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REVIEW ARTICLE

HLA Immune Response Genetics WILEY

Determination of unacceptable HLA antigen mismatches in kidney transplant recipients

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With the introduction of the virtual allocation crossmatch in the Eurotransplant (ET) region in 2023, the determination of unacceptable antigen mismatches (UAM) in kidney transplant recipients is of utmost importance for histocompatibility laboratories and transplant centers. Therefore, a joined working group of members from the German Society for Immunogenetics (Deutsche Gesellschaft für Immungenetik, DGI) and the German Transplantation Society (Deutsche Transplantationsgesellschaft, DTG) revised and updated the previous recommendations from 2015 in light of recently published evidence. Like in the previous version, a wide range of topics is covered from technical issues to clinical risk factors. This review summarizes the evidence about the prognostic value of contemporary methods for HLA antibody detection and identification, as well as the impact of UAM on waiting time, on which these recommendations are based. As no clear criteria could be

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determined to differentiate potentially harmful from harmless HLA antibodies, the general recommendation is to assign all HLA against which plausible antibodies are found as UAM. There is, however, a need for individualized solutions for highly immunized patients. These revised recommendations provide a list of aspects that need to be considered when assigning UAM to enable a fair and comprehensible procedure and to harmonize risk stratification prior to kidney transplantation between transplant centers.

KEYWORDS

HLA antibodies, immunological risk, kidney transplantation, unacceptable antigen mismatches, waiting time

1 | INTRODUCTION

Antibodies against non-self, incompatible HLA in renal transplantation can lead to humoral rejection and premature graft failure. These alloantibodies can be induced by sensitizing events such as pregnancy, transfusion of blood products and organ transplantation.

The determination of possible donor specific HLA antibodies (DSA) that may cause humoral rejections have become a routine procedure before and after kidney transplantation. Therefore, a test for antibodies against HLA (HLA-A, -B, -C, -DR, -DQ, and -DP) is performed upon registration to the waiting list and subsequently every 3 months or after potentially sensitizing events.

HLA antibodies are detected using sensitive solid phase methods and by means of the complement-dependent lymphocytotoxicity test (LCT). Subsequently, an assessment is made which of these HLA antibody specificities may be associated with a high risk of antibody-mediated rejection (ABMR) and/or worsened graft survival, and are therefore reported to Eurotransplant (ET) as "unacceptable HLA antigen mismatches" (UAM). From the cumulative antigen frequency of the UAM in a representative population, the "virtual panel reactivity (vPRA)" as an important allocation-relevant parameter is calculated. The vPRA indicates the proportion of potential organ donors in a virtual population carrying at least one UAM and thus not suitable for the patient from an immunological perspective.

In the standard ET allocation process (ET kidney allocation system, ETKAS) as well as in the Acceptable Mismatch (AM) program, only UAM-negative organs are allocated to the respective recipients.[1] This ensures that positive crossmatch results at the recipient center, with the consequence of reallocation of the organ and prolongation of cold ischemia times (CIT), are avoided in the majority of cases. For the allocation in the ET-Senior-Program (ESP) for organs and recipients aged 65 years and older, UAM can only be considered if the HLA typing of the donor is already available at the time of allocation. In general, organs within the ESP are allocated regionally with short CIT, but without HLA matching. Therefore, this group of patients (20%–30% of kidney transplantations in Germany) needs special consideration regarding DSA and UAM. Moreover, in the case of kidney allocation in combination with other organs, UAM may not be considered at all.

Harmonization of the assignment and reporting of UAM at German transplant centers is expected to improve both equity of allocation chances and optimal use of deceased donor organs across Germany.

The current project started in July 2019 in order to revise the previous recommendations of the German Society for Immunogenetics (DGI) from 2015[2] according to current scientific knowledge. Therefore, the present recommendations are based on the previously published manuscript and were modified by the working group to better reflect recent evidence in the literature and to harmonize the determination of UAM in deceased donor kidney transplant recipients across German transplant centers. Meetings of the joint working group from DGI and German Transplantation Society (DTG) were held between July 2019 and July 2021. The final recommendations were approved by the boards of DGI and DTG in September 2021.

2 | RECOMMENDATIONS

2.1 | Timing of the determination and review of UAM

UAMs are determined when the patient is registered on the waiting list at ET (active ET listing) and are regularly updated according to the specifications of the guidelines of the German Medical Association (Bundesärztekammer,

BÄK).[1] It must be ensured that the sensitization history of patients on the active waiting list is reviewed at least annually. In this context, a review of the reported UAM must take place. The transplant center is responsible for organizing documentation and reporting of sensitizing events. Key sensitizing events to be considered include blood transfusions, pregnancies, and previous transplantations.

