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Research Article

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Apheresis Platelets Reduce De Novo HLA Antibody Formation After Heart Transplantation: Real World Data from a Retrospective Multicenter Study

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Abstract

De novo development of human leukocyte antigen (HLA) antibodies remains a significant challenge in heart transplant (HTx) recipients. Despite advances in perioperative management and immunosuppressive therapy, HLA sensitization continues to contribute to graft dysfunction, rejection, and reduced survival. Beyond investigating the immunogenic impact of pooled versus apheresis-derived platelets, this study provides real-world epidemiological data on the current transfusion practices in this high-risk cohort.

Design: *We conducted a retrospective, multicenter cohort study with approval from the ethics committees of all participating institutions.*

Setting: *The study took place at three German university hospitals (Heidelberg, Munich and Düsseldorf) between December 2011 and December 2021.*

Participants: *Eligible participants were adult patients aged 18 years or older who underwent HTx and provided written informed consent.*

Interventions: *The study among others investigates the type of platelet transfusion received, evaluating its association with the development and progression of HLA antibodies within one year following HTx.*

Measurements and Main Results: *A total of 223 patients were included (median age 54 [IQR 46–60] years; 37% female). Ten patients received pooled platelet concentrates (PPC), and 167 received apheresis platelet concentrates (APC) intraoperatively. Within one year, 70% (n = 7) of PPC recipients developed de novo HLA antibodies compared to 28% (n = 46) of APC recipients.*

Conclusions*: Analysis of real-world hospital data suggests an increased risk of HLA sensitization associated with pooled platelet concentrate transfusions. These findings might indicate that, in routine clinical practice, the preferential use of apheresis platelets during heart transplantation may help reduce alloimmunization risk and potentially may improve long-term outcomes.*

1. Introduction

Despite the development of numerous new and groundbreaking therapeutic approaches in the field of immunosuppression, rejection of transplanted organs - especially in heart transplantations (HTx) - remains a significant risk for patients. Donor-specific human leukocyte antigen (HLA) antibodies are associated with antibody-mediated rejection (AMR) of the transplanted organ and might play an important role in the development and the progression of cardiac allograft vasculopathy (CAV) [1-3]. Alloimmune sensitization can be triggered by any process that involves contact with foreign antigens, such as by blood transfusions, pregnancies, implanted homografts and durable mechanical circulatory support devices (dVAD) and is defined as the development of antibodies against, for example, non-self-HLA or other cellular antigens present on grafted cells [4-6]. Different types of blood products present various HLA-characteristics on their cell surfaces, leading to various risks of alloimmune sensitization. In particular, the repeated administration of platelet concentrates has been identified as a risk factor, since platelets for example express significantly more HLA class I antigens on their cell surface compared to erythrocytes [7].

To minimize exposure to allogeneic HLA antigens, some transplant centers prefer to transfuse apheresis platelet concentrates during and after HTx. The advantage of apheresis platelet concentrates is that each unit contains platelets from a single donor only, whereas multiple-donor ("Buffy-coat") platelet concentrates are derived from 4–6 different donors. However, a major disadvantage of apheresis platelets is the significantly more complex and expensive production.

The debate regarding whether heart transplant patients should receive apheresis-derived thrombocytes (single-donor platelets) versus pooled thrombocytes (multiple-donor platelets) continues. Limited data exist specifically comparing the outcomes of apheresis versus pooled thrombocytes in HTx patients. However, studies in other populations suggest that single-donor platelets may be associated with fewer adverse immunological and infectious complications [8,9]. Although previously it was discussed that benefits of single-donor platelets might not be as clinically relevant as assumed [10].

While the theoretical risk of alloimmunization varies between blood product types, there is a paucity of data regarding the current clinical utilization patterns of platelet concentrates in HTx. Over the last decade, many centers have shifted predominantly toward single-donor apheresis platelets to mitigate donor exposure, rendering pooled platelets a less frequently chosen option. Therefore, beyond assessing the immunogenic impact, this study aims to reflect the real-world epidemiological landscape and standard of care in a multicenter cohort, documenting the extent of this shift in transfusion practices.

