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Full Length Article
Analysis

Transfusions in Aplastic Anemia Patients Cause HLA Alloimmunization: Comparisons of Current and Past Cohorts Demonstrate Progress

Katja Julen[1,2], Thomas Volken[3], Andreas Holbro[1,2], Laura Infanti[1,2], Jörg P. Halter[2], Stefan Schaub[4], Caroline Wehmeier[4], Tamara Diesch[5], Alicia Rovó[6], Jakob R. Passweg[2], Andreas Buser[1,2], Beatrice Drexler[1,2,*]

[1] *Blood Transfusion Center, Swiss Red Cross, Basel, Switzerland*
[2] *Division of Hematology, University Hospital Basel, Switzerland*
[3] *School of Health Professions, Zurich University of Applied Sciences, Winterthur, Switzerland*
[4] *Clinic for Transplantation Immunology and Nephrology, University Hospital Basel*
[5] *Division of Hematology /Oncology, University Children's Hospital Basel, Switzerland*
[6] *Division of Hematology, University Hospital Bern, Switzerland*

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Transfusions are the mainstay of supportive therapy in patients with aplastic anemia (AA) and may lead to anti- HLA alloimmunization, thereby also increasing the risk for donor-specific antibodies in the setting of HLA-mismatched transplantation. Historically, AA patients were thought to be at particularly high risk for HLA alloimmunization. In past decades, blood product manufacturing (leukoreduction) and HLA antibody testing have improved significantly by single antigen bead (SAB) technology. It is currently unknown how those developments have impacted HLA alloimmunization and treatment outcome in patients with AA. We retrospectively investigated 54 AA patients treated by immunosuppressive therapy or allogeneic hematopoietic cell transplantation after the introduction of the SAB assay at our center. We compared the HLA antibody results to a historical AA cohort (n = 26), treated before introduction of leukoreduced blood products from 1975 to 1995. HLA alloimmunization was detected in 43 of 54 (80%) recently treated patients. Past pregnancy, female gender, disease severity, age, and a history of other transfusions were significantly associated with a larger number or higher intensity (mean fluorescence intensity) of HLA antibodies. Treatment outcome including bleeding episodes, response to treatment, engraftment, graft-versus-host disease, and overall survival was not associated with HLA alloimmunization. In the historical cohort a significantly higher number of HLA antibodies ($P < .01$) with a higher mean fluorescent intensity ($P < .01$) was observed. HLA alloimmunization remains frequent in AA tested by current techniques, but it has significantly decreased since prior decades and does not affect treatment outcome.

Aplastic anemia (AA) is a rare disease defined as bicytopenia or pancytopenia with hypocellular bone marrow and is classically treated by intensive immunosuppressive therapy (IST) or allogeneic hematopoietic stem cell transplantation (HCT). Transfusion support with RBC and platelets are the mainstay of supportive care [1]. However, transfusions, as well as previous pregnancies and transplantation, may trigger antibodies against non-self HLA [2–6]. HLA antibodies in turn can cause platelet transfusion refractoriness and are associated with graft failure after HCT, eventually impacting therapy response and clinical outcome [7,8].

Immune dysregulation is known to play a major role in the pathogenesis of AA [9], and AA patients may be particularly at risk for HLA alloimmunization considering that Holohan et al. [10] already documented in 1981 a higher frequency of alloimmunization in AA compared to other hematological malignancies and solid tumors. Most of the older literature dates back before the general introduction of leukoreduction of blood products significantly decreasing HLA alloimmunization [11]. In addition, detection of HLA antibodies was with cell-based assays, which has been replaced by the solid-phase single antigen bead (SAB) technology in the past years. SAB technology allows the detection of HLA antibodies with a high sensitivity, allowing a detailed

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*Correspondence and reprint requests: Beatrice Drexler, MD, Division of Hematology, University Hospital Basel, Petersgraben 4, 4031 Basel, Switzerland.
E-mail address: Beatrice.drexler@usb.ch (B. Drexler).