2.2 | Methods for the assessment of UAM

In addition to the LCT with unseparated lymphocytes or isolated T-lymphocytes, sensitive solid phase methods are used for final determination of UAM. Sensitive solid phase methods include techniques for detecting HLA antibodies (screening) and determination of HLA antibody specificities (identification or differentiation) and are usually employed in a stepwise approach. Here, commercial test formats based on xMAP™ technology, based on bead microarrays for designated flow cytometers (Luminex™) or based on Microspot ELISA technology are currently available.

The "Single Antigen Test" in bead-based test format (Single Antigen Bead [SAB] method) and the "HLA Single Antigen Microspot ELISA"[2] (Single Antigen ELISA [SA-ELISA]) are currently the most sensitive and comprehensive methods for the determination of HLA antibody specificities because of the high number of available HLA antigen preparations and the advanced resolution of antibody specificities. The HLA Single Antigen Microspot ELISA differs significantly from the ELISA methods commonly used in HLA antibody diagnostics in the past. Instead of HLA isolated from human cells, the HLA Single Antigen Microspot ELISA uses recombinant HLA fixed on a microtiter plate as in bead-based single antigen assays. In this way antibodies against a large number of individual HLA can be differentiated in one well.[3] It is recommended that, at a minimum, all patients with positive results in the screening test at the time of registration on the waiting list for renal transplantation and at annual follow-up should be tested for HLA class I and class II antibodies of the IgG isotype using a single antigen solid phase (SA) test. If quarterly screening tests show significant changes in antibody profile or signal strength, additional testing by an SA test should capture and document the change (addition or disappearance) of antibody specificities in order to change UAM when appropriate. Because positive results in the SA test have occasionally been observed even with negative results in the screening test,[4,5] it may be useful to perform a SA test for clarification in other cases as well.

For patients with sensitizing events, retesting for SA may be required in addition to the intervals described in Section 2.2.

For the determination of UAM, LCT techniques using isolated B-lymphocytes (B LCT) can also be applied as a supplementary tool. For inactivation of interfering IgM autoantibodies, an additional test with addition of dithiothreitol (DTT) should be performed.

The following precautions should be taken when performing SA testing:

Minimize the complement-interference/prozone effect by either of the following methods[6]:

1. Freezing.
2. Heat inactivation at 56°C for 30 min.
3. Addition of 0.2 M EDTA at a ratio of 1:20 to the serum. Subsequently, filtration can be performed before use in the test systems.

Compensation of the varying antigen density on the solid phase, for example, by means of the suggested correction functionality of the respective analysis software.

In the case of allele-specific antibodies to HLA exhibited by the patient, 2-field typing of the patient's corresponding HLA loci is required unless the allele specificity of the antibody can be determined to be irrelevant based on the patient's population genetic background. Specificities directed against alleles of the patient have to be excluded when defining UAM.

In patients who show a reduction in panel reactivity (PRA) in LCT after the addition of DTT, autologous LCT with and without DTT should be performed to confirm IgM autoantibodies. IgM-HLA alloantibodies have not previously been shown to confer an increased immunological risk in the setting of renal transplantation.[7] Therefore, the performance of an additional SA test for the detection of IgM-HLA alloantibodies cannot be recommended.

With the addition of further reagents, complement-binding HLA antibodies can be determined by SA test, which showed clinical relevance both pre- and post-transplantation.[8] Determination of complement-binding antibodies may be of value in assessing clinical risk and defining UAM, so this method may be used as an adjunct for clarification in SA test-positive patients, considering the known technical limitations.[9]

3 | CRITERIA FOR DETERMINING UAM

All highly immunized patients who meet the ET AM program[3] criteria should be registered in the AM program (current criteria are provided on https://etrl.eurotransplant.org/abouteurotransplant/organization/).

All antibody specificities clearly identified by LCT using DTT are considered a contraindication to renal transplantation and must be reported as UAM.

