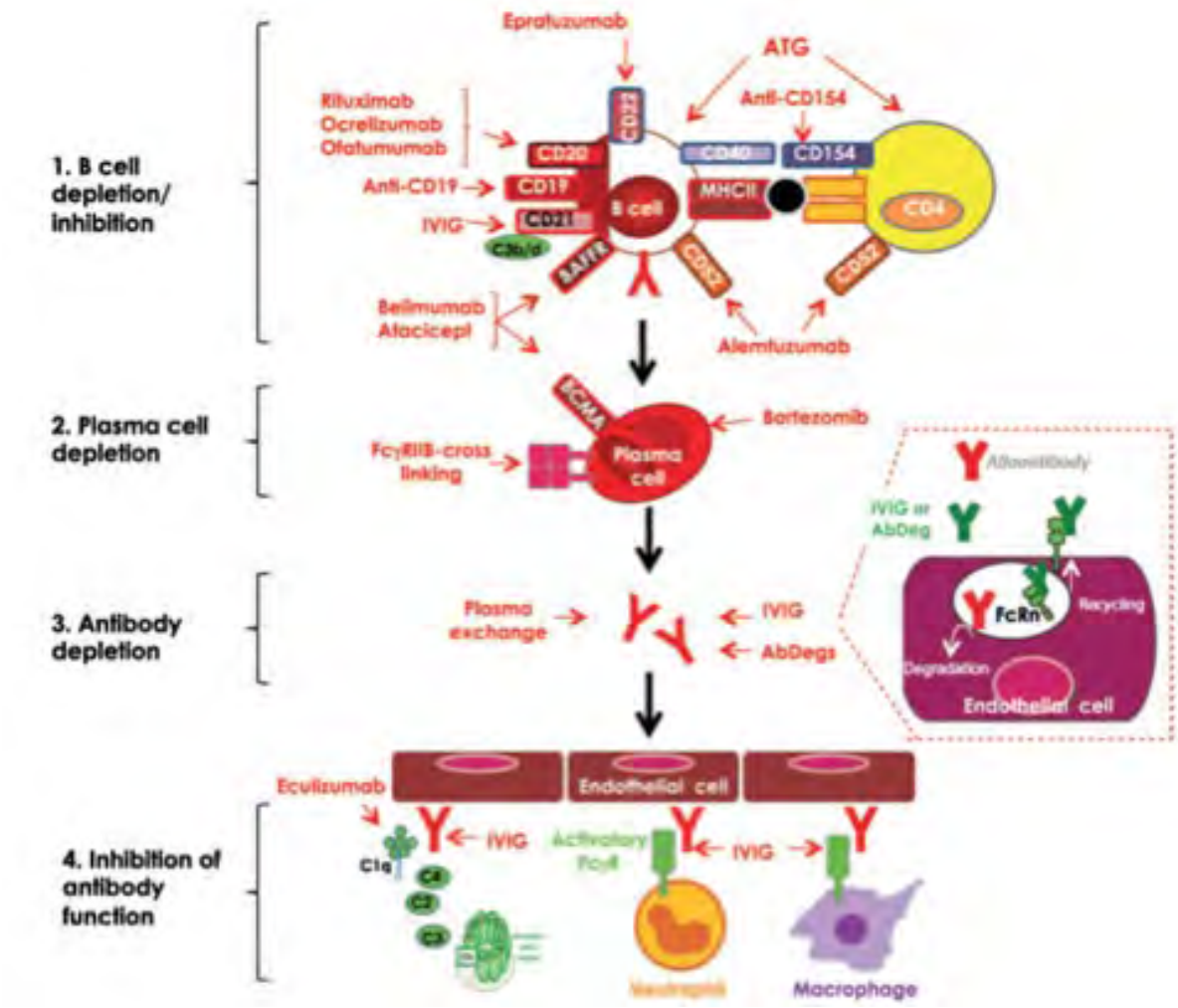


Figure 3: Representation of the mode of action of drugs and monoclonal antibodies interacting with B cells



Recommendations

Organ allocation

- We recommend all countries have an active policy of prioritising highly sensitised patients for organ transplantation

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Chapter 5: Outcomes after HLA incompatible transplantation

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HLA incompatible transplantation (HLAi) is defined as transplantation where the baseline CDC or flow cytometric cross-match is positive. Evidence regarding outcomes after HLAi is

limited. Two studies from the US have shown a clear survival advantage for those having HLAi, compared with matched patients who remain on the waiting list, but a large UK registry study found no survival advantage (or disadvantage). One US study found a lower rate of hospitalisation after three years when HLAi recipients were compared with those who remained on the waiting list (RR 0.74; 95% CI 0.66–0.84; p<0.001). We found no data comparing quality of life in these groups.

Although HLAi has become less popular in recent years, the data does not support the avoidance of this approach if the only alternative is the waiting list. Studies on quality of life after HLAi are urgently needed.

Introduction

There are two key issues which have proved problematic when considering outcomes after HLA incompatible transplantation (HLAi) - the definition of HLAi and the comparison group. Most would accept that those with a positive flow cytometric or CDC cross-match with their donor would fall into the category of HLAi. However, it is less certain whether those who have donor-specific antibodies (DSA) to their donor in solid phase assays, but a negative flow or CDC cross-match should be considered as HLAi. Such patients are considered separately below, but it is important to realise that many studies reporting outcomes after HLAi include these patients within their groups. For this chapter, HLAi is defined as patients who are cross-match positive. Published studies including DSA positive, cross-match negative patients, but where the data from these patients could not be separated from cross-match positive patents, have been excluded.