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Table 1
Patient, Sensibilization, Disease, and Therapy Characteristics

				Total number	%
Number of patients					
Recent cohort				54	100
Females				21	39
Historical cohort				26	100
Females				10	38
Age at diagnosis, median (IQR)					
Recent cohort	48 years (22-64 years)				
Historical cohort	20 years (14-28 years)				
Further characteristics of the recent cohort:					
Race					
Caucasian				53	98
Non-Caucasian				1	2
Pregnancy				34	100
Miscarriage				12	35
Live birth				22	65
Transfusions, median (IQR)					
Before first therapy					
RBC	8 (2-14)				
PC	8 (3-17)				
Before first HLA antibody testing					
RBC	5 (0.3-11)				
PC	6 (1-13)				
Diseases					
Acquired AA				50	93
Inherited AA				4	7
Fanconi anemia				3	6
Dyskeratosis congenita				1	2
Severity of aAA					
Non-severe				3	6
Severe				34	63
Very severe				17	32
Days diagnosis to first treatment, median (IQR)	55 days (24-141)				
IST				29	54
HCT				13	24
IST + HCT				12	22
HCT details					
Donor					
Matched related				20	80
Matched unrelated (at least 10/10)				0	0
Mismatched				5	20
9/10 match				4	16
Haploidentical				1	4
Conditioning					
Cy/Flu				2	2
Cy/Flu/ATG				12	48
Cy/Bu				1	4
Cy/Bu/ATG				5	20
Cy/Flu/Alemtuzumab				3	12
Flu/Bu				1	4
Flu/Bu/ATG				1	4
GvHD prophylaxis					
MTX and/or cyclosporine				21	88
MMF/cyclosporine +/− MTX				3	12

(*continued*)

Table 1 (*Continued*)

				Total number	%
Graft source					
PBSC				11	44
BM				14	56
AB0-Incompatibility D/R					
No Incompatibility				13	52
Minor Incompatibility				7	28
Major Incompatibility				3	12
Bidirectional				2	8
CMV Status D/R					
−/−				10	40
+/−				1	4
−/+				7	28
+/+				7	28

IQR indicates interquartile range; RBC, red blood cell concentrate; PC, platelet concentrate; AA, aplastic anemia; IST, intensive immunosuppression; GvHD, graft-versus-host disease; HCT, hematopoietic stem cell transplantation; Cy, cyclophosphamide; Flu, fludarabine; Bu, busulfan; MTX, methotrexate; MMF, mycophenolate mofetil; PBSC, peripheral blood stem cells; BM, bone marrow; D/R, donor/recipient.

characterization of HLA antibodies [2,5,6,12]. There is limited knowledge about incidence and significance of HLA antibodies measured by SAB assays in AA. Therefore this study aims to analyze HLA alloimmunization in consecutive AA patients treated by IST or HCT at our center since the introduction of this technique in 2008. Likewise, we aim to compare the results to a historical validation cohort treated from 1975 to 1995, at a decade when leukoreduced blood products had not been introduced for which samples had been stored. We also intend to identify associated risk factors for HLA alloimmunization and possible effects on treatment and patient outcome.

METHODS

Study population

All consecutive AA patients treated with HCT or IST at the University Hospital and the Children's University Hospital, Basel, Switzerland, between 2008 and 2018 were included in this retrospective study (n = 54). Patients without HLA antibody testing were excluded.

As a validation cohort we tested sera for HLA antibodies using current techniques of 26 historical patients, treated at the University Hospital Basel from 1975 to 1995 for whom stored samples were available. The samples were collected before start of the first treatment and stored at −75°C.

Data on baseline characteristics, diagnosis, treatment, HLA typing, and antibody testing results and outcomes were collected from patients' history and added to a chart for further statistical evaluation [13]. For the historical cohort, the exact number of transfusions and pregnancies was not available. The local ethic committees approved the study (EKNZ Project-ID 2019-01614).

Treatment

The majority of patients underwent HCT with bone marrow from a matched related donor (MRD), less frequently from a 9/10 mismatched or haploidentical donor. The FCA regimen (fludarabine, cyclophosphamide (Cy), ATG) was used as conditioning in the majority of cases; less frequently CyBuATG, the FCC regimen (fludarabine, Cy, alemtuzumab), or reduced-intensity conditioning schemes were given. Cyclosporine/methotrexate was the graft-versus-host disease (GvHD) prophylaxis in most of the patients (Table 1).