TABLE 1 Frequency of positive reactions in single antigen tests of sera from healthy male blood donors without sensitizing event according to Morales-Buenrostro et al.[15]

Specificity	Frequency, %
A*30:02	**18.9**
A*31:01	**11.3**
A*80:01	8.5
A*34:01	6.8
A*66:02	6.6
A*43:01	5.9
A*66:01	5.9
A*01:01	5.7
A*25:01	5.7
A*33:01	5.2
A*11:02	4.5
A*02:03	4.2
A*24:02	4.0
A*26:01	3.5
A*30:01	3.5
A*33:03	3.5
A*24:03	3.3
A*29:02	3.1
A*74:01	3.1
B*15:12	**11.1**
B*82:01	**10.4**
B*15:16	9.9
B*37:01	7.8
B*44:02	6.1
B*45:01	5.9
B*81:01	4.7
B*08:01	4.5
B*54:01	4.2
B*42:01	3.8
B*56:01	3.8
B*07:02	3.3
B*55:01	3.3
B*57:03	3.3
B*67:01	3.3
B*15:02	3.1
C*17:01	**11.3**
C*02:02	5.0
C*03:02	4.7
C*06:02	4.2
C*03:03	4.0
C*05:01	4.0
C*01.02	3.8
C*04.01	3.8
C*15:02	3.5
C*18:02	3.1
DRB1*04:04	5.4
DQA1*05:03/DQB1*03:01	**10.8**
DQA1*06:01/DQB1*03:01	**10.6**
DQA1*03:03/DQB1*03:01	9.4
DQA1*05:05/DQB1*03:01	8.3
DQA1*03:01/DQB1*03:01	6.1
DQA1*05:01/DQB1*02:01	3.5
DQA1*01:02/DQB1*05:02	3.3
DPA1*02:01/DPB1*01:01	**20.5**

Note: Bold values are alleles reacting frequently positive (in at least 10% of sera).

All HLA antibody specificities that are detectable in solid phase tests but negative in LCT are risk factors for ABMR and reduced graft survival after kidney transplantation. Mean fluorescence intensity (MFI) thresholds between 500 and 1500 have been reported for SAB tests to distinguish background reactions from positive test results.[10,11] It is important to keep in mind that the MFI value depends on many factors, for example, on the manufacturer or on the number of different beads carrying an epitope. Thus, for antibodies against high-frequency epitopes (e.g., Bw4) at the same antibody concentration, the measured MFI values are significantly lower because the antibodies are distributed over a larger number of beads.[10]

At present, there are no adequate compensation mechanisms for reported UAM in the ET allocation algorithms. Therefore, when deciding which specificities to report as UAM, the consequences of reporting (increased waiting time but reduced immunologic risk) must be weighed against the consequences of not reporting (shorter waiting time but increased immunologic risk). In particular, the following factors must be taken into account:

3.1 | Plausibility of the reactions

HLA antibody specificities that are plausible based on the patient's sensitization history should generally be reported as UAM.

In women with a history of pregnancy, HLA characteristics of the child or the child's father should be determined to establish the potential sensitizing HLA antigen differences. The likelihood of clinically relevant sensitization by

transfusion has been significantly reduced by the introduction of leukocyte-depleted erythrocyte and platelet concentrates,[12] but can still occur. Donors of blood products are not necessarily HLA-typed which makes plausibility checks in case of transfusions oftentimes unrealistic.

Checking the positive reacting HLA for common epitopes can be helpful in plausibility testing.[13,14]

If HLA antibody specificities cannot be substantiated by the patient's sensitization history, nonspecific response patterns because of either "natural" antibodies or denatured antigens on the solid phase[15–18] must be considered. In this case, the respective HLA should generally not be reported as UAM. Tables 1–3 provide an overview of the currently known frequent non-specific response patterns. However, it should be noted that these specificities vary from batch to batch and a non-specific pattern does not exclude specific sensitization.

For the determination of UAM in cases of unclear sensitization history, the course of HLA antibodies at different sampling times must be considered. Repeatedly detectable antibodies should be reported as UAM because there is no evidence that antibodies without a clear sensitization event are not clinically relevant.

3.2 | vPRA Level

Since the prolongation of waiting time is to some extent proportional to the vPRA, the reporting of rare HLA (e.g., B76) has little effect. However, beyond a vPRA of approximately 95%–98% in the standard ETKAS allocation scheme, the transplant probability is significantly reduced. In ESP, because of only regional organ availability, transplant probability may be limited even at a lower vPRA.[19,20]

3.3 | Immunological risk

The higher a patient's risk for graft loss because of high plausibility of the anti-HLA antibodies (such as re-transplantation, positivity for HLA class I and II antibodies in combination, HLA antibodies because of pregnancy, transfusions), the more likely antibody specificities should be defined as UAM.