The second issue is a comparison group. Results from HLAi are often compared with those from compatible transplants, but the problem is that many HLAi patients will never have the option of a compatible transplant, as the chance for the most highly sensitized to receive a deceased donor kidney, or matching in a kidney sharing scheme, is essentially nil [1,2]. It is important, therefore, when considering outcomes, to also include patients who remain on dialysis and who are waiting for an organ offer as comparators.

The easiest outcome to consider is mortality, and although one would expect that this should be a straightforward outcome to determine, there are difficulties here. One problem is that of immortal time bias [3] (incidentally, a concept initially described in cardiac transplantation) - that is, if, for example, we consider those who have had an HLAi

transplant, they have, by definition, not died prior to the transplant, while those on the waiting list who weren't transplanted might have died. This tends to overestimate the benefit of transplantation.

Many will be aware that patients undergoing HLAi may experience serious perioperative morbidity, and this should be easy to capture, although may be poorly reported. It is much harder to capture morbidity whilst remaining on dialysis, and to compare it. For example, would a post-transplant wound infection be of comparable importance to a patient as an infected aneurysm in a fistula, requiring admission and treatment? Thus, one might expect underestimation of the benefit of transplantation.

Patients undergoing HLAi are usually subjected to more powerful immunosuppressants, and therefore will be at higher risk of infections. Anecdotally, serious or unusual infections are a feature of transplanting such patients, so data on infections is important.

Finally, many patients who have been on dialysis for many years due to sensitisation, invoke a poor quality of life as the reason for wanting an HLAi. They may be willing to accept increased risks due to the perception of significant benefit in their quality-of-life post-transplant, and therefore it is important to weigh this when considering outcomes.

This section will therefore consider the following:

i) A comparison of mortality rates between HLAi and those who remain without a transplant
ii) A comparison of morbidity between HLAi and those who remain without a transplant
iii) A comparison of quality of life between HLAi and those who remain without a transplant

Mortality

There are only three studies comparing mortality in those who have undergone HLAi with those who remain on the waiting list, and these are detailed in **Table 1**. The study by Montgomery [4] compared outcomes from a single centre with those in patients taken from the United Network for Organ Sharing (UNOS) database, matching them from the date of the transplant of the index patient in a 5 to 1 ratio. Matching criteria were well considered. There was a clear survival advantage for those who underwent HLAi compared with remaining on the waiting list, and this applied even when those who subsequently

underwent a compatible transplant were considered (the 8-year patient survival rate for this group was 49%).

However, it might have been possible that the survival benefit shown for HLAi was due to the approach in this (expert) centre, so in 2016, a study by Orandi [5] considered HLAi in 1025 patients from 22 centres in the US (these included 185 DSA positive, cross-match negative patients). The results were strikingly similar.

However, the results from these studies have been partly contradicted by a UK registry study, which found no difference in survival when comparing 213 HLAi patients with 852 well-matched controls who remained on the waiting list [2]. It is unclear why findings differ between the US and Europe, but one explanation may be a generally lower survival rate on dialysis in the US.

Nevertheless, no survival disadvantage for HLAi was found, suggesting that at the least HLAi may offer these patients a better quality of life, and at best, an improved quantity of life.

Table 1: Mortality in HLAi versus no transplantation

	Country	**Time (years)**	**Patient survival, %**		**p-value**
			HLAi transplant	**No transplant, but on waiting list**	
Montgomery, 2011 [4]	USA	8	**80.6%** n=211	**30.5%** n=1050	p<0.001[a]
Orandi, 2016 [5]	USA	8	**76.5%** n=1025	**43.9%** n=5125	p<0.001[a]
Manook, 2017 [2]	UK	7	**78.3%** n=213	**76.9%** n=852	p=NS[b]

NS, not significantly different
a, Kaplan Meier; b, Kaplan Meier and log rank test

Morbidity

There are no studies which compare morbidity in those undergoing HLAi with those who remain on the waiting list. This is an important gap in our knowledge, particularly given the statements above regarding survival. There is one study by Orandi [6], which compared hospital readmissions in 379 HLAi transplants with matched controls who remained on the waiting list, using registry data from the US. Those who underwent HLAi, unsurprisingly, had a higher readmission rate in the first month (RR 5.86; 95% CI 4.96–6.92; $p<0.001$), but interestingly, had lower rates of hospitalisation subsequently (at 3 years: RR 0.74; 95% CI 0.66–0.84; $p<0.001$). The data on reasons for admission do not permit detailed comparisons of morbidity, but the implication is that after an expected increased perioperative risk, those who undergo HLAi suffer fewer complications in the long term than those who remain on the waiting list.

A report by Kim [7] compared 56 HLAi (positive T cell flow cytometric cross-matches were excluded) with 274 compatible transplants, providing data on infectious complications, which may help in considering the risk. Urinary tract infections (41% vs 7.7%), cytomegalovirus (CMV) viraemia (54% vs 14%) and pneumocystis jiroveci pneumonia (PJP) (5% vs 0%) were all significantly higher in the HLAi group ($p<0.001$). However, perhaps with the exception of PJP, it is difficult to be clear about the severity of these complications, and hence the cost to the patient. Another study which compared 27 HLAi patients with 69 ABOi patients, found no significant difference in viral, bacterial or fungal infections between the two groups, although of note, 6% of the ABOi group had PJP, compared with none of the HLAi group [8].