The aim of our study is to identify the presence and dynamics of HLA antibodies before and after HTx, particularly in the context of transfusions using pooled and non-pooled platelets. Furthermore, given the varying availability and institutional protocols regarding blood products, this study also seeks to provide epidemiological data on the actual distribution and usage frequency of pooled versus apheresis-derived platelets in a current high-risk transplant setting.

2. Methods

2.1. Study Design and Patient Population

We conducted a retrospective multicentric cohort study involving adult patients (18 and older) who underwent HTx at three German HTx centers. Study period was from December 2011 until December 2021 and varied from each center (Heidelberg:

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January 2020 until December 2021; Munich: December 2011 until October 2021; Düsseldorf: December 2018 until December 2021). Study period variation was due to organizational reasons: patients transplanted at the University Hospital of Heidelberg before January 2020 were not included, due to the first implementation of systemically measuring HLA antibodies perioperatively only starting in January 2020. Data for this research was accessed at all hospitals starting on 15.01.2022.

The study was approved by the Medical Ethical Committee of Heidelberg and individually of the ethic committees of each participating center (Heidelberg: S-191/2022, München: 22-0839, Düsseldorf: 4567).

All research was conducted in compliance with the latest version of the Helsinki Declaration standards.

2.2. Inclusion Criteria

We included all patients who underwent HTx at the University Hospital of Heidelberg, the LMU University Hospital of Munich and the University Hospital of Düsseldorf, in the periods specified above. Patients were included if information about perioperative hemotherapy and repetitive HLA antibody screening was available.

2.3. Exclusion Criteria

Patients with missing HLA antibody screening, those who underwent multiorgan-transplantations, and children were excluded from the study.

2.4. Data collection

Patient demographics, as well as clinical data were obtained from the electronic health records of each center, and information on hemotherapy from the databases of the respective blood banks. Patient data was initially pseudonymized and later anonymized as soon as it was possible according to the research or statistical purpose. Deceased patients were also included in the study, and their available data is analyzed, even if the cause of death was not HTx-related.

2.5. Study variables

Patient demographics, including age, gender, comorbidities, reason for HTx, waiting time until HTx and perioperative HLA-antibody status as well as perioperative blood component transfusion were collected.

The following criteria for perioperative blood component transfusion were defined:

- Preoperative blood transfusions;
- Blood transfusions in the month of HTx;
- Intraoperative blood transfusions;
- Blood transfusions up until 10 days after HTx;
- Blood transfusions within 52 weeks of HTx.

Platelet concentrates were either apheresis-derived (single-donor platelets) or pooled (multiple-donor platelets, derived from 4-6 buffy coats), each unit containing 200 to 350 ml.

2.6. HLA Sensitization Assessment and detection of HLA antibodies

HLA typing and antibody diagnostics are essential components in HTx management at the University Hospitals of Heidelberg, LMU Munich, and Düsseldorf. All centers utilized Luminex Single Antigen Bead (SAB) assays to detect IgG antibodies and define sensitization based on mean fluorescence intensity (MFI) cutoffs and panel reactive antibody (PRA) percentages, although specific protocols and additional methods varied between sites.

All antibody screening and HLA sensitization assessment procedures at the participating centers were conducted in accordance with international guidelines such as those from Eurotransplant, the European Federation for Immunogenetics, the International Society for Heart and Lung Transplantation, and other national transplantation authorities [11-13].

These guidelines provide recommendations on screening intervals, assay selection, interpretation criteria, and clinical management of sensitized patients to optimize transplant outcomes.

2.7. General Methods Overview

All three centers performed pre- and post-transplant HLA antibody screening using Luminex SAB assays. Sensitization was assessed by calculating PRA percentages, and MFI cutoff values were applied to determine the clinical relevance of detected antibodies. Additional methods such as complement-dependent cytotoxicity (CDC) testing and complement-fixing antibody detection using C1q-binding assays were used variably. Molecular HLA typing methods ranged from PCR-based approaches to next-generation sequencing (NGS) for high-resolution typing.