IST consisted of cyclosporine and equine antithymocyte globulin (hATG, ATGAM; Pfizer, New York, NY), during a period when hATG was unavailable rabbit ATG was used. Since 2016 patients received eltrombopag when included in a prospective trial. All patients were transfused with γ-irradiated (30 Gy), leucocyte-depleted RBC since 1999, and pathogen-reduced platelets (Intercept) since 2011. RBC were transfused at a hemoglobin level below 80 g/L and platelet concentrates at a platelet count less than 10 g/L or 20 g/L in case of fever, mucositis, or GVHD, respectively. Patients received HLA-matched or antigen-negative platelet units if available in case of platelet refractoriness [14]. Standard supportive care also included infectious disease prophylaxis and broad-spectrum intravenous antibiotics as published elsewhere.

Outcome

Treatment response to IST was classified according to NIH criteria at the most recent follow-up [15,16]. Neutrophil engraftment after HCT was defined as the first of 3 consecutive days of an absolute neutrophil count (ANC)

Table 2
HLA Antibodies

		Total number	%
Number of HLA antibodies, median (IQR)	8 (1-27)		
Class 1	2 (0-9)		
Class 2	2 (0-6)		
MFI, median (range)	1975 (874-5385)		
HLA antibody testing before first therapy, median (range)			
Number of patients		38	70
Number of HLA antibodies	8 (1-27)		
HLA antibody testing during/after first therapy, median (range)			
Number of patients		28	52
Number of HLA antibodies	7 (2-22)		
Number of days from diagnosis till first HLA antibody testing, median (range)	73 (30-317)		
Number of HLA antibodies in females, median (range)			
With pregnancy	11 (5-46)		
Without pregnancy	7 (4-14)		

Table 3
Associations With HLA Alloimmunization in the Study Cohort: Regression Results

Variable	Number of antibodies*			Average MFI†			Highest MFI†		
	IRR	*P*	95% CI	exp (b)	*P*	95% CI	exp (b)	*P*	95% CI
Age	0.98	.000	0.96-0.99	1.01	.174	1.00-1.03	1.01	.512	0.99-1.03
Gender (ref = male)									
Female	1.77	.158	0.80-3.94	2.70	.003	1.41-5.17	4.65	.001	1.87-11.56
Pregnancy (ref = no)									
Yes	4.26	.002	1.72-10.55	1.89	.022	1.10-3.25	1.60	.167	0.82-3.10
Disease severity (ref = severe)									
Very severe	2.58	.002	1.42-4.68	0.59	.145	0.29-1.20	0.87	.808	0.28-2.70
RBC transfusions‡	1.00	.943	0.98	1.01	.046	1.00-1.02	1.01	.170	1.00-1.03
PLT transfusions‡	1.01	.527	0.98-1.02	1.00	.845	0.99-1.02	1.00	.947	0.98-1.02
Days§	1.00	.162	1.00-1.00	1.00	.813	1.00-1.00	1.00	.621	1.00-1.00
Constant	11.15	.000	4.92	567.11	.000	207.4-1550.4	1080.64	.000	225.0-5189.5
Alpha	1.65								

MFI indicates mean fluorescent intensity; IRR, incident rate ratio; exp(b), exponentiated coefficient; 95% CI, 95% confidence interval; *P*, Probability
* Negative binomial regression with robust standard errors.
† Generalized linear model of the Gaussian family with log link and robust standard errors.
‡ Number of transfusions.
§ Days between HLA antibody testing and diagnosis.

exceeding 0.5 g/L and platelet engraftment as a platelet count exceeding 20 g/L without transfusion for 7 days. Acute and chronic GvHD were diagnosed and graded as published elsewhere [17,18].

In this retrospective cohort, including patients referred from external centers, data on all blood counts and confounding factors (fever, sepsis, etc.) at the time of transfusion were not available. We therefore assessed response to platelet transfusions by using bleeding as a clinical outcome parameter instead of the corrected count increment, which is based on the platelet increment after 2 consecutive transfusions. Bleeding was classified according to the World Health Organization (WHO) bleeding grades.

HLA antibody testing

Serum samples were centrifuged for 10 minutes at 2558*g* (4000 rpm) and stored at −30°C until further testing. HLA antibody Class I- and Class II-testing was performed by using the One Lambda LABScreen Single Antigen class I (LS1A04, Lot no. 11 or Lot no. 12) and LABScreen Single Antigen class II (LS2A01, Lot no. 13) with microbead based assay technique (Luminex, Austin, TX). Anti-HLA antibodies with a mean fluorescent intensity (MFI) >500 are considered to be positive in our center. This cutoff can be supported by a recent study evaluating different definitions for a positive SAB result in solid organ transplantation [19]. For the purpose of this study, we either considered an MFI >500 as positive in most cases or the highest MFI of a bead coated with a self-HLA. The testing was performed after a median of 73 days (interquartile range [IQR] 30-317) after diagnosis and before therapy with IST or HCT.