Quality of Life

We were unable to find any studies which compared quality of life in those undergoing HLAi, with that of remaining on the waiting list and hoping for a compatible transplant. This is clearly a major gap in our knowledge, since, given the statements regarding mortality above, will be the prime determinant for the most appropriate choice for patients.

New agents

There have been several recent studies in HLAi using new agents for desensitization. Two studies considering the use of rituximab to allow deceased donor HLAi were stopped early

due to unacceptable rejection rates. The first found higher rejection rates in those given rituximab compared with an IL2 inhibitor [9], whilst the second study found higher rejection in those given IviG and placebo, compared with those receiving IviG and rituximab [10].
One small Phase I/II study considered the use of C1-INH for HLAi, and found comparable results with standard care [11].
Two large studies evaluating the use of eculizumab have been published, showing good efficacy in allowing highly sensitized patients to undergo deceased donor transplantation, [12], but no clear benefit in living donation transplants [13], although this is debatable.
A recent report of the use of imlifidase in a Phase II study of 18 HLAi patients showed promising results, with 88.9% graft survival at 6 months – nevertheless, the rate of antibody-mediated rejection appeared to remain high [14].

Summary

We have found no evidence of increased mortality after HLAi compared with remaining on the waiting list, and, in the US, HLAi conferred a survival advantage.
There are few data concerning morbidity after HLAi in comparison with remaining on the waiting list, but there is some evidence that after the initial perioperative period, subsequent morbidity is lower.
There are no data on the quality of life after HLAi compared with remaining on the waiting list.

Recommendations

Areas for further research

We recommend that data be collected prospectively for sensitised patients, in order to compare the effect of an HLA incompatible transplant with remaining on the waiting list.
This data should include:

- Mortality
- Morbidity
- Quality of Life (Level 1C) (Chapters 2 and 5)

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Chapter 6: The place of kidney paired donation for sensitized patients

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The current situation with kidney paired donations

Kidney Paired Donation (KPD) is a promising innovation in kidney transplantation, consisting of a considerable range of strategies developed for patients with a willing, but immunologically incompatible donor. One third of all potential live kidney donors are not suitable to donate a kidney to their intended recipients, due to human leukocyte antigen (HLA) or ABO blood group incompatibility. Incompatible living donor kidney transplantation

(LDKT) is feasible using various desensitization protocols, but their outcomes are inferior compared with those from transplants with compatible living donors. Higher infection risk, major incidence of acute rejection and the need for stronger immunosuppression may compromise long-term graft outcome. There are also increased monetary costs involved and an increased length of hospitalization due to desensitization.

Rapaport first proposed the concept of KPD in 1986 [1], but it was first performed later, in 1991, in South Korea [2]. This first KPD consisted of a simple, two-way swap between two incompatible pairs in a single transplant center. Over the years, KPD has shown encouraging results and is popular worldwide. Further expansion of KPD has led to the development of more complex systems and innovative solutions in order to maximize the number of exchanges.

The simplest form of KPD is a **two-way exchange** involving two incompatible pairs who swap their donors to achieve a compatible transplant for both recipients.

The **closed loop** between three or more incompatible pairs whose recipients find a compatible kidney by exchanging their donors, represents another basic form of KPD. It consists of multiple surgeries (nephrectomies and transplants) that should be performed simultaneously allowing each pair to benefit from the swaps at the same time and preventing the risk of donor reneging.

These two forms of KPD work efficiently for pairs with blood type A/B incompatibility or for less immunized recipients. Unfortunately, for highly sensitized patients with a wide range of anti-HLA antibodies or for blood type O recipients, it is very hard to find a compatible match for each pair involved in a closed loop.

The option of a non-directed altruistic donor (**NDAD**) who is willing to donate his/her kidney with no intended recipient, is a real solution to the problem of reciprocal matching and avoids the need to 'close the loop'. The NDAD's kidney is matched with the recipient of an incompatible pair whose living donor donates to another incompatible recipient, initiating a **domino-paired kidney exchange**. The chain ends with the donor of the last pair donating to a recipient on the waiting list (WL) or waiting for another suitable match, starting another sequence of paired donations later (non-simultaneous extended altruistic donor chain, **NEAD**), thus becoming a bridge donor. This model of KPD intrinsically includes non-

simultaneous surgeries and it is associated with an incremented risk of donor reneging. Donors might decide not to donate once their intended recipients have been transplanted. This risk increases significantly for bridge donors, who usually have to wait a while before donating.

In 2016, Melcher, et al [3] proposed that a deceased donor organ should start a chain of living donor kidney transplants among incompatible pairs, but the first report of a successful deceased donor-initiated chain was published by Furian, et al in 2019 [4]. In the **DECeased donor kidney paired exchange (DEC-K)** program, the chain-initiating kidney, selected from the deceased donor pool, is allocated to a recipient with an incompatible living donor and, at the end of the domino-chain, the living donor of the last pair donates to a WL patient. Recipients of incompatible pairs are given priority in the allocation of a chain-initiating kidney from a deceased donor only in the absence of urgent, highly sensitized patients or candidates for combined transplants, according to the Italian policy for graft allocation (please see: http://www.trapianti.salute.gov.it/imgs/C_17_cntPubblicazioni_344_allegato.pdf). The program requires appropriate management of the ethical, allocation and logistic issues brought up by the very nature of the exchanges, but it is feasible. The major advantage of the DEC-K program is the ability to offer an opportunity for transplantation to recipients of incompatible donor pairs, but it also benefits WL candidates by allocating chain-ending kidneys from a living donor to them, prioritizing sensitized patients and those who have waited a long time for immunological reasons.

