How the definition of acceptable antigens and epitope analysis can facilitate transplantation of highly sensitized patients with excellent long-term graft survival

Sebastiaan Heidt[1], Geert W. Haasnoot[1], Frans H.J. Claas[1]

[1]Eurotransplant Reference Laboratory, Leiden University Medical Center, Leiden, the Netherlands

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Corresponding author: Dr. Sebastiaan Heidt
Eurotransplant Reference Laboratory
Leiden University Medical Center
Albinusdreef 2 – 2333 ZA Leiden, the Netherlands
e-mail: s.heidt@lumc.nl

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Abbreviations

AM: acceptable mismatch

CDC: complement dependent cytotoxicity

cRF: calculated reaction frequency

DSA: donor specific antibody

HLA: human leucocyte antigen

MMC: minimal match criteria

NIMA: non-inherited maternal antigen

NIPA: non-inherited paternal antigen

PRA: panel reactive antibody

SAB: single antigen bead

SAL: single antigen line

Abstract

Purpose of review

Highly sensitized patients awaiting a renal transplant have a low chance of receiving an organ offer. Defining acceptable antigens and using this information for allocation purposes can vastly enhance transplantation of this subgroup of patients, which is the essence of the Eurotransplant Acceptable Mismatch program. Acceptable antigens can be determined by extensive laboratory testing, as well as on basis of HLA epitope analyses.

Recent findings

Within the Acceptable Mismatch program there is no effect of HLA mismatches on long-term graft survival. Furthermore, patients transplanted through the Acceptable Mismatch program have similar long-term graft survival to non-sensitized patients transplanted through regular allocation. While HLA epitope analysis is already being used for defining acceptable HLA antigens for highly sensitized patients in the Acceptable Mismatch program, increasing knowledge on HLA antibody – epitope interactions will pave the way towards the definition of acceptable epitopes for highly sensitized patients in the future.

Summary

Allocation based on acceptable antigens can facilitate transplantation of highly sensitized patients with excellent long-term graft survival.

Keywords

AM program, HLA, kidney transplantation, minimal match criteria

Introduction

Exposure to foreign human leucocyte antigens (HLA) through either pregnancy, blood transfusions or organ transplants can result in sensitization in the form of HLA antibodies [1-3]. Sensitized patients in need of a kidney transplant have a lower chance of receiving a compatible donor organ since organ donors bearing the HLA antigens to which the patient has made antibodies are excluded. The higher the level of sensitization, the lower the chance that a compatible organ offer will be made, resulting in what could be indefinite waiting times for patients with the highest levels of sensitization [4]. Therefore, one of the biggest challenges in kidney transplantation is to transplant highly sensitized patients in a timely manner.

Several options to enhance transplantation of highly sensitized patients exist, such as kidney paired donation [5], increased priority in regular allocation [6], desensitization [7], and special programs such as the Eurotransplant Acceptable Mismatch (AM) program. The latter program is based on the definition of acceptable mismatches, and the use of this information in the allocation process. This review will describe the concept of the AM program, its results, novel developments, as well as future directions, such as an increased use of epitope analyses for highly sensitized patients.

What are acceptable mismatches?

For sensitized patients, unacceptable antigens are routinely defined and used for exclusion of donors with an HLA type, which corresponds to the unacceptable antigens listed [8]. Unacceptable antigens are based on a presumed clinical relevance of antibody specificities determined by either complement dependent cytotoxicity (CDC) or solid phase assays, such as luminex single antigen bead (SAB) assays [8]. The relative level of sensitization of an individual patient can be calculated based on the unacceptable antigen profile in relation to the frequency of these HLA antigens in the donor population, which is referred to as calculated or virtual PRA (panel reactive antibody), or calculated reaction frequency (cRF) [9]. This value gives an indication towards which percentage of possible donors from the actual donor pool a patient has made antibodies [10]. In most allocation programs, highly sensitized patients are defined as having a cRF of 85% to 100%.