2.8. University Hospital Heidelberg

Pre- and post-transplant antibody screening relied on Luminex SAB assays for IgG antibody specificity. Sensitization was expressed as PRA percentages postoperatively. MFI cutoff values of >1000 pre-transplant and >500 post-transplant was applied to identify clinically relevant antibodies. In cases of high antibody reactivity, C1q-binding SAB assays are additionally performed upon clinician request (cutoff >300 MFI) to assess complement-fixing capacity.

Screening frequency was differentiated by patient status: annual testing for patients without ventricular assist devices (VAD) and biannual testing for VAD patients. Serum samples were also sent to specialized transplantation immunology laboratories twice yearly and four weeks after immunization events such as blood transfusions. Post-transplant monitoring included monthly antibody testing or testing upon signs of graft dysfunction, with immunoglobulin therapy administered until antibodies were undetectable or for six months post-transplant. Virtual and confirmatory warm crossmatches were performed when donor typing and recipient serum were available.

2.9. University Hospital LMU Munich

Prior to transplantation, patient serum was screened for HLA

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antibodies using Luminex LAB Screen Mixed assays (One Lambda, USA) in combination with complement-dependent cytotoxicity (CDC) testing. If either test was positive, antibody specificity was determined using LAB Screen Single Antigen assays (One Lambda, USA). After transplantation, only Luminex-based assays were applied for continued antibody monitoring; CDC testing was not routinely used post-transplant.

HLA typing of donors and recipients was conducted via molecular genetic methods. MFI cutoffs matched Heidelberg's criteria (>1000 pre-transplant, > 500 post-transplant), and sensitization was expressed as PRA percentages. Screening frequency included biannual testing pre-transplant, quarterly testing during the first year post-transplant, and annual testing thereafter.

2.10. University Hospital Düsseldorf

HLA typing combined classical serologic cytotoxicity testing with molecular methods, including PCR with sequence-specific primers (PCR-SSP) and sequence-specific oligonucleotide probes (PCR-SSOP). For high-resolution typing, amplicon-based NGS using Illumina technology targeted exons relevant to class I and II HLA genes.

Antibody screening was performed using Luminex SAB assays, with interpretation considering cross-reactive epitope groups to distinguish clinically relevant antibodies. Sensitization was primarily defined by antibody specificity rather than PRA percentages, with MFI values above 2000 considered clinically significant. Standardized intervals for antibody screening were not explicitly reported; screening is performed according to clinical indications and in accordance with established international guidelines.

2.11. Outcomes

Primary outcome was the development or progression of HLA antibodies within one year after HTx. Furthermore, specificities and quantity of HLA antibodies detected in the transplant recipient before and after HTx were analyzed. Secondary outcomes were previous transfusions and surgeries, such as prior transplantations or dVAD-implantations, previous pregnancies, immunosuppressive therapy regimes, mortality after HTx within 52 weeks and the occurrence of postoperative complications, especially including postoperative infections.

2.12. Statistical Analysis

Statistical analyses were performed using Excel and SPSS version 30.0.0. Descriptive statistics were computed to summarize patient and disease characteristics as well as clinical patient information. Categorial data were expressed as a total number (n) and percentage (%). The non-normally distributed data was displayed as median and interquartile range, continuous variables were expressed as mean and standard deviation (SD). To compare means between two independent groups-specifically, patients receiving pooled platelets versus single-donor apheresis platelets-a chi-square-test was performed. The test assessed differences in HLA antibody levels. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Study population

The total study population from all three participating centers comprised 223 patients who underwent HTx (Heidelberg: n= 35, Munich: n= 113, Düsseldorf: n= 75). The median patient age was 54 (46–60) years, and 37 % were female (see Table 1).