List exchange is another form of KPD, proposed by Delmonico et al, to prevent the issue of donor reneging [5]. In this scheme, the donor of the incompatible pair donates before the recipient has received their compatible transplant from the deceased donor pool but, after donation, the paired recipient acquires priority over the WL candidates.

Other novel schemes of KPD take place in the setting of “chronological incompatibility” and constitute the **advanced donation programs (ADPs)** where a living donor donates his/her kidney at his/her convenience to a recipient of an incompatible pair in need of transplant while his/her intended recipient received the reciprocal compatible kidney later on.

A modification of ADP is the **voucher system**. A living donor donates their kidney as a NDAD, starting a domino chain or donates directly to a candidate on the WL, while the intended

recipient gets a voucher for the future. When the recipient is in need of a transplant, he/she will have priority for graft allocation on the deceased donor WL. This model seems to work efficiently in the case of pairs whose donor might become too old to donate by the time the recipient really needs transplantation.

A strong correlation between the number of pairs enrolled in a kidney exchange program and the success of matching more possible pairs has been widely demonstrated. That said, **participation of ABO and HLA compatible pairs** appears to be a brilliant strategic move for further expanding the pool and, therefore, the number of successful matches. This model provides undeniable benefits for recipients of incompatible pairs, whereas advantages for compatible pairs seem more questionable. Receiving a kidney from a younger donor, with negative serology for cytomegalovirus (CMV) or Epstein-Barr virus (EBV) or getting a better HLA match or weight match might represent an appealing gain for recipients of compatible pairs, encouraging their participation in exchange programs.

Although KPD was conceived as an alternative for incompatible pairs to avoid their recipients undergoing expensive and risky desensitization protocols, another strategy to improve KPD results is **combining exchange programs with desensitization**. Given the excellent results extensively published in the literature regarding ABO incompatible transplantation in the absence of donor specific antibodies (DSA), it could be a good strategy to accept ABO incompatible living donors against whom recipients have lower anti-blood group antibody titers, in the setting of KPD. This strategy has been successfully applied in the Australian program and by Montgomery, at the John Hopkins Institute [6].

Trans-organ paired exchange represents the most innovative concept of KPD. It can be helpful in circumstances when a donor is not suitable for donating a kidney but is still fit to donate other organs for exchange. For example, a living kidney donor who is not eligible for renal donation but can donate his/her liver to a liver recipient of a pair whose donor is ruled out from liver donation but is suitable for kidney donation. Torres, et al published the first case of trans-organ exchange, attracting many criticisms related to the surgical risk of donation that is very different for different organs [7].

Desensitization versus KPD: outcome in ABO and HLA incompatible patients

KPD and desensitization have traditionally been considered competing strategies to solve the immunological incompatibility that can mitigate living donation. Published literature assists physicians in making decisions regarding the choice of KPD over desensitization or vice versa, when encountering an incompatible pair for LDKT. Risks and benefits relating to the possible strategies - KPD or desensitization - should be outlined and discussed with patients.

Outcomes of ABO incompatible (ABOi) transplantation after desensitization have proved to be excellent over the years [8–10], but have highlighted the fact that blood type O recipients have low match rates and long wait list times [11–13]. There is also a lack of blood type O donors - with blood type O being predominant among recipients but underrepresented in the domino-donor population [14,15]. Blood type O donors are always blood group compatible with their intended recipients, so the only reason why they would join a KPD program is the presence of unacceptable DSA in the recipient, causing a positive cross-match.

With graft outcomes following ABOi LDKT being comparable with ABO compatible LDKT [16,17] probably the most convenient option for a blood type O recipient who has an ABOi living donor is desensitization, especially if they present with an acceptable baseline antibody titer and are more likely to be desensitized. For these patients, KPD should be offered only when desensitization is unsuccessful or in the case of very high antibody titer, which may require aggressive immunosuppressive therapy and intensive desensitization protocols, thus increasing the risk-benefit ratio.

Things are very different for HLA incompatible (HLAi) pairs. Desensitization protocols have been applied in cases of sensitized recipients who have a willing, but incompatible living donor due to the presence of DSA [6,18] Certainly, HLAi LDKT after desensitization provided a significant survival benefit for these patients, compared with remaining on dialysis. Montgomery, et al [6] demonstrated in a cohort of 211 HLA-sensitized patients who were desensitized and subsequently transplanted thanks to their incompatible donor, that patient survival was 90.6% at 1 year, 85.7% at 3 years, 80.6% at 5 years, and 80.6% at 8 years, as compared with rates of 91.1%, 67.2%, 51.5%, and 30.5%, respectively, for patients in the dialysis group. However, poor outcomes have been reported after HLAi LDKT

compared with HLA compatible LDKT. A 1.64-fold and 5.01-fold increased risk of graft loss at 1-year for recipients with a positive flow cytometric crossmatch and positive cytotoxic crossmatch, respectively, is reported in the literature [19]. This may be a consequence of increased post-operative complications, including delayed graft function (DGF) and acute rejection [20–22] given the potential risk of post-desensitization rebound of DSA. Moreover, need for intensive immunomodulation (infusion of intravenous immunoglobulin, anti-B-cell agents, other agents such as eculizumab, bortezomib, a C1 esterase inhibitor, sessions of plasmapheresis or rescue splenectomy), in addition to post-transplant immunosuppressive medications exposes these patients to a serious risk of infection [23].