Despite their high cRF values, most highly sensitized patients have not made antibodies to every possible foreign HLA antigen [11]. In the AM program, these apparent holes in the

patient's antibody repertoire are used to enhance the chance of receiving a compatible organ offer. By extensive laboratory testing, the absence of HLA antibody specificities is actively explored. Through defining those HLA specificities towards which the patient did not develop any antibodies, one can predict with high certainty that crossmatches with donors carrying the corresponding antigens will be negative. HLA antigens towards which the patient has not made antibodies are therefore considered acceptable antigens, and when these antigens are present on a donor organ these are acceptable mismatches.
The true benefit for highly sensitized patients in the AM program comes from the addition of acceptable antigens to their own HLA type, creating an extended HLA phenotype which is used for allocation purposes. Since matching is now based on a higher number of HLA antigens, the chance of receiving a matched organ offer increases substantially [12]. Furthermore, instead of AB0 identity which is adhered to for regular kidney allocation within Eurotransplant, AM allocation is based on Eurotransplant AB0 compatibility. When a compatible donor organ based on the aforementioned criteria becomes available for a patient on the AM waiting list there is mandatory shipment of the organ to the corresponding recipient center.

Which patients are eligible?

To be eligible to enter the AM program, the patient needs to have a wide range of transplant-relevant antibodies. Therefore, the main inclusion criterion is having at least 85% panel reactive cytotoxic antibodies. Antibodies detectable by luminex only are taken into consideration, only if these are attributable to a defined immunizing event. This is to prevent the AM program from losing its exclusivity for the most difficult to transplant patients [13]. Furthermore, the patient needs to be on dialysis for at least 2 years before being allowed to enter the AM program.

How are acceptable antigens defined?

Initially, acceptable antigens were defined in CDC panel screening assays. In these assays, the serum of the patient was tested for reactivity with a panel of 50 to 100 healthy blood donors, with HLA types corresponding to those most prevalent in the donor pool. Interpretation of the pattern of negative reactions in these screening panels by means of deduction informed on which HLA antigens were not recognized by the antibodies in the

patient's serum [14]. However, for highly sensitized patients with the highest level of panel reactivity the number of negative reactions is so low that determining acceptable antigens becomes virtually impossible. Therefore, patients-specific panels were devised, making use of a collection of 20.000 HLA-typed blood donors in the Leiden laboratory. The cells in these panels were selected such that only one HLA antigen was different from the patient. By making panels of sufficient numbers of different blood donors, acceptable antigens could be determined. The obvious downside of patient-specific panels is the huge effort of designing and compiling these panels. Therefore, a more off-the-shelf source of cells was developed that could be used for many patients, independent of their HLA typing. Transfection of plasmids coding for single HLA molecules into K562 cells resulted in Single Antigen Lines (SALs), that solely express one HLA antigen [15]. These SALs can then be used to determine (the lack of) HLA antibody reactivity against defined HLA molecules [16].

The advent of highly sensitive solid phase assays such as luminex single antigen bead (SAB) assays has further simplified the definition of the absence of reactivity against certain HLA alleles. Negative reactions in these solid phase assays finely pinpoint which HLA antigens are acceptable. Especially for HLA class II, luminex SAB assays are extremely useful. Finally, HLA epitope analysis is being used to further fine-tune the definition of acceptable antigens, which will be discussed later in this review.

Results of the AM program

From its inception in 1989 up to 2017, a total of 2539 highly sensitized patients have been included in the AM program, of which 1463 received a transplant (figure 1). We have previously shown that waiting times for highly sensitized patients to receive a transplant are significantly lower than those of highly sensitized patients awaiting a kidney through regular allocation within Eurotransplant [17,18].