The most frequent underlying condition causing terminal heart disease leading to HTx was non-ischemic cardiomyopathy (n= 138; 62%), followed by ischemic cardiomyopathy (n= 75; 34%). The patients with non-ischemic cardiomyopathy were mostly suffering from dilated cardiomyopathy (n= 104, 47%), other non-ischemic cardiomyopathies (n= 25, 11%), and arrhythmogenic right ventricular cardiomyopathy ARVC (n= 19, 4%).

	Study population, patients (n)	Age, years Median (Q1-Q3)	Gender, female patients, n (%)	Non-ischemic cardio-myopathy, n (%)	DCM (dilated cardiomyo-pathy), n (%)	HCM (hyper-throphic cardio-myopathy), n (%)	ARVC (arrhyhthmo-genic right ventricular cardiomyopathy), n (%)	Restrictive cardiomyopathy n (%)	Other NIC (non-ischemic cardiomyopathy), n (%)
Total study population	223	54 (46–60)	82 (37)	138(62)	104 (47)	5 (2)	10 (4)	2 (0,9)	25 (11)
Heidelberg	35	55 (43–60)	8 (23)	22 (63)	19 (54)	1 (3)	0 (0)	1 (3)	2 (6)
München	113	51 (44–57)	37 (33)	84 (74)	58(51)	2 (2)	10 (9)	1 (0,9)	18 (16)
Düsseldorf	75	59 (53–62)	37 (49)	32 (43)	27 (36)	2 (3)	0 (0)	0 (0)	5 (7)

Table 1: Baseline Demographics

The mentioned other non-ischemic cardiomyopathies included, for example, patients with toxic cardiomyopathies after chemotherapy and due to alcohol abuse or Becker muscular dystrophy.

All patients with amyloidosis (n= 8, 4 %) underwent HTx at the University Hospital in Heidelberg. Over half of the study population (n= 135, 61 %) was listed high urgent (HU) (see Table 2). In Germany, high-urgency status is governed by Eurotransplant's allocation rules and national transplant policies. Patients who are classified as HU receive priority due to life-threatening conditions that cannot be managed with alternative therapies. The criteria for HU-status include among others the dependence on

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inotropic agents or short-term mechanical circulatory support and severe heart rhythm instability despite combined antiarrhythmic medication.

Three patients underwent redo HTx, two of these performed in Munich. Ninety-six patients had undergone previous cardiac surgeries. Some of them underwent more than one cardiac surgery prior to HTx, which leads to a total of 120 prior cardiac procedures (see Table 2). Regarding the type of previous surgeries: 71 patients (32%) had undergone implantation of a dVAD, such as Berlin Heart Excor, Heartware, Heartmate II and Heartmate III.

Seven patients required short-term mechanical circulatory support, such as veno-arterial ECMO prior to HTx and were transplanted directly from the device.

The median waiting time for HTx was 167 (57– 462) days in the total study population. At the University Hospital of Heidelberg, the median time on the waiting list before HTx was 380 (81–1089) days (see Table 2).

	ICM, n (%)	Valvular, congenital heart defect n (%)	Amyloidosis, n (%)	Re-HTx, n (%)	Patients with previous cardiac surgeries, n (%)	Total number of previous cardiac surgeries, n	VAD, n (%)	Temporary assist prior to Htx, n (%)	Time on waiting list, days Median (range Q1–Q3)	HU, patients n (%)
Total study population	75 (34)	7 (3)	8 (4)	3 (1)	96 (43)	120	71 (32)	7 (3)	167 (57–462)	135 (61)
Heidelberg	6 (17)	0 (0)	8 (23)	1 (3)	20 (57)	25	18 (51)	1 (3)	380 (81–1089)	23 (66)
München	28 (25)	5 (14)	0 (0)	2 (2)	30 (27)	46	18 (16)	3 (3)	135 (49–347)	81 (72)
Düsseldorf	41 (55)	2 (55)	0 (0)	0 (0)	46 (61)	49	35 (47)	3 (4)	178 (67–482)	31 (41)

Table 2: Clinical Characteristics of the Study Population

3.2. Preoperative HLA Values

The median time between the most current eligible laboratory work to determine HLA antibody presence and HTx varied among the study centers, ranging from 170 days in Düsseldorf, to zero days in Heidelberg. Preoperative HLA antibodies were present in 65 patients (29 %). Preoperatively, most patients showed the presence of HLA-B antibodies (n= 35, 16 %) (see Table 3).