3.4 | Possibility and risks of longer dialysis treatment

A longer dialysis time (or time on the waiting list) is associated with increased mortality and lower transplant success and must be carefully weighed against the risks of an earlier transplantation, possibly accepting an increased immunological risk in each individual case.

3.5 | Possibility and risks of increased immunosuppression

Transplantations with increased immunological risk require more intensive immunosuppressive therapy of the recipient. The increased risk of adverse drug effects, infections, and malignancies because of the therapy must be carefully weighed against the risk of remaining on the waiting list in each individual case.

Overall, the final determination of the UAM can only be made in close cooperation between the HLA laboratory and the transplant center. The laboratory findings and the documentation of sensitizing events in the medical history form the basis for the further decision, which is made by the treating physicians of the transplant center depending on the patient's situation (initial or re-transplantation, ESP or ETKAS allocation, comorbidities, urgency, etc.). The recommendations are intended to harmonize the definition of UAM across centers. The definition of UAM may nevertheless require an individualized approach. In these cases, the reasons should be documented.

4 | DISCUSSION

4.1 | Prognostic value of different MFI thresholds

There has been a considerable debate whether a uniform MFI threshold can be applied to differentiate harmful from harmless DSA and therefore can be used to define UAM.[21] While most retrospective studies have found an increasing risk of early ABMR in patients with increasing MFI,[22–27] only some studies have reported an increasing risk of graft loss,[22,23,25] whereas others have not.[11,26,28] Tambur et al. recently advocated antibody titration to be superior to MFI levels in assessing antibody strength.[29] However, this approach is extensive in labor and cost and might be reserved for specific clinical circumstances such as desensitization or living donor transplantation. Most MFI-based studies used the DSA with the highest MFI (immunodominant DSA) or the cumulative MFI of all DSA for risk stratification. It is well established that there is an association of high MFI values with other risk patterns such as broadness of sensitization and sensitization against both HLA class I and class II.[23,26,28,30,31] Therefore, these studies are unable to assess the risk associated with individual DSA and direct translation to the

TABLE 2 Frequency and mean fluorescence intensity (MFI) of positive reactions in single antigen tests of sera from patients without sensitizing events on the kidney waiting list according to Gombos et al.[17]

Specificity	Median MFI (Range)	Frequency of positive reactions, %	Allele frequency in population, %
A*24:02	4193 (1178–12,197)	8.8	8.7
A*31:01	3377 (1036–8710)	7.8	2.4
A*24:03	4487 (1010–11,025)	6.9	0.1
A*25:01	2426 (1235–5004)	6.9	1.9
A*43:01	2019 (1557–3738)	6.9	0.0
A*66:01	2501 (1259–5226)	6.9	0.3
A*23:01	4974 (1141–7108)	6.9	1.7
A*30:01	2386 (1253–5497)	5.9	1.3
A*80:01	3416 (2312–8012)	5.9	0.0
A*30:02	2547 (1183–4959)	4.9	0.9
A*11:02	2562 (1007–7126)	3.9	0.0
B*15:12 (B76)	**2289 (1001–6022)**	**21.6**	**0.0**
B*37:01	**2211 (1026–5130)**	**13.7**	**1.4**
B*08:01	2119 (1003–9862)	7.8	12.5
B*44:02	2617 (1060–10,427)	7.8	9.0
B*15:11 (B75)	4749 (1365–14,400)	6.9	0.0
B*15:16 (B63)	2016 (1067–5441)	6.9	0.0
B*45:01	5452 (1012–11,899)	6.9	0.4
B*82:01	1575 (1441–5410)	6.9	0.0
B*49:01	6007 (1091–7569)	5.9	1.3
C*17:01	**2960 (1185–8979)**	**11.8**	**0.7**
C*05:01	1345 (1005–3962)	7.8	9.1
C*03:03 (Cw9)	1474 (1041–4937)	5.9	5.5
C*01:02	1995 (1217–4065)	4.9	2.9
C*18:02	1680 (1029–4727)	4.9	Unknown
DRB1*13:01	1434 (1078–2348)	4.9	6.3
DQA1*05:03/DQB1*03:01	1855 (1286–9804)	7.8	Unknown/18.5
DQA1*02:01/DQB1*03:03	1001 (1026–8004)	6.9	Unknown/4.5
DQA1*01:03/DQB1*06:03	2173 (1025–6614)	5.9	Unknown/6.5
DQA1*03:02/DQB1*03:02	2126 (1021–10,081)	4.9	Unknown/9.5
DQA1*03:02/DQB1*03:03	2001 (1117–10,124)	4.9	Unknown/4.5
DQA1*02:01/DQB1*03:01	1880 (1136–3641)	3.9	Unknown/18.5
DQA1*03:03/DQB1*04:01	1442 (1009–2240)	3.9	Unknown/0.0
DPA1*01:03/DPB1*20:01	1535 (1014–5107)	5.9	Unknown/unknown
DPA1*02:01/DPB1*01:01	1612 (1042–2069)	5.9	Unknown/unknown
DPA1*02:01/DPB1*02:02	1533 (1073–10,391)	5.9	Unknown/unknown