It is well known that the modern era of transplantation matching for HLA still matters for transplant outcome. Both data from the Collaborative Transplant Study (CTS), as well as regular allocation within Eurotransplant show that an increase in HLA antigen mismatches results in inferior long-term graft survival [18,19]. For the acceptable mismatch theory to hold true, no match affect should be present for patients transplanted through the AM program. Indeed, both analyses on the role of HLA mismatches at the broad antigen level and the split antigen level did not reveal any HLA match effect [12,18]. In other words, when

mismatches are proven acceptable, the number of HLA mismatches is not affecting graft survival.

Another important aspect is how graft survival in the AM program compares to patients being transplanted through regular allocation. Obviously, if graft survival would be significantly inferior, one could doubt whether such a program would be justifiable. To this aim, we recently analyzed the 10-year graft survival of highly sensitized patients transplanted through the AM program compared to their highly sensitized counterparts transplanted through regular allocation. We demonstrated that long-term graft survival was significantly better for patients transplanted through the AM program (72.8% versus 62.4%). When focusing on repeat transplant recipients (the vast majority of patients transplanted through the AM program), the difference was even more profound (72.6% versus 55.0%). No difference in graft survival was observed between AM patients receiving a first or subsequent transplant. Multivariate analysis showed that receiving a transplant through the AM program was an independent predictor of superior graft survival. Interestingly, when comparing highly sensitized patients receiving a repeat transplant through the AM program to non-sensitized patients receiving a repeat transplant through regular allocation, graft survival was identical [18]. This important finding indicates that highly sensitized patients can be transplanted with graft survival outcomes similar to non-sensitized patients. When taken into consideration that pregnancy is an important route of sensitization, it is no surprise that around 60% of patients transplanted through the AM program is female [18]. Upon analyzing the 10-year graft survival of female patients transplanted through AM, we found no difference to that of male recipients (84.2% versus 80.6%, p=0.13). However, when also taking into consideration the donor gender, it becomes clear that female donor organs transplanted to patients in the AM program have a significantly inferior graft survival compared to male donor organs, irrespective of the gender of the recipient (figure 2).

Minimal match criteria

For regular allocation it has been shown that matching for HLA-DR has a more pronounced effect on acute rejection episodes and graft survival than matching on HLA-A and HLA-B [4]. An effect of the latter only becomes evident in case of full HLA-DR match [20]. Therefore, the AM program was initiated with a requirement of minimal match criteria (MMC) consisting of two HLA-DR matches or one HLA-DR and at least one HLA-B match. For

patients with the status high urgency, and the most difficult to transplant patients (a chance of receiving an organ offer within the AM program of <0.1%) these MMC are abandoned. Nevertheless, in 2016 and 2017, a total of 417 kidney offers were not made based on the donor HLA type not fulfilling the MMC requirement.

Previously, we have shown that acceptable mismatches truly are acceptable, since for patients transplanted through the AM program, no effect of HLA mismatches was found [12,18]. This suggested that the adherence to MMC may actually not be required. To analyze whether adhering to MMC results in a graft survival benefit, we compared 10-year graft survival of patients transplanted with either two HLA-DR matches or one HLA-DR and one HLA-B match to graft survival of patients not having this degree of matching. When we analyzed the effect on graft survival based of the abovementioned match levels on kidneys allocated through regular allocation (only excluding unacceptable antigens), we clearly observed that minimal sharing of either two HLA-DR matches or one HLA-DR and at least one HLA-B antigen results in superior graft survival. In contrast, we found no difference in graft survival between patients transplanted according to the MMC or not within the AM program population, showing that the MMC do not have any beneficial effect for patients in the AM program and can be abandoned (Figure 3).

Why are some HLA mismatches acceptable?

There are several possible reasons that a highly sensitized patient does not form antibodies to certain HLA antigens. The first possibility is that that the acceptable antigens are (part of) the non-inherited maternal antigens (NIMA), with which the patient has been in contact during fetal development [21]. Acquired neonatal tolerance towards foreign antigens encountered in fetal life has already been described in cattle by Ray Owen in 1945 [22]. In the setting of kidney transplantation, it has been shown that sibling transplants carrying the NIMA haplotype had superior graft survival compared to sibling transplants carrying non-inherited paternal antigens (NIPA) [23]. In line with these data, it was shown that acceptable antigens defined for patients in the AM program often were NIMA, which confirms that the patients are less reactive to these HLA mismatches. [24].