	HLA-A, n (%)	HLA-B, n (%)	HLA-C, n (%)	HLA-DR, n (%)	HLA-DQ n (%)	HLA-DP, n (%)
Total study population	22 (10)	35 (16)	8 (4)	21 (9)	17 (8)	9 (4)
Heidelberg	4 (11)	7 (20)	3 (9)	12 (34)	4 (11)	6 (17)
München	8 (7)	7 (6)	0 (0)	3 (27)	3 (3)	NA
Düsseldorf	10 (13)	21 (28)	4 (5)	6 (8)	10 (13)	3 (4)

Table 3: Preoperative Presence of HLA Antibodies

3.3. History of Previous Pregnancies and Transfusions

Twenty-nine patients had been pregnant once or multiple times prior to HTx, adding up to 56 reported pregnancies in total. There was no systematical documentation of data regarding pregnancy history in any of the three centers.

Overall, 66 patients of the study population received allogenic blood transfusions (ABT) preoperatively (see Table 4). In the cohort of the University Hospital of Düsseldorf, only four patients underwent preoperative ABT (see Table 4).

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	Patients with pregnancies prior to HTx n (%)	Total number of pregnancies prior to HTx n	Patients with prior pregnancies to HTx and preoperatively HLA antibodies n (%)	Patients who had ABT prior to HTx n (%)	Patients who had ABT prior to HTx and preoperatively HLA antibodies n (%)	Patients with prior pregnancies to HTx and ABT with preoperatively HLA antibodies n (%)	VAD patients, n	VAD patients with HLA antibodies before HTx, n (%), one-sided p-value	VAD patients with HLA antibodies after HTx, n (%), one-sided p-value
Total study population	29 (35)	56	8 (28)	66 (30)	29 (44)	4 (45)	71	39, <0.001	21, 0.783
Heidelberg	5 (62)	11	3 (60)	21 (60)	18 (85)	2 (50)	18	17 (94), 0.028	11 (61), 0.826
München	22 (59)	41	5 (23)	41 (36)	9 (22)	2 (40)	18	4 (22), 0.120	4 (22), 0.830
Düsseldorf (n=37, due to missing data)	2 (5)	4	0 (0)	4 (5)	2 (50)	0 (0)	35	18 (51), 0.309	178 (67-482)

Table 4: History of Previous Pregnancies, ABT or VAD and Impact on HLA Antibodies

3.4. Impact of Transfusion History and Prior Pregnancies on HLA Antibodies

Of 66 patients with history of ABT, 29 patients (44 %) screened positive for HLA antibodies. Among 29 female patients who had pregnancies prior to HTx, HLA antibodies were preoperatively detected in 8 of them.

HLA antibodies were detected in four female patients who had history of both pregnancies and ABT prior to the HTx , two of them treated at the University Hospital of Heidelberg and two at the University Hospital of Munich (see Table 4).

3.5. Durable VAD and its Association with HLA Antibodies

A total of 71 patients had a dVAD prior to HTx. HLA antibodies were detected in 39 of them prior to HTx, whereas 21 patients with a prior dVAD developed de novo antibodies only after HTx.
Patients with dVAD were more likely to have pre-formed HLA antibodies before HTx than the patients without it ($p< 0.001$). There was no statistically significance in de novo development of post-HTx HLA antibodies in patients with dVAD ($p= 0.783$).

3.6. Intraoperative Platelet Transfusions and Their Association with HLA Antibodies

In our total study cohort, 10 patients received pool platelets and 167 patients received apheresis platelets. A group of 31 patients received both types and 15 patients did not receive any platelet transfusions at all.

