Transplantation in highly sensitized patients: challenges and recommendations

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Abbreviations

AM: acceptable mismatch

CDC: complement dependent cytotoxicity

cPRA: calculated panel reactive antibody

cRF: calculated reaction frequency

DSA: donor specific antibodies

$F(ab')_2$: fragment antigen-binding

Fc: fragment crystallizable

HLA: human leucocyte antigen

IdeS: IgG-degrading enzyme derived from *Streptococcus pyogenes*

IVIg: intravenous immunoglobulins

KPD: kidney paired donation

SAL: single antigen line

vPRA: virtual panel reactive antibody

Abstract

Introduction: Highly sensitized patients awaiting a kidney transplant accrue on the transplant waiting list. The breadth of HLA antibodies in this subpopulation of patients precludes receiving a compatible organ offer through regular allocation within an acceptable time-frame.

Areas covered: Several alternative options to receive a transplant exist for these patients, including additional priority in regular allocation, special programs based on allocation through acceptable antigens, kidney paired donation programs, desensitization protocols, or a combination of the latter two. In this review, these options and their outcomes are discussed, as well as some possibilities to further enhance transplantation of this disadvantaged group of patients.

Expert commentary: For highly sensitized patients, special attention is required, for which several strategies may apply. A step-wise approach may be the optimal strategy to facilitate successful transplantation of highly sensitized patients

1. Introduction

An ever-growing problem in the field of kidney transplantation is the accumulation of highly sensitized patients on the transplant waiting list. These patients have made antibodies against almost all frequent human leucocyte antigen (HLA) types present in their donor population, making it extremely difficult to transplant them with a crossmatch negative donor organ. Sensitization towards HLA can occur through pregnancies, blood transfusions, and prior transplants. Since the presence of pre-transplant donor-reactive cytotoxic antibodies is associated with hyper-acute rejection [1,2], potential donors bearing HLA antigens towards which a patient has made antibodies are excluded for this patient (unacceptable antigens). Most commonly, patients are regarded highly sensitized when having an HLA antibody profile that reacts to ≥85 - 100% of donors in the donor population, usually expressed as calculated PRA (cPRA), virtual PRA (vPRA) or calculated reaction frequency (cRF).

Within Eurotransplant, around 20% of the patients awaiting a kidney transplant is sensitized, and around 5% is highly sensitized. Highly sensitized patients experience prolonged waiting times associated with high mortality rates on dialysis [3]. Therefore, extra effort needs to be taken to transplant this group of patients. In the present review, we will discuss the options, that are currently available to facilitate successful transplantation of this extremely difficult to transplant patient group.

2. HLA sensitization

The HLA system is extremely polymorphic due to its role as antigen presenting moiety. Different HLA molecules have different peptide binding motifs, allowing for a wide range of peptides to be presented. Due to HLA polymorphism, the likelihood of being able to present pathogen-associated peptides to the immune system is high on the population level. Through evolutionary pressure and human migration history, many different HLA molecules exist, with a striking difference in allele frequencies in different populations [4]. Due to this high level of polymorphism, the likelihood of finding a fully matched unrelated donor is extremely slim. For example, within Eurotransplant in the period 2008 - 2017, only 11% of patients received a zero-mismatched kidney, when only considering HLA-A and -B on the broad, and HLA-DR on the split antigen level.

Since the immune system is not educated for any other HLA molecule than self, foreign HLA molecules can elicit an immune response. Already in the normal situation of pregnancy, the inherited paternal HLA antigens present on the foetus result in sensitization in the form of

alloantibodies in about 30% to 75% of the mothers, depending on the HLA antibody detection technique used [5]. In addition, exposure to foreign HLA through blood transfusions or organ transplants can also result in HLA antibody formation. Sensitization in these different scenarios may not be equally strong, as immunization by blood transfusion is generally less broad than immunization by pregnancy [5], and less often results in memory B cell responses [6].