In 2005, Segev, et al proved, by a simulation based on UNOS data, the superiority of KPD over desensitization, guaranteeing better graft outcomes and higher transplantation rates for HLAi pairs [24]. Interestingly, 47% of the HLAi pairs could have been matched through an optimized national KPD program. The authors clearly stated that KPD should be the preferred treatment for patients who have HLA incompatibilities with their willing donors, as it is less expensive compared with desensitization and requires less immunosuppression [24].

Bingaman, et al identified three disadvantaged patient categories who would particularly benefit from KPD programs: highly sensitized recipients, multiparous females and retransplant patients [25]. The KPD program improved their access to transplantation and offered them excellent graft outcomes and low rejection rates.

However, despite the implementation of KPD strategies, in the United States, patients with a PRA of 99.9% remain the most disadvantaged transplant candidates with prolonged waiting times and high waiting list mortality [26]. This is why some transplantation centers still promote desensitization as a valid and needed approach to increase the probability of transplantation in highly sensitized patients [27]. Others have proposed KPD only in cases of failed desensitization procedures, as a kind of “rescue” therapy [28].

To resolve the dilemma of whether to use a desensitization protocol versus KPD for incompatible pairs, an Italian group proposed a decisional algorithm including and integrating both strategies in a unique flowchart [29]. They analyzed the outcomes of 54 patients transplanted at Pisa Transplantation Centre, between 2005 and 2017, applying KPD

or desensitization therapy. Results achieved with KPD versus those achieved with desensitization for the main groups of incompatibility (ABO and HLA) were compared. No significant differences among the groups were recorded in terms of patient and graft survival. However, DSA+ desensitized patients proved to be more prone to produce de-novo DSA after LDKT and when the DSA titer was high (>3000 mean fluorescence intensity, MFI), recipients showed a higher risk of acute rejection (50% vs 14%). Furthermore, desensitization strategies were more expensive, with a cost equal to 3 months of dialysis. The authors concluded that for HLAi couples, a KPD strategy should always be preferred. For ABOi pairs, desensitization protocols or KPD offer comparable results, differing only by cost, but KPD requires a 3-month prolongation of dialysis while waiting for a compatible match.

Strategies to expand the KPD pool

KPD match rates are dependent on the number of incompatible pairs enrolled in a program [24, 30]; hence, expanding the pool size is critical to the implementation of KPD. ABOi pairs represent an important source for the overall KPD pool; if the number of ABOi registered pairs is lower than those with HLA incompatibility, match probability decreases [31]. Furthermore, a KPD pool with several ABOi pairs would potentially offer the opportunity of a higher match probability by accepting ABO incompatibilities in the exchanges. The Australian KPD program has applied this strategy since 2013 [32], showing an enhancement in transplant rate for all KPD enrolled patients. Ferrari, et al confirmed the same result later with 17 out of 92 transplanted patients from the Australian registry receiving a kidney from an ABOi matched donor. These recipients were distributed across 15 domino chains, which could only have been realized if ABOi matching was accepted [33].

The integration of desensitization protocols in KPD programs can also consist of allowing low-risk crossmatch-incompatible kidney transplantation in highly-sensitized patients, in the setting of paired donation. This is another promising approach, strongly endorsed in the literature [34,35], and developed to improve KPD efficiency by increasing the transplant rate of highly sensitized patients and reducing their wait time for a LDKT. In 2013, Blumberg, et al proposed a protocol including acceptable crossmatch-incompatible donors and the administration of intravenous immunoglobulin to transplant 12 HLA-sensitized patients

(median calculated PRA: 98%) with allografts from the KPD program. In the Californian experience, KPD was successfully performed across crossmatch-incompatible transplants, representing another viable chance of an organ for very sensitized recipients [36]. A more complex model of integration is represented by CIAT (Computerized Integration of Alternative Transplantation Programs), recently developed in The Netherlands to integrate paired exchange, altruistic donation and ABO/HLA-desensitization [37]. To compare CIAT with reality, a simulation was performed on data from the Dutch Living Donor Kidney Program and included difficult-to-match and highly immunized patients (virtual PRA >85%). HLAi matching with DSA-MFI <8000 was allowed, as well as ABOi matched for long-waiting blood group O or B patients. Compared with reality, the simulation results showed that CIAT would have led to better transplant opportunities for difficult-to-match patients and highly sensitized patients, and more ABO compatible matches, without compromising the total number of matches.

Another concept to increase KPD pair numbers and, accordingly, the number of successful matches within the same pool, is the Unbalanced Paired Donation, consisting of the inclusion of ABO/HLA compatible pairs in KPD programs [38,39]. This strategy was initially proposed as an attempt to facilitate transplantation for patients in the most disadvantageous categories in a KPD program: blood group O recipients and those who are highly sensitized [40–42]; and a mathematical analysis conducted in 2007 found that including compatible pairs (CC) in a KPD program would correct the blood group imbalance that usually characterizes a pool of ABOi/HLAi pairs [43]. In fact, all recipients of incompatible pairs benefitted: their chance of receiving a kidney from a compatible donor doubled from 28% to 65% for a single-center program and from 37% to 75% for a national one. Looking at real data from the analysis of the first 9 years of the National Kidney Register, the participation of CC facilitated 146 transplants, including 43 recipients with PRA>80% [44].