High resolution HLA typing

High-resolution HLA-A/B/C/DRB1 typing of donors and patients was performed by either SSO DNA-typing (LABType HD; One Lambda, West Hills, CA) or sequencing-based typing (Histogenetics LLC, Ossining, NY) [20] for the study cohort.

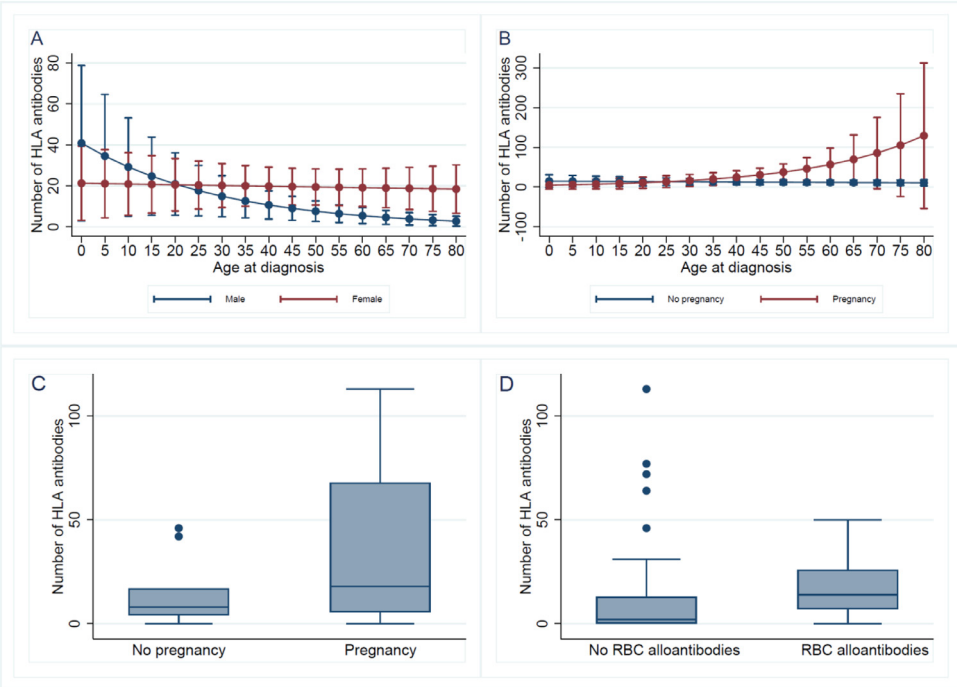

Figure 1. (A) Number of HLA antibodies according to age and gender. (B) Association between pregnancy and number of HLA antibodies over age. (C) Number of HLA antibodies in women with or without pregnancies. (D) Number of HLA antibodies in patients with or without red cell alloantibodies.

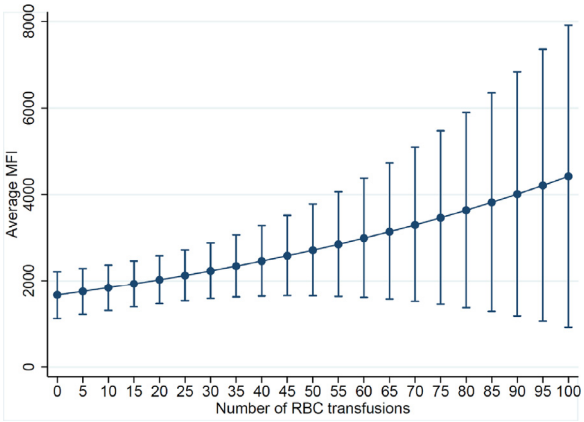

Figure 2. Association between MFI and RBC transfusions.

Statistical analysis

The first available HLA antibody test result in each patient was used to assess the number of HLA antibodies (class I and II), as well as the average and highest MFI. Negative binomial regression models with robust standard errors were applied to assess the association between the number of HLA antibodies, gender, age at diagnosis, disease severity (severe, very severe), pregnancy (no/yes), and the number of previous RBC transfusions and platelet transfusions in the study cohort. We adjusted for the number of days between HLA antibody testing and diagnosis. Similarly, generalized linear models of the Gaussian family with a log link and robust standard errors were used to assess the association between average and highest MFI and the respective covariates. All statistical analyses are described in detail in the supplemental material. All calculations were made with Stata Version 15.1 (StataCorp, College Station, TX).