The second reason why a patient does not form antibodies against particular HLA antigens is that the immune system of the patient does not see any foreign structures on these HLA antigens. HLA molecules are proteins consisting of amino acids as building blocks. While

many amino acids are identical between different HLA molecules, polymorphisms do exist at various locations on the molecule [25]. When different from self and present on antibody accessible locations, the humoral immune system may recognize these polymorphic amino acids, which can be referred to as epitopes [26]. While the combination of epitopes on an HLA antigen is unique for this particular antigen, individual epitopes are shared between different antigens [27]. Thus, whether and to which extent the immune system of a given patient recognizes epitopes on mismatches HLA antigens as foreign, depends on the epitopes present on the own HLA molecules of the patient . In case a mismatched donor HLA antigen contains only epitopes that are present on the total HLA antigen makeup of the recipient, no foreign epitopes are seen by the immune system of this patient [28]. In this case, the antigen mismatch is not associated with an epitope mismatch. Therefore, all HLA antigens that solely carry epitopes that are present on one or more of the HLA antigens of the highly sensitized patient can be considered acceptable antigens, since the immune system of the patient will regard these epitopes as 'self' and will not mount an antibody response [29]. It has indeed been shown for both HLA class I and HLA class II that minimizing the number of epitope mismatches results in a vast reduction of donor specific antibody (DSA) formation [30-32].

But even in the presence of one or a few accessible foreign epitopes a patient may not be able to produce clinically relevant antibodies [33]. Most of the antibodies affecting graft outcome are of the IgG type. In order to be able to develop IgG antibodies, there is a need for T cell help to the B cell recognizing the foreign antibody epitope [34]. T cell help requires T cell recognition of foreign peptides derived from the allogeneic HLA molecule presented by the HLA class II molecules on the B cell. Only in case both B cells and T cells recognize epitopes derived from the allogeneic HLA molecules, IgG antibodies will be produced. A first algorithm has been developed to predict the presence and number of such immunogenic peptides [35]. Two recent studies showed a correlation between low match grades for indirectly recognizable T cell epitopes and the formation of de novo donor-specific antibodies [36], as well as inferior long-term graft survival [37]. It is to be expected that application combining an algorithm to predict B cell epitopes and an algorithm to predict T cell epitopes will be instrumental for the prediction of the immunogenicity of a particular HLA antigen for an individual patient.

How to use epitope analyses for highly sensitized patients

Epitope knowledge is already being used to define acceptable HLA antigens for highly sensitized patients in the AM program since 2004, making this the first transplant program to use epitope analyses in the clinical setting [38]. HLAMatchmaker is used to define HLA class I antigens with maximally one epitope mismatch with the HLA repertoire of the patient. The reason that until now the epitope analysis for the acceptable mismatch program has been restricted to HLA class I is because the most immunogenic epitopes for HLA class I have been experimentally verified, resulting in a reliable data set on which the epitope analysis is performed. Verification of HLA class I epitopes was done by using human monoclonal antibodies directed against HLA class I, as well as absorption elution studies [39-41]. Considering the recent clinical data on the relevance of HLA class II epitope matching on DSA free survival [31,32], it is to expected that the analysis of HLA class II epitopes will get more attention in the near future. Actually, we are currently validating a strategy to produce human monoclonal antibodies directed against epitopes on HLA-DR and -DQ, which will be instrumental for the definition of the most immunogenic HLA class II epitopes.