Note: Bold values are alleles reacting frequently positive (in at least 10% of sera).

individual patient level is problematic. Until now, there has only been one prospective study using an MFI cutoff of 5000 (10,000 for HLA DQ) for pretransplant risk stratification. This study reported low rates of early ABMR (8%) and graft loss (12%) within 24 months in DSA-positive patients.[32] Although these results are intriguing, they cannot be generalized because risk stratification also involved flow cytometric crossmatch and the follow-up period was short. Results from a multicenter study integrating sensitization history with an upper MFI threshold

TABLE 3 Specificities, frequency of positive reactions (% pos.) and mean fluorescence intensity (MFI) of the 10 most frequent positive reactions in single antigen tests of sera from men without sensitizing events according to Wehmeier et al.[18]

Class I						Class II					
Lot	Antigen	Allele	% pos.	Mean MFI	Max MFI	Lot	Antigen	Allele	% pos.	Mean MFI	Max MFI
9	**B76**	**B*15:12**	**11.4**	**1824**	**6726**	11	**DP1**	**DPB1*01:01/ DPA1*01:03**	**12.2**	**1719**	**4286**
	B8	B*08:01	8.9	1547	3995		**DP5**	**DPB1*05:01/ DPA1*02:02**	**11.6**	**1364**	**3398**
	Cw17	C*17:01	8.9	1568	3295		**DR4**	**DRB1*04:04**	**11.0**	**1047**	**2856**
	Cw4	C*04:01	8.1	1054	2476		DP1	DPB1*01:01/ DPA1*02:01	7.7	1238	2589
	A34	A*34:01	7.3	668	829		DP11	DPB1*11:01/ DPA1*02:02	7.7	1026	1750
	A66	A*66:02	7.3	1170	1902		DR16	DRB1*16:02	7.7	1078	2155
	B46	B*46:01	7.3	968	3094		DR16	DRB1*16:01	7.2	1376	2903
	B57	B*57:01	7.3	1308	5460		DQ6	DQB1*06:03/ DQA1*01:03	6.6	1334	2894
	B73	B*73:01	6.5	1064	1933		DR18	DRB1*03:02	6.1	958	1837
	A11	A*11:02	6.5	2543	6659		DQ2	DQB1*02:01/ DQA1*05:01	5.5	1756	7551
10	**B76**	**B*15:12**	**14.3**	**1708**	**6454**	12	**DP1**	**DPB1*01:01/ DPA1*02:01**	**18.6**	**1253**	**4701**
	Cw17	C*17:01	8.3	1629	5945		**DP5**	**DPB1*05:01/ DPA1*02:02**	**16.8**	**1285**	**4660**
	A66	A*66:02	7.5	1557	3432		**DP1**	**DPB1*01:01/ DPA1*01:03**	**15.0**	**1257**	**4269**
	B37	B*37:01	6.8	2413	6499		**DP19**	**DPB1*19:01/ DPA1*01:03**	**15.0**	**915**	**2202**
	B57	B*57:03	6.8	1756	4410		**DR16**	**DRB1*16:02**	**13.2**	**1120**	**2482**
	B73	B*73:01	6.0	1160	2201		**DR4**	**DRB1*04:04**	**12.3**	**913**	**2031**
	Cw12	C*12:03	6.0	1661	3490		**DQ4**	**DQB1*04:01/ DQA1*02:01**	**10.5**	**1090**	**3771**
	B75	B*15:11	5.3	1115	2457		DP14	DPB1*14:01/ DPA1*02:01	9.1	1291	3625
	Cw4	C*04:01	5.3	1026	1581		DQ7	DQB1*03:01/ DQA1*05:03	9.1	3271	9998
	A25	A*25:01	4.5	1607	5903		DR53	DRB4*01:01	9.1	1070	2671
11	B76	B*15:12	9.6	1327	3010	13	**DP1**	**DPB1*01:01/ DPA1*02:01**	**15.4**	**1944**	**4587**
	Cw12	C*12:03	7.4	2981	8733		**DR53**	**DRB4*01:01**	**15.4**	**855**	**1662**
	Cw17	C*17:01	7.4	1846	4480		**DP5**	**DPB1*05:01/ DPA1*02:02**	**12.8**	**1212**	**2748**
	B63	B*15:16	6.7	1932	4947		**DR4**	**DRB1*04:01**	**12.8**	**917**	**1578**
	Cw4	C*04:01	6.7	1339	4854		**DP19**	**DPB1*19:01/ DPA1*01:03**	**10.3**	**801**	**1221**
	A80	A*80:01	5.2	1343	3067		DP3	DPB1*03:01/ DPA1*01:03	7.7	724	927
	B44	B*44:02	5.2	1443	3500		DQ7	DQB1*03:01/ DQA1*05:03	7.7	787	926