RESULTS

Baseline characteristics of the study cohort

The study cohort consisted of 54 AA patients, including more males (33 [61%]) than females (21 [39%]). The median age at diagnosis was 48 years (IQR 22-64). The majority of patients were diagnosed with acquired AA (50 [93%]); in 4 (7%) patients the diagnosis of inherited AA was made. Most patients suffered from severe AA (34 [63%]), less frequently from very severe AA (VSAA) (17 [32%]) and non-severe (3 [6%]). Patients were treated after a median of 55 (IQR 24-141) days after diagnosis. Data on therapy response and complications is listed in the supplemental material. The median transfusion number before the first HLA antibody testing was 5 (IQR 0.3-11) RBC and 6 (IQR 1-13) platelet concentrates. The median number of days from diagnosis until the first HLA antibody testing was 73 (IQR 30-317). Further baseline details are listed in Table 1.

HLA alloimmunization of the study cohort

Number of HLA antibodies

HLA antibodies class I or II were detected in 43 of 54 (80%) patients. Thirty-eight (70%) patients were tested for HLA antibodies before the first treatment; in these patients a median of 8 (IQR 1-27) HLA antibodies were found. In patients with HLA antibody testing during or after treatment showed a median number of HLA antibodies of 7 (IQR 2-22) (Table 2). In the adjusted model (Table 3), past pregnancy (Incidence rate ratio (IRR) = 4.26; 95% confidence interval [CI]: 1.72-10.55; $P = .002$) and very severe disease (IRR = 2.58; 95% CI: 1.42=4.68; $P = .002$) were associated with a higher number of HLA antibodies, whereas older age (IRR = 0.98; 95% CI: 0.96-0.99; $P = .000$) was associated with a lower number of HLA antibodies. Further analysis revealed that the latter was particularly the case in men (Figure 1A). Gender, the number of previous RBC and platelet transfusions, and the number of days between diagnosis and HLA antibody testing were not statistically associated with HLA antibody number. When analyzing separately for HLA antibody class I and II, the covariates—except age and past pregnancy—were not statistically significant (results not shown). Interestingly, VSAA patients did not receive more RBC and platelet transfusions before HLA antibody testing. The median number of antibodies was higher in previous pregnant females than in nulliparous females (18 (IQR 6-68) versus 8 (IQR 4-17), Figure 1C). In a subgroup of

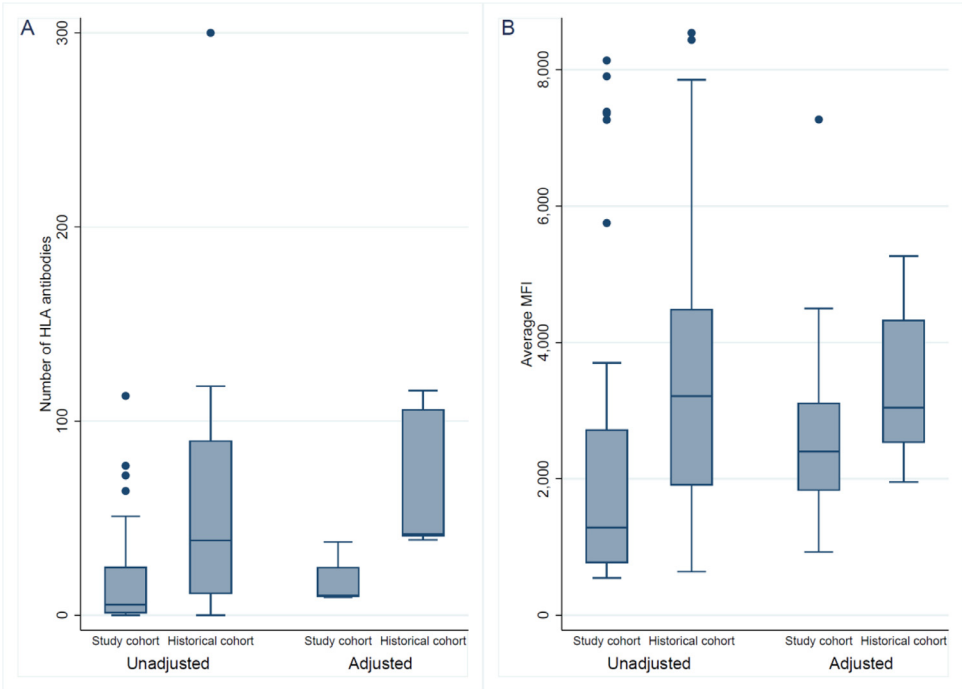

Figure 3. (A) Number of HLA antibodies and (B) Average MFI in historical cohort compared to study cohort.