The mean number of RBC transfusions performed during HTx was seven (SD 7.9).

A look at the formation of de novo HLA antibodies and its correlation to perioperative platelet transfusion during HTx shows that 70 % of the patients (n= 7) receiving pool platelets developed HLA antibodies, while only 28 % of the patients (n= 46) receiving apheresis platelets did. In 39 % (n= 12) of the patients who received both types of platelets, de novo HLA antibodies could be detected.

No significant difference was observed, potentially due to limited statistical power in the pooled group. Nevertheless, the observed trends suggest a potential association between platelet transfusion type and the risk of HLA sensitization.

3.7. Pooled Vs Apheresis Platelets

For exploratory purposes, a chi-square test was performed. Patients were classified into four groups: only pooled concentrates, only apheresis concentrates, both product types, or no transfusion.

The distribution of new HLA antibody formation across transfusion groups was as follows:

Blood product type	New antibodies n (%)	No new antibodies n (%)
Only pooled	7 (70.0%)	3 (30.0%)
Only apheresis	46 (27.5%)	121 (72.5%)
Both	12 (38.7%)	19 (61.3%)
No transfusion	4 (26.7%)	11 (73.3%)

A significant difference between groups was observed (Pearson's $\chi 2 = 9.045$, $p = 0.029$). The highest proportion of new HLA antibody formation occurred in the group receiving only pooled concentrates (70%). However, interpretation is limited by the presence of low expected cell counts; therefore, these findings should be considered exploratory.

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3.8. Logistic Regression Analysis

To control for potential confounders, a binary logistic regression model was fitted including transfusion group, age, sex, number of pooled and apheresis concentrates transfused, VAD status, and number of previous pregnancies. A derived grouping variable ("pool group_vs_apheresis") was created accordingly.

In the final multivariable model, none of the predictors reached statistical significance. The number of transfused pooled concentrates was not associated with antibody formation (p = 0.840). Model performance was limited, with a R^2 of 0.081 and a sensitivity of 15.9%.

A second model included only patients who had received either exclusively pooled or exclusively apheresis concentrates (n = 177). In this analysis, patients receiving only pooled concentrates had a significantly higher risk of developing new HLA antibodies than those receiving only apheresis concentrates (B = -2.082, p = 0.004, OR = 0.125). Model fit was moderate (Nagelkerke R2=0.108, R^2 = 0.108), but sensitivity remained low (15.1%).

In a simplified model examining pooled concentrates alone, the number of transfused pooled concentrates was significantly associated with new HLA antibody formation (B = 0.600, p = 0.025, OR = 1.82). Each additional pooled concentrate was associated with an estimated 82% increase in the odds of antibody formation. However, explanatory power was weak (Nagelkerke R2=0.031R^2 = 0.031R2=0.031), and sensitivity was low (7.2%).

3.9. Immunosuppressive Therapy:

Table 5 shows the immunosuppressive regimens of the three centers. Almost all patients were treated with Tacrolimus, Mycophenolat-Mofetil (MMF) and a Glucocorticoid. All patients from Heidelberg received ATG as well.

	Tacrolimus, n (%)	Ciclosporin, n (%)	Prednisolon, n (%)	MMF, n (%)	ATG, n (%)	Other immunosuppressive drugs (f.e Sirolimus), n (%)
Total study population	220 (99)	3 (1)	218 (98)	219 (98)	37 (17)	12 (5)
Heidelberg	33 (94)	2 (9)	31 (89)	34 (97)	35 (100)	10 (29)
München	112 (99)	1 (1)	112 (99)	110 (97)	2 (2)	2 (2)
Düsseldorf	75 (100)	0	75 (100)	75 (100)	NA	NA

Table 5: Immunosuppressive Therapy

3.10. Postoperative Complications and Death

Overall mortality within the study period of 52 weeks was 7 % (n= 15), from which Munich had the lowest mortality, namely 1 %. Only 4 % of the study population had a transplant failure, defined individually and taken from the medical patients´ report (see Table 6). The rate of Acute Cellular Rejection (ACR) within the first year was 25 % (n = 57) (see Table 7). The most common postoperative complication was infection (n= 28, 13 %), followed by thromboembolic and bleeding complications (n= 15, 7 %) and others, such as acute kidney failure or CMV-reactivation (n= 15, 7 %).