Since an increasing number of unacceptable antigens decreases the chance on an organ offer for a patient, accurate classification of serum HLA antibodies for the definition of unacceptable antigens is essential [7]. In the past, screening for HLA antibodies and subsequent unacceptable antigen definition was rather black and white. By using complement dependent cytotoxicity (CDC) assays to test the reactivity of patient serum against a panel of healthy blood donors representing the HLA makeup of the local donor population, a panel reactive antibody (PRA) level could be determined [8]. This type of screening made sure that the antibodies identified would most probably cause a positive crossmatch, and therefore were likely to cause hyperacute rejection [9]. With the advent of new highly sensitive bead-based luminex assays, the definition of unacceptable antigens for highly sensitized patients has become much more dependent on the interpretation and expertise of the individual laboratories (Chen et al, in preparation). The output of luminex single antigen bead (SAB) assays is mean fluorescence intensity (MFI) for the individual beads, which is a very sensitive, but crude measure of antibody level and/or binding strength. For proper interpretation, is of importance to realise that many factors affect the interpretation of these assays such as prozone effect, antibody titre, antibody affinity, competition for shared epitopes on different beads, complement factors and immune complex interference, as well as irrelevant antibody reactivity against denatured HLA molecules [10-12]. By taking every luminex signal seriously, many patients would end up being highly sensitized, with the accompanying lower chance on an organ offer through regular allocation [9]. Therefore, a per-patient risk assessment with an individualized threshold for positivity, correlation to previous sensitizing events, HLA epitope analysis, and taking into account the chance of receiving a transplant is advised. In the next sections, we will discuss the possibilities to increase transplantation rates for highly sensitized patients.

3. Priority points in regular allocation

A straightforward way to increase transplant rates of highly sensitized patients is to give them a higher priority in the regular allocation system. The new Kidney Allocation System (KAS) in the

United States has indeed resulted in increased transplant rates for sensitized patients by using a point system with a sliding scale for patients with >20% cPRA. Highly sensitized patients (in KAS defined as cPRA ≥98%) receive further priority by regional allocation to those patients with a cPRA of 99% and national allocation to those patients with a cPRA of 100% [13-15]. Impressively, the transplant rate of highly sensitized patients was reported to have increased from 2.4% to 13.4% in the first year of implementation, albeit with a strong bolus effect in the first half year [16]. This increase in transplant rates rendered the median waiting time for highly sensitized patients from >19 years to 3.2 years [17]. Nonetheless, there remains debate about the true impact of for highly sensitized patients, for which a different chance of a donor organ for patients was reported within the 100% cPRA group when stratifying the level of sensitization into decimals [7,18,19]. This suggests that the patients with the highest level of sensitization are still difficult to transplant and remain on the waiting list. Indeed, the patient group with 99.95-100% cPRA is transplanted at the lowest level even in the new system [16]. It is also important to realize that the success for transplanting truly highly sensitized patients in the new KAS is very much dependent on the centres' unacceptable antigen listing policies, as Houp and colleagues noted that intermediately sensitized patients could become listed as highly sensitized by including low level luminex antibodies in the unacceptable antigen list, thereby competing for organs with patients who are truly highly sensitized [7].

While an increase in transplantation rates for highly sensitized patients is a very favourable result, an increased number of organs is shipped over longer distances, thereby increasing cold ischemia times. In the first year, the proportion of organs sent to highly sensitized patients with a cold ischemia time of over 24 hours increased from 21.4% to 29.2% with the accompanying increase in delayed graft function rates [16]. While 6-month graft survival was not affected, the effect on the longer term needs to be determined. With long-term graft survival data not available for the coming years, it is of importance to note that we have recently shown that allocation of organs to highly sensitized patient based on the absence of unacceptable antigens leads to relatively poor 10-year graft survival rates, indicating that merely providing priority to highly sensitized patients in regular allocation may not be sufficient [20]. Additionally, the rate of zero HLA-A, -B, -DR and zero -DR mismatches significantly declined in the new KAS, increasing the chance of HLA immunization in case of graft loss [16].