patients (n=7), we could not show a difference regarding HLA number before first-line IST or shortly after IST treatment versus directly before HCT (Figure 1c in supplemental material).

Characteristics of HLA antibodies

The median MFI was 1975 (IQR 874-5385) in the study cohort. The median MFI was 4365 (IQR 1705-7357) in previous pregnant females and 1192 (IQR 720-4725) in nulliparous females. In the adjusted model (Table 3), female gender (exp (b) = 2.70; 95% CI: 1.41-5.17; P = .003), past pregnancy (exp (b) = 1.89; 95% CI: 1.10-3.25; *P* = .022), and the number of previous RBC transfusions (exp(b) = 1.01; 95% CI: 1.00-1.02; *P* = .046) were associated with higher average MFI (Figure 2). The remaining covariates (age at diagnosis, previous platelet transfusions, disease severity, and the number of days between diagnosis and HLA antibody testing) were not significant. One RBC transfusion was associated with an average predicted MFI increase of 22 (95% CI: 0.06-43), whereas pregnancy led to a predicted increase of 2209 in average MFI (95% CI: 523-3895). Female gender per se was associated with a predicted increase of 1898 in average MFI (95%CI: 630-3167). Furthermore, adjusted highest MFI (Table 3) were substantially higher in women (exp(b) = 4.65; 95% CI: 1.87-11.56; *P* = .001). Remaining covariates were not significant. The predicted highest MFI in women and men was 9062 (95% CI: 5532-12591) and 1949 (445-3455), respectively.

HLA antibody specificities

Overall, 209 different HLA antibodies specificities were detected (95 class I, 114 class II). HLA antibodies against HLA-A and HLA-B were most frequent. In the majority of the patients, antibodies of both class I and II (26%) were detected; 9 patients (22%) had only class I, and 7 patients (17%) only class II. The number of antibody specificities in females was much higher than in male patients (569 antibodies in 17 females versus 249 antibodies in 24 males). In 5 patients undergoing mismatched HCT there were no donor-specific antibodies (DSA).

HLA antibodies and treatment outcome

There was no significant difference in the number (*P* = .487 and *P* = .384) or MFI (*P* = .560 and *P* = .836) of HLA antibodies between patients with or without relevant bleeding (WHO grade $\geq$ 2) during and after treatment. In the setting of HCT neither neutrophil nor platelet engraftment was significantly associated with the number (Sub-distribution hazard-ratio (SHR) = 1.01; 95% CI: 0.99-1.04; *P* = .284 and SHR = 1.03; 95% CI: 0.99-1.06; *P* = .052) or MFI (SHR = 1.00; 95% CI: 0.99-1.00; *P* =.729 and SHR = 1.00; 95% CI: 0.99-1.00; *P* = .110) of HLA antibodies. Acute GvHD and chronic GvHD were not significantly associated with the number (*P* = .604 and *P* = .565) and MFI (*P* = .664, *P* = .253) of HLA antibodies. In the total cohort (*P* = .767 and *P* = .565), as well when assessing patients with IST (*P* = .793 and *P* = .366) versus HCT (*P* = .978) separately, we found that therapy response was not influenced by the number and MFI of HLA antibodies. Neither HLA antibody number nor MFI affected survival in the first year after diagnosis, when comparing the groups divided by median HLA antibody number (*P* = .967) or median MFI (*P* = .999).