	Deaths, n (%)	Transplant failure, n (%)	Infection, n (%)	Malignancy, n (%)	Thromboembolic and bleeding complications, n (%)	Others, n (%)
Total study population	15 (7)	8 (4)	28 (13)	4 (2)	15 (7)	15 (7)
Heidelberg	5 (14)	3 (9)	3 (9)	0	3 (9)	4 (11)
München	1 (1)	4 (4)	21 (19)	4 (4)	9 (8)	10 (9)
Düsseldorf	9 (12)	1 (1)	4 (5)	0	3 (4)	1 (1)

Table 6: Postoperative Complications

The median time elapse between ACR and HTx was 120 days (36–180) (see Table 7). Fifty-seven patients (25 %) had an ACR within the first postoperative year (see Table 7).

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	ACR >1 within 1-year postoperatively, n (%) and association to mortality, p-value	Time after HTx with ACR >1-year, days (range)
Total study population	57 (25), <0.011	120 (36–180)
Heidelberg	16 (45), 0.21	15 (8–53)
München	40 (35), 0.457	143 (96–190)
Düsseldorf	1 (1)	NA

Table 7: Acute Cellular Rejection Within 1-Year

There is a significant correlation between ACR > 1 (according to the ISHLT-definition more than a mild rejection) within the first postoperative year and the overall mortality (p<0.011).

4. Discussion

Historically, the debate between pooled whole-blood-derived versus single-donor apheresis platelets focused on availability and cost. In the context of solid organ transplantation, however, the priority has shifted toward minimizing antigen exposure. This evolution in clinical practice has led to a widespread preference for apheresis products in many institutions, resulting in a scarcity of modern comparative data. This study addresses this gap by analyzing HLA sensitization rates within the context of these established, albeit asymmetrical, utilization patterns.

HLA sensitization can occur after blood transfusions, particularly in individuals who receive multiple transfusions (4, 6). The results of our study suggest that a prior ABT can be a risk factor for developing HLA antibodies. The transfused blood contains HLA antigens from the donor, which might be recognized as foreign by the recipient's immune system. Thus, it can lead to the production of antibodies against the donor's HLA antigens. HLA-sensitization can complicate HTx (but also other solid organ transplants), as the presence of these antibodies increases the risk of allograft rejection and reduces the likelihood of finding a compatible donor. Blood transfusions, especially repeated ones, are therefore an important factor in the development of HLA antibodies and should be carefully managed in transplant candidates [14]. It is crucial to emphasize that different blood products are associated with varying immunization rates, making it essential to carefully determine the indication for transfusion. Platelet transfusions are particularly relevant because platelets express significantly more HLA antigens than red blood cells, leading to a higher risk of HLA sensitization [7,15] . Even though red blood cell transfusions carry a lower risk of HLA sensitization, they can still contribute to non-HLA immune responses, such as alloimmunization against minor blood group antigens [16]. Avoiding unnecessary transfusions reduces the risk of developing donor-specific antibodies (DSAs), which are linked to AMR and CAV in transplant recipients.

Our findings suggest that pooled platelet concentrates may be associated with a higher likelihood of new HLA antibody formation, particularly in comparison with apheresis concentrates. The chi-square analysis indicated a significant group difference, with the highest antibody rate observed in the pooled-concentrate group. However, this association was not retained in the multivariable model, suggesting that the effect may be influenced by other covariates or reduced by model complexity.

Interestingly, 27 % (n=15) patients receiving no platelet transfusions still developed de novo HLA antibodies, underscoring the multifactorial nature of sensitization, including exposure to other blood products (RBC/FFP) and surgical trauma.