Overall, if there is a striking gain for incompatible pairs, CCs participating in a KPD program are disadvantaged, waiting for a match and postponing the transplant surgery that would have been otherwise performed. The altruism behind their participation should be balanced by giving them a potential benefit. From published data, the main reasons why CCs join a

KPD program are size/age mismatch, CMV or EBV serology mismatch between donor and intended recipient, the opportunity to receive a better HLA match, avoiding complex donor kidney anatomy or pure altruism [44–46].

Successful single-center experiences of KPD including CC, have been able to provide recipients of CC with kidneys from younger donors [25] or kidneys with a better Living Donor Profile Index score (LKDPI), as a predictor of long-term graft survival [45]. This "gain" of receiving a "better matched kidney" for a CC entering KPD programs is considered, by some authors, to be risky, as it might require time to find a better match, thus delaying transplantation, and the estimated quality of the graft might misrepresent the real outcome of the transplant. To answer these points, Gill, et al introduced the concept of the "reciprocity-based strategy" in which the recipient of a CC acquires priority for a repeat deceased donor transplant in case the LDKT fails [47]. The authors highlight how this strategy would be embraced more willingly by CC, since it guarantees them a significant and concrete benefit. Despite the ethical concerns about the inclusion of CC in a KPD program, the **Unbalanced Paired Donation**, by expanding the pool, results in increasing the overall number of LDKTs and facilitating access to transplantation for the most sensitized candidates enrolled in the program.

The last way to widen the number of patients enrolled in a KPD program, is the creation of transnational registers. Indeed, the inclusion of international pairs offers a higher probability of finding a compatible donor, especially for difficult-to-match recipients who have less opportunities to be transplanted within a national or local program. Some authors hypothesized that differences in HLA antigen prevalence across different ethnicities may play an important role in KPD matching [48]. Hence, sensitized recipients presenting with DSA against several donors available in their national pool, may find a compatible match more easily among donors of another race or from other countries.

National KPD programs may differ on ethical viewpoints, legal and financial frameworks, clinical practices and population size depending on the country. Whenever a collaboration is established for a transnational KPD, the different regional models need to merge together and reach a compromise that suits all the cultures involved in the cooperation for it to be successful. The goal is for the collaboration to appropriately benefit all populations, recipients and donors involved [49].

Transnational kidney exchanges have been successfully realized across Europe and USA. The international cooperation between Italy, Spain and Portugal has led to 2 two-way exchanges [50], the Czech-Austrian KPD program, involving patients from Austria, Germany, Slovenia and Ukraine facilitated 81 transplants [51] while 38 LDKTs were performed through 6 chains and 2 cycles between 30 US patients and 8 non-US patients, of whom 11 presented with a PRA>80% [52]. At the Mayo Clinic, in 10-years of KPD, a small group of pairs were from outside of the US [45]. Their participation enabled highly sensitized patients to receive a compatible transplant because 75% of chains/swaps included an international pair, and also a recipient with a PRA of at least 90%.
These results demonstrate the feasibility of merging small national KPD programs to increase the pool size and encourage the development of international registries to optimize the KPD resource.

Logistical issues of KPD programs

A KPD program, before starting, requires an extensive assessment of all logistic, legal and ethical issues, including concerns regarding increased times of cold ischemia (CIT) and the risk of donor renege, which might affect the outcomes of the program.

When a kidney exchange involves two or more different transplant centers, shipping the organs rather than asking donors to travel to the recipient center seems to be the favored choice. Usually, both donors and recipients feel much more comfortable undergoing surgery at their trusted transplant centre. However, a shipped kidney implies a longer CIT compared with standard LDKT, and a possible increase in the probability of graft failure has been historically a cause for concern. In a 2020 study, conducted on 10-years of transplant activity in the National Kidney Register, extended CIT proved not to be predictive of DGF or graft outcome [53].

The Italian DEC-K experience confirms that shipping kidneys is safe and does not affect graft or patient survival even when the graft comes from a deceased donor, as in the case of a chain initiating kidney, which requires time for organ procurement as well as travel time (mean CIT 7 hours) [14]. It has to be mentioned that distances in the Italian territory are generally conducive for short CIT. However, even when shipped distances increase and CITs

increase accordingly, as in the US [54,55] or Australia [56] or in transnational KPD programs [50,57], no association between extended CIT and DGF or graft loss were found.

Certainly, a KPD program limited to a single center would succeed in keeping CIT as short as possible but it would only realize a very small number of transplants, given the availability of a small pool size. Pushing the boundaries for acceptable prolonged CIT has helped to expand and optimize KPD by making the utilization of kidneys originating from distant transplant centers possible.

The other major topic when discussing KPD, is the risk of a donor reneging, and the probability of this may vary according to the type of paired donation. Performing surgical procedures simultaneously within a closed loop is logistically difficult and requires a great deal of careful coordination, but it does minimize the risk of donors reneging. The complexity grows proportionately with the number of pairs involved in the loop since all donors should undergo nephrectomy concurrently, ensuring that no donors withdraw after their paired recipient received their kidney transplant. List exchange and Advanced Donation Programs likewise prevent the issue of donor renege, but also require a bigger contribution from donors who have to donate before their intended recipient gets a compatible transplant.