Once the most important antibody epitopes have been verified, this knowledge can be used for donor selection in the acceptable mismatch program. Current definition of acceptable mismatches is based on the absence of antibody reactivity against the allogeneic HLA molecules. However, it is virtually impossible to test the lack of antibody reactivity against all (>15,000) HLA alleles. Therefore, the future strategy should be based on the definition of acceptable epitopes rather than alleles. An acceptable epitope is defined by the lack of reactivity of the patient' sera with this particular antibody epitope. Once the acceptable epitopes are known for a highly sensitized patient, one can use this knowledge for the definition of acceptable antigens, without the need to test all foreign HLA alleles for potential antibody reactivity. All allogeneic HLA alleles consisting of the combination of self and acceptable epitopes can be registered as acceptable antigens. It is to be expected that such strategy will facilitate the definition of acceptable mismatches and increase the chance to find a compatible donor for highly sensitized patients.

Conclusion

Allocation of organs to highly sensitized patients on basis of acceptable mismatches is a highly effective way of transplanting the most difficult to transplant group of patients. Long-

term graft survival of patients transplanted through the AM program is excellent, and equal to the graft survival of non-sensitized patients. Importantly, the number of patients transplanted through the AM program can potentially be even higher when the MMC are abandoned. Whereas the AM program is the first transplant program to have incorporated HLA epitope analysis, in the future epitope knowledge will be increasingly used for both defining what HLA antigens are acceptable and unacceptable for highly sensitized patients.

Key points

- Acceptable mismatches do not negatively affect long-term graft survival
- Long-term graft survival of highly sensitized patients transplanted through the AM program is similar to that of unsensitized patients
- Minimal match criteria for AM patients can be abandoned
- Further incorporation of HLA epitope analysis will facilitate the definition of acceptable epitopes for highly sensitized patients

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Figure Legends

Figure 1. The number of patients included and transplanted in the AM program in the last 28 years.

Figure 2. Kaplan-Meier analysis on 10-year graft survival of male and female patients transplanted with either male of female donors through the AM program. Death censored 10-year graft survival is depicted. P-value is calculated by using log rank test.

Figure 3. The effect of HLA matching for either two HLA-DR antigens or one HLA-DR antigen plus at least one HLA-B antigen. (A) Kaplan-Meier analysis on 10-year graft survival of matching for either two HLA-DR antigens or one HLA-DR antigen plus at least one HLA-B antigen in regular allocation. (B) Kaplan-Meier analysis on 10-year graft survival of matching for either two HLA-DR antigens or one HLA-DR antigen plus at least one HLA-B antigen within the AM program. Death censored 10-year graft survival is depicted. P-value is calculated by using log rank test.

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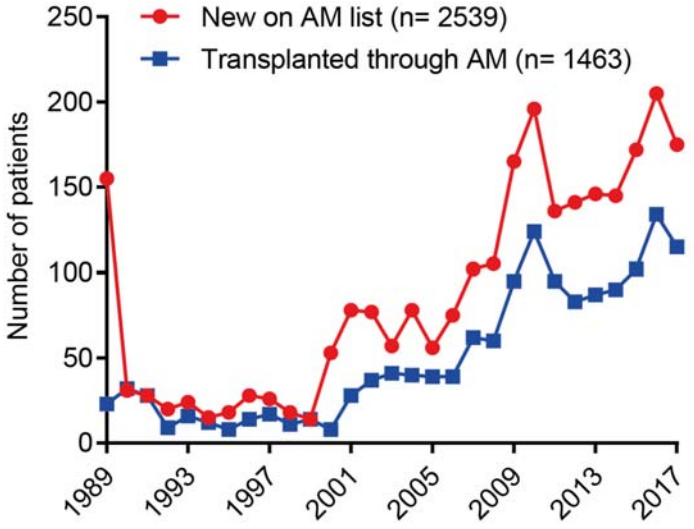
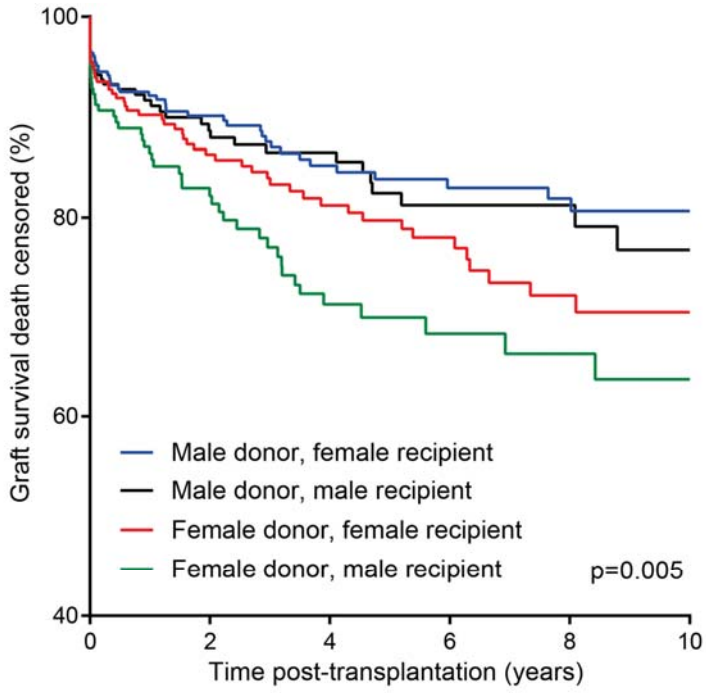