Considering the advantages and disadvantages of apheresis platelets, it might be worth administering them to HTx patients or potential transplant candidates despite the higher cost- as it is shown through our data already widely practice- while having a lower risk of developing HLA antibodies on the long run. HLA-selected platelets might even further decrease the risk of developing de novo HLA antibodies [17].

Although the sample size is low, our evidence suggests that pool-derived platelets may have a higher likelihood of triggering de novo HLA antibodies, making the use of apheresis-platelets a more favorable option in HTx recipients or potential transplant candidates. Therefore, it is essential to establish transfusion protocols for potential transplant candidates and recipients.

In our three study centers, this has been implemented and has become a standard procedure. The most common postoperative complications in our study were infections, followed by thromboembolic and bleeding complications. The CASH study found acute kidney injuries to be the most common complication after HTx [18].

Different risk factors for the development of HLA sensitization have been identified based on our data, including among others transfusions, dVAD and prior pregnancies. Among 29 female patients who had pregnancies prior to HTx, HLA antibodies were detected preoperatively in 8 of them. Pregnancy can increase the risk of developing HLA antibodies, as it exposes the mother's immune system to fetal antigens inherited from the father. This exposure may trigger the maternal immune system to produce antibodies against the paternal HLA antigens present on fetal cells. In some cases, these antibodies can persist after pregnancy, increasing the risk of complications in HTx [19-21].

Our findings demonstrate that patients supported with a dVAD prior to HTx were significantly more likely to have pre-formed

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HLA antibodies compared to those without. This suggests that mechanical circulatory support may contribute to sensitization, potentially due to repeated blood transfusions, infections, or the device itself acting as an immunogenic stimulus as shown in previous studies [22].

5. Limitations

Although our study contributes to the understanding of HLA sensitization during HTx, several limitations must be acknowledged. Despite including three transplant centers, our total study group remains relatively small and we acknowledge the disparity in group sizes as a limitation. The small number of patients receiving pooled platelets (n=10) limits statistical power and generalizability. The overall predictive performance of the regression models was poor, and sensitivity was low across analyses. Accordingly, these results should be interpreted as exploratory and hypothesis-generating rather than definitive. . While the unequal group sizes constitute a methodological limitation, they simultaneously underscore the real-world nature of this cohort, capturing current transfusion practices in adult heart transplantation. This enhances the external validity of our findings, although at the expense of reduced power for analyses involving the smaller group, which is transparently addressed in the limitations.

While RBC transfusions carry a lower risk of HLA sensitization than platelets, the mean 7 units transfused per patient may have contributed to cumulative immune exposure. FFP and cryoprecipitate, though less studied, may also contribute [23]. Future prospective studies with larger cohorts are needed to validate these findings.

The multicenter study design holds a limitation, as the differing thresholds and overall strategies for antibody detection across the three centers make direct comparison challenging. The three study centers do not have the same approaches of platelet transfusion strategies. Consequently we focused on assessing the centers separately instead of combining the datasets.

This study was conducted retrospectively, limiting the ability to establish a direct causal relationship between transfusion of blood products and HLA sensitization. Prospective randomized trials would provide much stronger evidence. Due to organizational reasons, patient screening in Heidelberg started only in 2020, which is why fewer patients were included from this study center. There might be confounding factors, such as the fact that HTx recipients often not only receive multiple RBC and platelet transfusions, but also plasma and additional medication. These co-administered components may contribute to, or prevent HLA sensitization, making it challenging to isolate the effect of platelet transfusions and RBC transfusions alone.

6. Conclusion

In this cohort, transfusion of pooled platelet concentrates was associated with an increased risk of new HLA antibody formation in HTx patients, especially relative to apheresis concentrates. The findings, however, are limited by modest model performance and small subgroup sizes. Larger prospective studies are needed to confirm whether pooled platelet products confer a higher immunological risk than apheresis products in HTx patients.

Data Availability

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

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