The risk of donor renege is much higher in domino-paired donation. Unavoidably, surgeries cannot be simultaneous within a chain and a donor withdrawal would cause a premature break in the chain, leaving a recipient orphaned, despite the fact that his/her paired donor has already donated. This risk increases when donors wait a long time before donating [58], as in the case of bridge donors or when it takes too much time to schedule a continuous chain. Loss of donor motivation or changes in the donor's state of health might occur, affecting the success of the entire KPD program and reducing interest in pursuing this option for kidney transplantation. In addition to a deep psychological assessment, donors and recipients should go through an educational process to fully comprehend the principles and functioning of domino kidney paired exchange before giving their consent to participate in the program. As highlighted by Furian, et al in the first report of the DEC-K experience, this is essential to prevent the risk of donor renege [4]. Other strategies to avoid premature ending of kidney chains are scheduling donor nephrectomy soon after the paired recipient

has received their transplant and, as suggested by the Dutch experience, to construct short length chains [59].

Recommendations
Organ allocation

- Increase access to the donor pool, through greater use of:
  - Increased access to and harmonisation of Kidney Exchange Programmes, with greater and standardised sharing of outcomes (Chapters 2 and 6)
  - If a particular country does allow unspecified kidney donations, consider including these in kidney sharing schemes (Chapters 2 and 6)
  - Kidney paired donations should have an option to include compatible pairs and deceased donor organs
- Kidney Paired Donation is the preferred initial option over desensitization given the better transplant outcomes and cost-effectiveness, in both ABO and HLA incompatible pairs, unless there is a need for desensitization, there is clinical urgency or a low chance of a transplant

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Appendix Bibliographic searches

Chapter 1 Definition of sensitization

Clinical question (1)

In renal transplantation what is the effect of the number of eplet mismatches (eplet load) on the induction of donor specific antibodies (DSA) after transplantation.

Search strategy and results

- The Transplant Library (www.transplantlibrary.com) was searched from inception to January 11, 2021 using the search strategy below:

1. (eplet or epitope).ti,ab.
2. Epitopes/
3. HLAMatchmaker.ti,ab.
4. or/1-3
5. ("donor specific antibod$" or DSA).ti,ab.
6. 4 AND 5
7. Limit 6 to kidney transplant

This search identified 4 potentially relevant references.

- MEDLINE and EMBASE were searched from inception to January 11, 2021. In order to create a manageable search set, the search criteria above were refined to:

1. (eplet or epitope).ti,ab.
2. Epitopes/
3. HLAMatchmaker.ti,ab.
4. or/1-3
5. ("donor specific antibod$" or DSA).ti,ab.
6. 4 AND 5
7. KIDNEY TRANSPLANTATION/
8. ((kidney or renal) adj5 (graft$ or allograft$ or transplant$)).ti,ab.
9. Organ transplantation/
10. or/6-8
11. 6 and 10

This search yielded 387 potentially relevant references.

Clinical question (2)

In renal transplantation what is the clinical relevance of donor specific HLA antibodies present before transplantation, detected by solid phase assays only.

Search strategy and results

- The Transplant Library (www.transplantlibrary.com) was searched from inception to January 8, 2021 using the search strategy below:

1. ("donor specific antibod$" or DSA).ti,ab.
2. Limit 1 to kidney transplant

Searches identified 78 potentially relevant references.

- The last systematic review has a search date to 2016. Searches were therefore expanded to include non-randomised studies from 2016 to 8th January 2021 for completeness. We searched MEDLINE and EMBASE from 01/01/2016 to 08/01/2021. In order to create a manageable search set, the search criteria above were refined to:

1. (Luminex or "solid phase").ti,ab.
2. ("donor specific antibod$" or DSA),ti,ab
3. 1 and 2
4. (pretransplant$ or pre-transplant$),ti,ab.
5. 3 and 4
6. KIDNEY TRANSPLANTATION/
7. ((kidney or renal) adj5 (graft$ or allograft$ or transplant$)).ti,ab.
8. Organ transplantation/
9. or/6-8
10. 5 and 9
11. Limit 9 to yr="2016-current"

This search yielded 216 potentially relevant references.

Chapter 2 Comparison of practices across Europe

Clinical questions

1. What are the different approaches to blood group incompatible transplantation in Europe?

2. What are the different approaches to HLA antibody incompatible transplantation in Europe?

3. Which countries in Europe use desensitisation for antibody incompatible living or deceased donor transplantation?

4. Which countries in Europe have living donor sharing schemes?

5. What desensitisation techniques are used in Europe?

Search strategy and results

- The Transplant Library (www.transplantlibrary.com) was searched from inception to January 11, 2021 using the search strategy below:

1. Blood Group Incompatibility/
2. ABO incompatib$.mp.
3. ABOi.ti,ab.
4. HLA incompatib$.mp.

5. incompatible pair$.mp.
6. incompatible don$.mp.
7. Desensitization, Immunologic/
8. (desensitiz$ or desensitis$).ti,ab.
9. kidney chain.mp.
10. kidney exchange.mp.
11. kidney sharing scheme.mp.
12. paired exchange.mp.
13. paired don$.mp.
14. domino chain.mp.
15. sharing registry.mp.
16. or/1-15

Searches identified 48 potentially relevant references.