(Continues)

TABLE 3 (Continued)

Class I						Class II					
Lot	Antigen	Allele	% pos.	Mean MFI	Max MFI	Lot	Antigen	Allele	% pos.	Mean MFI	Max MFI
	B45	B*45:01	5.2	2276	4950		DQ7	DQB1*03:01/ DQA1*05:05	7.7	862	1109
	A11	A*11:02	4.4	2107	3362		DR103	DRB1*01:03	7.7	795	909
	B8	B*08:01	4.4	1983	5074		DR4	DRB1*04:02	7.7	1033	2026

Note: Bold values are alleles reacting frequently positive (in at least 10% of sera).

strategy, initiated by one of the coauthors (DZ), are pending. Given the numerous technical limitations of solid phase assays that preclude definitive interpretation of individual MFI values[10,18] and the limitations of the clinical studies mentioned above, a strategy of assigning unacceptable antigens based solely on upper MFI thresholds does not seem justified.

4.2 | Prognostic value of complement-binding antibodies

The capacity of HLA antibodies to bind or activate complement in vitro can be determined by two commercial assays (C1q-positive or C3d-positive antibodies, respectively), and several in-house assays (usually C4d-binding antibodies). This in vitro complement-binding activity is strongly associated with the MFI of the generic SAB test, while IgG subclass information has distinctly lower predictive value, likely because complement-binding IgG1 and IgG3 subclasses usually dominate regarding frequency and relative amounts.[33] The previous recommendations stated that detection of complement-binding HLA antibodies could be useful in assessing clinical risk and defining UAM. In 2018, Bouquegneau et al. performed a comprehensive meta-analysis of a total of 37 studies including 7936 patients tested for complement-activating anti-HLA antibodies. Most patients underwent kidney transplantation ($n = 5991$), but even liver ($n = 1459$), heart ($n = 370$), and lung recipients ($n = 116$) were included. Circulating complement-activating anti-HLA DSAs were associated with an increased risk of allograft loss and allograft rejection for both de novo and preexisting DSA.[8] However, most studies included in this meta-analysis examined de novo DSA or did not differentiate between preformed and de novo DSA. Even more important, most studies on preexisting DSA have compared patients with complement-activating DSA with patients without DSA.

Of the few studies on preexisting complement-activating DSA versus preexisting non-complement-activating DSA, two studies with a total of 120 patients reported an increased risk for ABMR in patients with preformed C4d-binding DSA compared with patients with non-C4d-binding DSA.[34,35] This contrasts to two studies on C4d-binding DSA and two further studies on C1q-binding DSA with a total of 179 patients, which did not detect an increased risk of ABMR in patients with complement-activating DSA versus patients with non-complement-activating DSA.[36–39]

Two studies found no difference in graft loss between 21 patients with C4d-positive DSA and 18 patients with C4d-negative DSA prior to kidney transplantation,[36] or 15 patients with C1q-positive DSA and 13 patients with C1q-negative DSA,[38] respectively. The last study of preformed DSA prior to kidney transplantation included in the meta-analysis compared 30 patients with C1q-positive DSA with 62 patients with C1q-negative DSA. In patients with C1q-positive DSA, the 7-year graft survival rate was significantly reduced (40.7% versus 73.4%), even when the analysis was restricted to patients with DSA of 10.000 MFI or more (38.4% versus 68.9%).[40] The results of the PROCARE study were published after the meta-analysis by Bouquegneau et al. In this study, the 10-year graft survival of 97 patients with C3d-positive DSA was similar to that of 470 patients with C3d-negative DSA (hazard ratio 1.02; 95% confidence interval 0.70–1.48).[41]