4. The Acceptable Mismatch approach

Regular allocation is based on avoidance of unacceptable HLA mismatches on the donor organ. For the highly sensitized patient, the breadth of unacceptable antigens therefore often precludes transplantation. Within Eurotransplant, a special program for highly sensitized kidney transplant candidates called the Acceptable Mismatch (AM) program has been running for almost 30 years, which is based on the positive identification of acceptable antigens [21]. These are defined as HLA antigens to which the patient has made no antibodies, as proven by extensive laboratory testing. Initially, acceptable antigens were solely defined by CDC, in which negative reactions in regular screening panels or patient-specific panels were interpreted to provide information on which HLA antigens were not recognized [22]. Later, single antigen lines (SALs) that solely express one HLA type by transfection or transduction were additionally used as off-the-shelf targets in flow cytometry [23,24]. Whereas for the inclusion in the AM program antibodies detected by CDC are still leading, the definition of acceptable antigens is increasingly relying on luminex SAB analysis, combined with epitope analysis through HLAMatchmaker for HLA class I [25]. Once acceptable antigens are defined, these are added to the HLA type of the patient, creating an 'extended HLA type' on which matching is performed [26]. If an organ donor with an HLA type compatible to this extended HLA type is reported to Eurotransplant, the kidney is immediately offered to the AM patient with mandatory shipment to the respective recipient centre. This approach has led to significantly decreased waiting times for highly sensitized patients in the Eurotransplant region [20,27].

From 1989 onwards, more than 2500 patients have been entered on the AM program waiting list, of which almost 1500 received a transplant. A recent analysis showed that antigens positively defined as being acceptable by laboratory testing are truly acceptable, since unlike regular allocation, no HLA match effect exists for patients who received a kidney through the AM program [20,26]. Moreover, we could demonstrate that highly sensitized patients transplanted through the AM program have a significantly better 10-year graft survival than their counterparts transplanted through regular allocation (72.8% versus 62.4%). When we focussed on repeat transplants (which are the majority of transplants in the AM program), then the difference in 10-year graft survival was even more outspoken (72.6% versus 55.0%), with the graft survival of AM patients comparable to that of non-sensitized individuals (69.3%) [20]. This clearly indicates that there is a benefit from allocation on the basis of proven acceptable antigens in comparison to the mere avoidance of unacceptable antigens. Besides excellent graft survival, the AM Program has also been shown to be a very cost-effective way of transplanting highly sensitized patients [28,29].

Unfortunately, also within the AM program there are patients with such a low chance of receiving a compatible organ offer, that they remain on the waiting list. Currently, around 25% of patients listed have a chance of <0.015% to find a donor via the AM program. When further looking into this particular patient group, it is clear that often these are patients with HLA types that are very uncommon in the Eurotransplant donor population [27]. Besides the decreased probability of finding an HLA match for these patients, their HLA antibody profile often contains antibodies directed at common HLA types in the Eurotransplant donor population. One of the ways to increase the chance for these patients is to extend the search for a compatible donor organ to outside their own donor population, since HLA frequency distribution differs between different populations, as mentioned above. To this aim, the European Union FP7 funded project EUROSTAM was initiated in 2012, with the mission to determine whether a Europe-wide AM program would benefit this patient group. Preliminary results indeed indicated that a significant proportion of patients with an extremely low chance to receive an organ in their original donor population had a vast increase in the chance of receiving a compatible organ in another donor population (Claas et al., in preparation). These results warrant a follow-up study in which the ethical, legal and logistical barriers of organ exchange between different national and international allocation programs need to be addressed.