Figure 1.



Number of transplants						
Male → female	335	199	132	95	66	48
Male → male	225	131	93	60	38	21
Female → female	266	159	113	74	43	28
Female → male	183	104	62	41	27	16

Figure 2.

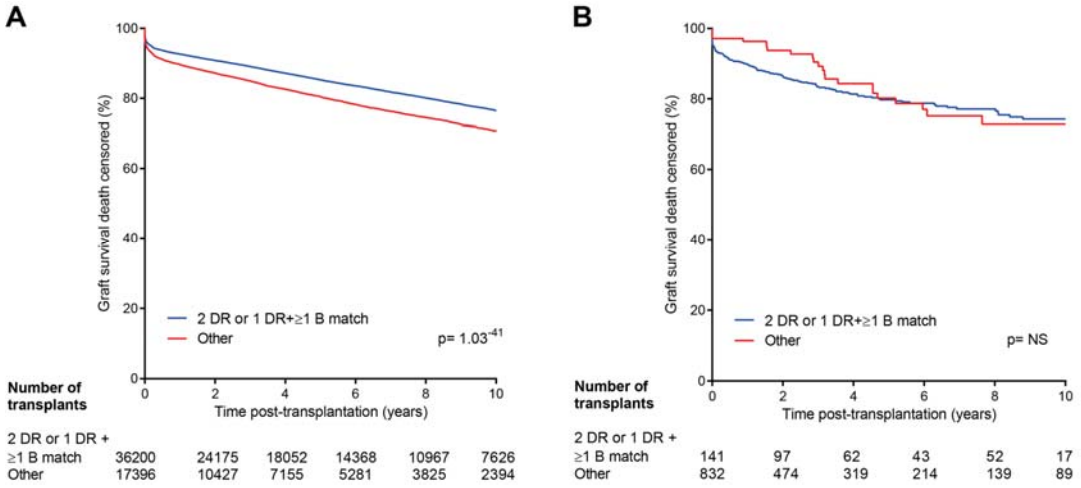

A
Graft survival death censored (%)
100
80
60
40
20
0
2 DR or 1 DR+≥1 B match
Other
p= 1.03-41
0
2
4
6
8
10
Time post-transplantation (years)
Number of transplants
2 DR or 1 DR + ≥1 B match 36200 24175 18052 14368 10967 7626
Other 17396 10427 7155 5281 3825 2394
B
Graft survival death censored (%)
100
80
60
40
20
0
2 DR or 1 DR+≥1 B match
Other
p= NS
0
2
4
6
8
10
Time post-transplantation (years)
Number of transplants
2 DR or 1 DR + ≥1 B match 141 97 62 43 52 17
Other 832 474 319 214 139 89

Figure 3.