Because of this inconclusive evidence, the working group felt that there is insufficient evidence to make more precise recommendations on how the complement-activating ability of antibodies should impact the assignment of unacceptable antigens. Nonetheless, these assays might supplement the determination of complement-fixing antibodies as suspected by LCT in highly sensitized patients.[42]

4.3 | Prognostic value of pretransplant IgM DSA

From a conceptual point of view, pretransplant DSA of the IgM isotype could cause direct complement-mediated

injury to the graft. Alternatively, IgM-expressing B cells might undergo class switching to secrete pathogenic IgG DSA upon allogeneic (re)-stimulation following transplantation. However, there is no clinical evidence so far that pretransplant DSA of the IgM isotype mediate an increased immunological risk.[7,43] Therefore, the Working Group does not recommend pretransplant anti-HLA IgM testing for risk stratification.

4.4 | Prognostic value of IgG DSA against HLA DQA and DP

Until very recently, HLA donor typing in Germany was restricted to HLA A, B, C, DR, and the gene encoding the beta chain of the DQ molecule, that is, DQB1. Donor typing for DQA1, encoding the DQ alpha chain, and DP (both DPA1 and DPB1), however, was not routinely available. In addition, HLA DQA1 and DP cannot be entered as UAM in the ET database. Therefore, UAM against these loci are currently not considered for allocation. Antibodies against these loci are commonly found in patients awaiting retransplantation.[44,45] There is no strong linkage disequilibrium between HLA-DQ or -DR, and -DP. Because of incomplete donor typing in the past, transplant physicians face the problem that these antibodies cannot be unequivocally related to a previous transplant, which would render them unacceptable for many transplant programs. There are numerous case reports associating the presence of anti-DP DSA with ABMR and subsequent graft loss[46,47] with some of the reported cases being completely matched for all other HLA loci.[48,49] For DQ in particular, there is an extra layer of complexity as the individual combination of α- and β-chain can be relevant for antibody specificity.[50] Some, but not all, frequent combinations of DQA1 and DQB1 are coated on individual beads in the SAB assay, leaving some ambiguities unresolved.[18]

Literature on the prognostic relevance of isolated preformed anti-DQA antibodies, however, is scarce. Given the clinical evidence for a causal role of anti-DQ and -DP DSA, especially in patients awaiting retransplantation, the working group strongly recommends to include antibodies against these loci in UAM algorithms and to only accept donor organs for sensitized patients after complete donor typing is available.

4.5 | Antibody differentiation of samples with negative results in screening tests

The density of HLA on single antigen beads is distinctly higher than the density of each HLA antigen attached to the screening beads, especially for HLA-Cw, -DQ, and -DP.[4] Therefore, it is not surprising that some sera produce positive results in single antigen testing despite negative results of the screening test.[4,5] Typically, a modified cut-off value is used for the screening test to avoid false-positive results and the overuse of expensive single antigen tests.[51,52]

Some of the positive reactions of screening-negative sera with single antigen beads could be because of denatured antigens on the single antigen beads. However, Snanoudj et al. demonstrated that 28 of 46 sera with clearly positive reactions in the single antigen assay (more than 3000 MFI) reacted with native HLA despite negative screening results when using a low cut-off (LSM ratio less than 2).[4] Interestingly, these sera showed weaker reactions in the T-cell flow crossmatch than sera with similar MFI but positive screening. Therefore, some of these antibodies might not recognize their cellular HLA target or at least with a lower affinity. Unfortunately, the authors do not report graft survival data for DSA-positive, but screening-negative patients. The ratio for ABMR was uniformly low for all DSA-positive patients (14% for patients with negative screening result vs. 16% for patients with positive screening result, $p = 0.44$), and not compared with DSA-negative patients. To the best of our knowledge, no large studies have evaluated the prognostic value of HLA-antibodies in screening-negative sera.

Therefore, and in view of the additional costs involved, the general use of single antigen tests for all sera cannot be recommended at present. However, one must be aware that a negative screening test does not completely exclude the presence of HLA-antibodies. Hence, the performance of single antigen testing may be indicated as an individual case decision.