5. Kidney Paired Donation

Transplantation with a kidney from a living donor is associated with superior long-term graft survival and has the advantage that the procedure can be performed pre-emptively. However, in case a highly sensitized patient has access to a living donor, the chance that this donor is HLA incompatible is substantial. When direct donation is not possible, an alternative option is to enter a kidney paired donation (KPD) program to find an HLA compatible donor. The first KPD program was initiated around 30 years ago in South Korea, where lack of a deceased donor program forced alternative ways of donation [30]. Since then, many KPD programs evolved, both on the national level as on the regional level with several similarities but also differences in policies [31]. In KPD programs, HLA and/or AB0 incompatible patient-donor couples are entered into dedicated match rounds to find the highest number of compatible combinations [31]. Initial match rounds are generally based on virtual crossmatching with a physical crossmatch preformed for every couple thereafter [32,33]. Logistically, there are several types of exchange combinations possible, such as direct exchange between two couples, circular exchange between three or more couples, or domino exchange made possible by an non-directed anonymous donor, with the final donor donating to a patient on the deceased donor waiting list [31].

Transplant outcomes for patients transplanted with a crossmatch negative donor kidney through KPD programs are excellent, and comparable to transplantation outcomes of non-sensitized patients [32,34]. However, although KPD programs are hugely successful, also in this setting highly sensitized patients remain the most difficult to transplant subpopulation [35]. It has been estimated that less than 15% of highly sensitized patients can find a compatible pair in a KPD match run [36]. While patients with 95-96% cRF have similar transplant rates to patients with lower immunization grades, patients with ≥97% cRF have significantly lower chance to find a suitable donor through KPD [31,37]. This is likely due to the relatively small number of eligible donors that participate in each round [35,36,38], advocating (inter)national, or at least multi-center KPD programs. Indeed, it has been reported that the relative number of highly sensitized patients in a KPD program affects the number of couples required to achieve match rounds with satisfactory match rates [31]. Since highly sensitized patients are not transplanted at a similar rate to other patients, they accumulate in the KPD pool [33,39], making it increasingly difficult to run successful match rounds.

As mentioned above, most KPD programs make use of an in initial virtual crossmatch based on the unacceptable antigens defined beforehand, most frequently by luminex SAB assays. Therefore, for a successful KPD match run there is a tradeoff between too little and too many unacceptable antigens defined. Too little unacceptable antigens defined may lead to a positive physical crossmatch at a later stage, frustrating the complete match round [35]. Indeed, for highly sensitized patients it has been reported that physical crossmatches were often positive, despite initial virtual crossmatches being negative [33]. On the other hand, too many unacceptable antigens defined can result in the inability to find a compatible couple to start with [40]. Again, a patient-specific approach to determine the truly relevant HLA antibody specificities in the light of contraindication versus risk is pivotal.

Whereas KPD is a very successful approach, the potential for highly sensitized patients to be transplanted through KPD with a living donor may not be fully utilized yet. There are several ways to potentially increase the chance for successful transplantation for highly sensitized patients in KPD programs. One is to include compatible pairs in the program, who themselves may benefit from a better match grade. Inclusion of such couples increases the possibilities for highly sensitized patients to find a compatible donor [41,42]. A second option is to cross the relatively lower AB0 blood group barrier for highly sensitized patients, again increasing the possibilities of an HLA compatible match [43]. Alternatively, enlarging the potential donor pool through international cooperation by creating trans-border KPD programs could benefit the highly sensitized patient who has access to a living

donor, similar to the notion of a Europe-wide AM program. Finally, the combination of KPD with desensitization strategies are used to allow for transplantation of seemingly incompatible pairs, as will be discussed in the next paragraph.

6. Desensitization

Desensitization is generally used to create a window of opportunity to transplant HLA incompatible kidneys with a negative crossmatch. As mentioned above, in case a sensitized patient has access to an HLA incompatible living donor, desensitization can be considered in conjunction with KPD, when the prospective donor obtained through KPD has a lower HLA barrier than the original donor, with DSA levels amenable to desensitization [44]. To this end, predefined criteria for what is regarded as DSA levels amenable to desensitization are required [45]. This is of importance since multiple reports showed that high level HLA antibodies are often refractory to desensitization [46-48]. Furthermore, high baseline HLA antibody levels are often associated with inferior outcomes [49-51]. In some centres, the majority of KPD procedures actually involve desensitization [36].