- Bibliographic searches were expanded to include non-randomised studies. We searched MEDLINE and EMBASE on January 11, 2021 using the search strategy below.

1. Blood Group Incompatibility/
2. ABO incompatib$.mp.
3. ABOi.ti,ab.
4. HLA incompatib$.mp.
5. incompatible pair$.mp.
6. incompatible don$.mp.
7. or/1-6
8. Desensitization, Immunologic/
9. (desensitiz$ or desensitis$).ti,ab.
10. kidney chain.mp.
11. kidney exchange.mp.
12. kidney sharing scheme.mp.
13. paired exchange.mp.
14. paired don$.mp.
15. domino chain.mp.
16. sharing registry.mp.
17. or/8-16
18. 7 and 17
19. organ transplantation/
20. kidney transplantation/
21. pancreas transplantation/
22. lung transplantation/
23. heart transplantation/
24. liver transplantation/
25. (pancreas$ transplant$ and kidney$ transplant$).tw.
26. simultaneous pancreas kidney transplant$.tw.
27. spk.tw.
28. lung transplant$.tw.
29. heart transplant$.tw.
30. liver transplant$.tw.
31. solid organ transplant$.tw.
32. kidney transplant$.tw.

33. pancreas transplant$.tw.
34. or/19-33
35. 18 and 34
36. remove duplicates from 35

This search yielded 1043 potentially relevant references.

A separate search was in MEDLINE and EMBASE conducted for Question 2 on January 11, 2021
1. HLA incompatib$.ti,ab.
2. anti-HLA.ti,ab.
3. Desensitization, Immunologic/mt [Methods]
4. (desensitiz$ or desensitis$).ti,ab.
5. transplant$.ti,ab.
6. 1 or 2
7. 3 or 4
8. 5 and 6 and 7
9. limit 8 to yr="2010 - 2021"

The search yielded 474 references.
All search results were limited to studies published from 2010 and only publications from one of the European countries were included. Non-English studies were excluded.

Chapter 3 How can we risk stratify patients

Searches were conducted by authors

Chapter 4 Desensitisation strategies

Searches were conducted by authors

Chapter 5 Outcomes after HLA-incompatible kidney transplantation

Clinical question

What is the outcome of HLA-incompatible transplantation compared to 'conventional' transplantation or dialysis?

Search strategy and results

- The Transplant Library (www.transplantlibrary.com) was searched from inception to January 28, 2021 using the search strategy below:

1. HLA incompatib$.ti,ab.
2. anti-HLA.ti,ab.
3. HLA abs.mp.
4. HLA antibod$.ti,ab.
5. incompatible kidney.ti,ab.

6. DSA.ti,ab.
7. donor specific antibodies.ti,ab.
8. or/1-7
9. limit 8 to kidney transplant

Searches identified 111 potentially relevant references.

- MEDLINE and EMBASE were searched from inception to January 28, 2021 using the search strategy below:

1. HLA incompatib$.ti,ab.
2. positive crossmatch.ti,ab.
3. ((kidney or renal) adj3 (transplant$ or graft$)).ti,ab.
4. 1 or 2
5. 3 and 4

This search yielded 736 potentially relevant references.

Chapter 6 Kidney sharing schemes for sensitised patients

Clinical questions

1. In HLA incompatible renal transplant recipients, is desensitization better than kidney paired donation (KPD)?
2. In KPD programmes, should ABO incompatible pairs be included?
3. In KPD programmes, should compatible pairs be included?
4. What is the impact of prolonged cold ischemia time and logistical issues in transnational KPD?
5. Are there immunological advantages for sensitized patients in transnational KPD versus national/local programs?

Search strategy and results

- The Transplant Library (www.transplantlibrary.com) was searched from inception to November 5, 2020 using the search strategy below:

1. kidney chain.mp.
2. kidney exchange.mp.
3. kidney sharing scheme.mp.
4. paired exchange.mp.
5. kidney paired donation.mp.
6. domino chain.mp.
7. paired don$.mp.
8. Blood Group Incompatibility/
9. ABO incompatible.mp.

10. HLA incompatible.mp.
11. incompatible pair$.mp.
12. incompatible don$.mp.
13. (sensitiz$ or sensitis$).ti,ab.
14. or/1-13
15. limit 14 to kidney transplant

Searches identified 25 potentially relevant references.

- MEDLINE and EMBASE were searched from inception to November 7, 2020 using the search strategy below:

1. kidney chain.mp.
2. kidney exchange.mp.
3. kidney sharing scheme.mp.
4. paired exchange.mp.
5. paired don$.mp.
6. domino chain.mp.
7. or/1-6
8. Blood Group Incompatibility/
9. ABO incompatible.mp.
10. HLA incompatible.mp.
11. incompatible pair$.mp.
12. incompatible don$.mp.
13. Desensitization, Immunologic/
14. (sensitiz$ or sensitis$).ti,ab.
15. or/8-14
16. living donors/
17. (liv$ adj3 kidney$).tw.
18. (liv$ adj5 donor$).tw.
19. or/16-18
20. 7 and 15 and 19

This search yielded 597 potentially relevant references.

For PICO questions 4 and 5 the search terms for KPD were combined with the additional terms below (search was run on November 17, 2020):

1. international cooperation/
2. Europe/
3. (transnational or international or multinational or European or global or world or cross$ border$).ti,ab.

This search yielded 109 potentially relevant references.