4.6 | Impact of UAM on waiting time

It is commonly accepted that increasing numbers of UAM are progressively limiting the available donor pool, thereby increasing waiting times for sensitized patients.[53] If the transplant program comprises adequate compensatory mechanisms, however, transplant rates could largely be independent of vPRA.[54] Because the predictive value of SAB-determined DSA at the level of an individual patient is low,[31] a liberal strategy of assigning UAM seems justified only if the waiting time is not excessively prolonged. Ziemann et al. reported a linear 1.3-week increase for every 1%-point increase in vPRA for a standard ETKAS patient in Germany, whereas sensitized patients in the ESP had to wait considerably longer.[19] Analysis of 2053 patients transplanted via ETKAS or ESP between 2019 and 2021 confirmed a moderate linear

increase with increasing vPRA, resulting in an additional 4 days of waiting time in ETKAS and 5.4 days in the ESP for each %-point increase in vPRA (unpublished data). However, as waiting time depends on many factors, sensitization only being one of them, there are large interindividual differences in waiting time for patients with moderate or even high vPRA levels. There is a large body of evidence from other kidney allocation systems that there is a population of "ultra-sensitized" patients (vPRA >95–99%) who are unlikely to find a suitable donor, likely because of additional factors such as the frequency of their own HLA.[55] As acceptance in the AM program will be limited to patients with a chance of receiving a compatible organ offer through regular allocation of lower than 2%,[56] increasing awareness of sensitization and more liberal assignment of UAM will result in increasing numbers of highly sensitized patients on standard waiting lists (ETKAS and ESP), with as yet unknown consequences on waiting times. Therefore, clinicians must continue to weigh the benefits of better HLA compatibility against the disadvantages of a longer dialysis vintage for individual patients.

4.7 | Transplanting against HLA antibodies not assigned as UAM

The problems of HLA-incompatible transplantation against preformed DSA are well described.[26,28,57,58] Preformed DSA are strongly associated with higher frequency of ABMR and premature graft loss. Currently, there are no approved ABMR therapies available and treatment guidelines are based on low-level evidence with unclear long-term success rates. In addition, costs and treatment-associated side effects may be problematic. If treatment is unsuccessful the patient has to return back to dialysis, may need graft nephrectomy and still suffer from the consequences of intense immunosuppression. As a consequence of a failed transplant, patients are even more sensitized and are less likely to receive another transplant. Therefore, clinicians need to weigh risks of prolonged dialysis treatment against likelihood of complications because of an incompatible transplant, as some patients may have only a single chance for a successful transplantation. It is important to remember that only successful (e.g., rejection-free, good renal function and quality of life) transplantations in the medium- or long-term provide a benefit for the patient and an optimal utilization of the scarce resource of deceased donor kidneys.

4.8 | Personalized solutions

As pointed out, a strict policy of UAM may lead to longer waiting times, which is of particular concern if patients suffer from severe dialysis-related side effects. For patients with an urgent need for a timely transplant (e.g., because they run out of dialysis access) the transplant team can decide for a more relaxed policy and to allow some specificities, which seem to be of lower clinical relevance. Another alternative is a desensitization strategy (e.g., with plasmapheresis/immunoabsorption[24] or the newly approved drug imlifidase[59–61]) together with more intense immunosuppression. While this may allow a successful transplantation in the short term, high ABMR rates of around 35%–40% and inferior graft survival in the long run limit this approach. Given the dramatic shortage of donor organs, ethical concerns arise whether such suboptimal results are justified. As a consequence, in each case in which a transplant center deviates from the standard UAM consensus, a thorough interdisciplinary individual risk–benefit assessment must be performed and should be documented. Most importantly, patients should get adequate information on the risks and limitations of this approach and give their informed consent.

4.9 | Future perspectives

These revised recommendations offer a framework for a fair and comprehensible assignment of UAM and harmonization of risk stratification between transplant centers in Germany. However, the increasing awareness of sensitization as a modifiable risk factor in kidney transplantation has to be better reflected in the current allocation algorithms. Extended donor HLA typing prior to organ allocation should become mandatory also for sensitized patients in the ET senior program. Moreover, one of the major challenges to be addressed in the near future is to identify the level of sensitization at which better compensation mechanisms have to be implemented for patients who do not qualify for the AM program but are to be transplanted via the ETKAS. Finally, long-term follow-up of HLA-incompatible transplantations will reveal whether the current strategies are justified.

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AUTHOR CONTRIBUTIONS

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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