Most desensitization strategies make use of either high dose intravenous immunoglobulins (IVIg) or low dose IVIg in conjunction with plasmapheresis [52,53]. In some protocols, these moieties are used in combination with the B cell depleting agent rituximab [54-56]. Despite the desensitizing effect of these agents, the subsequent transplants are subject to an increased risk of ABMR, both early and late [53,57]. In recent years, novel agents have been introduced, such as the proteasome inhibitor bortezomib and the complement component C5 inhibitor eculizumab. While initially these agents do not seem to have a clear benefit for desensitization purposes [58-60], bortezomib may be more potent when used in conjunction with plasmapheresis and rituximab [61]. More recently, a novel agent named the IgG-degrading enzyme derived from *Streptococcus pyogenes* (IdeS) has been used for desensitization purposes. This endopeptidase has the capacity to instantly and selectively cleave human IgG into $F(ab')_2$ and Fc fragments. In a study performed in the USA and in Sweden, a total of 25 patients were treated with IdeS just prior to HLA incompatible kidney transplantation. IdeS treatment resulted in a total loss of serum IgG within 6 hours after administration, a time in which all patients became DSA negative [62]. Currently, a multicenter, multinational, phase 2 trial of IdeS for desensitization is being performed (NCT02790437).

Many studies on the outcome of transplantation after desensitization are small, single centre studies without a proper control arm. Only few large studies on the benefit of desensitization compared to

remaining on the transplant waiting list have been performed, and their results remain controversial. The first large single centre analysis on the benefit of desensitization was from Johns Hopkins, where the patient survival of 211 desensitized patients was compared to patients who remained on the waiting list, and patients who either remained on the waiting list or received a transplant from a deceased donor [53]. This study showed a clear beneficial effect of the desensitization route on graft survival. A subsequent multicentre study on 1025 patients who were desensitized in the US confirmed this beneficial effect [50]. Interestingly, in a similar study performed in the UK on 213 desensitized patients, a completely different conclusion was drawn. In this study, no survival benefit was found for patients undergoing desensitization treatment compared to patients with the same degree of sensitization remaining on the waiting list, or patients that remained on the waiting list or were transplanted with either a deceased donor or through KPD [39]. Moreover, long-term graft survival was inferior to compatible transplants, either from living or deceased donors. The differences in outcome between these studies are striking and, besides methodological differences [63], bring to the attention the importance of acceptance criteria for these kinds of interventions. Other outcome measures such as quality of life may provide additional information on which patients benefit from desensitization [39].

7. Expert commentary

Sensitization towards HLA is one of the main obstacles in clinical organ transplantation. In the future, part of this problem could be minimized through prevention of sensitization by organ transplantation itself. HLA matching based on B cell epitopes has shown to allow for transplanting HLA mismatched organs that do not bear immunogenic epitopes for the specific patient, preventing DSA formation [64,65]. Whether such an approach is feasible for the majority of the patients remains to be established. Despite of this, sensitized patients will always be a part of the transplant waiting list due to immunization by pregnancy or blood transfusion. For these patients, special attention is required, for which all the above-mentioned strategies may apply. A step-wise approach may be the optimal strategy to facilitate successful transplantation of highly sensitized patients; making use of a potential living donor, increasing the chance in regular allocation, including patients in special programs such as the AM program, and finally, if none of the aforementioned strategies are successful, application of desensitization protocols.

8. Five-year view/key issues

- Highly sensitized patients will always be part of the kidney transplant waiting list due to immunization by pregnancy and blood transfusions.
- More allocation programs will introduce a sliding scale for increased priority based on sensitization level.
- Acceptable mismatch programs will expand to other donor populations to facilitate transplantation of the most-difficult to transplant patients.
- Kidney paired donation has the potential to be expanded, both in terms of donor populations, as well as the type of patient-donor couples included.
- Novel agents may allow for more rapid desensitiation, although residual plasma cell and memory B cell activity needs to be addressed.
- Sensitization due to transplantation should become a relatively infrequent complication with the introduction of HLA epitope matching.

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