Historical validation cohort

Baseline characteristics of the historical and study cohort were not significantly different with regard to gender (*P* = .935) and time between diagnosis and first HLA antibody testing (*P* = .444). However, patients in the historical cohort were significantly younger (*P*< .001) with a median age of 21 years at diagnosis (IQR: 17-28). Adjusting for age at diagnosis, gender, and the number of days between diagnosis and HLA testing, patients in the historical cohort nonetheless had significantly more HLA antibodies (IRR = 4.05; 95% CI: 2.04-8.05; *P* = .000) with a higher average (exp(b) = 2.08, 95% CI: 1.24-3.50; *P* = .006) and maximum (coef(b) = 2.21; 95% CI: 1.20-4.08; *P* = .011) MFI as compared to patients in the study cohort. The average number of predicted HLA antibodies was 66 (95% CI: 32-101) (Figure 3A). Similarly, average MFI and maximum MFI were higher in the historical cohort with predicted average MFI of 4582 (95% CI: 2933-6231) and predicted maximum MFI of 13273 (95% CI: 8588-17958) (Figure 3B).

DISCUSSION

HLA alloimmunization is a well-known phenomenon in AA patients [10,15,16,21], but data on the exact frequency and outcome of HLA alloimmunization tested by sensitive SAB assays and after introduction of leukoreduced blood products is still limited.

Our results demonstrate that HLA alloimmunization remains frequent in AA tested by sensitive SAB assays, but it has substantially decreased in the past decades, not only quantitatively but also in terms of MFI of HLA antibodies. This significant reduction of sensitization over the past years is best explained by transfusing leukoreduced blood products since 1999. In line with this, a pilot study in the United Kingdom first investigated the use of leukoreduced blood products in AA patients in 1997, comparing the results to a historical cohort without leukoreduction, also demonstrating a major reduction in HLA alloimmunization from 50% to 12% [22]. In this study HLA antibodies were tested by cell-based assays, probably underestimating the degree of alloimmunization. A smaller Egyptian study also confirmed the positive effect of leukoreduction in AA, reporting on a decreased rate from 40% to 0% [1]. Data on HLA antibodies tested by SAB assays in AA are scarce, but in a subgroup of patients from the NIH (n = 32) HLA alloimmunization was reported at a rate of 28% [23], which is quite low in comparison to our cohort. However, the study did not clearly state whether testing was performed for both HLA class I and II antibodies, and focused on a very selected patient group receiving granulocyte transfusions for severe infection. In a recent Chinese study with pediatric AA HCT recipients, the incidence of anti-HLA antibodies was also remarkably high (54.9%) tested by SAB assays for class I and II [24].

Overall, HLA alloimmunization remains a challenge in AA patients despite universal leukoreduction of blood products and the use of more sensitive tests, confirming a strong rationale for performing standardized HLA antibody testing in this patient group. The frequency of screening for HLA antibodies remains nevertheless unclear and has to be balanced against the clinical usefulness, because our results simultaneously demonstrate that the clinical outcome such as response to treatment, bleeding, and survival was not affected by HLA alloimmunization. From patients on the waiting list for kidney transplantation, we know that HLA alloimmunization, once detected, is relatively stable over time if no immunizing events occur, suggesting that the frequency of HLA antibody testing should be chosen individually rather than defining a specific interval for all patients [25]. This strategy might be difficult to transfer to AA, because the majority of AA patients are transfusion dependent at the time of diagnosis.

In the large multicenter Leukocyte Antibody Prevalence Study, the transfused volunteer blood donors did not appear to have a significantly higher prevalence of HLA antibodies than their nontransfused counterparts [26]. This is in line with our results, suggesting that blood transfusions could be less

important for HLA alloimmunization. However, the MFI was still significantly influenced by RBC transfusions in our study (Figure 2, Table 3), emphasizing the importance of this well-known immunizing event. In the context of transfusion, the rate of HLA alloimmunization can also be influenced by racial disparity between patients and blood donors. However, our patients and blood donors were predominantly of Caucasian origin.

In the setting of mismatched transplantation HLA antibodies are crucial because they can act as DSA, leading potentially to graft failure [27]. In our study cohort only 5 patients received mismatched HCT without detectable DSA. However, we regard DSA currently as less challenging in AA because MRD transplantation is most often performed in AA patients because of its superior outcome in comparison to mismatched unrelated donor or haploidentical HCT [28,29]. However, in patients without available MRD, the risk for HLA antibodies acting as a DSA should be taken seriously, emphasizing the importance of screening for HLA antibodies in this disease entity and preventing HLA alloimmunization in the first place. In this context, our finding of a higher HLA-antibody number in young male patients (Figure 1a) might be of particular importance because this can affect the outcome of mismatched transplantation in this young patient group, potentially leading to adaptions of conditioning and desensitization. One hypothesis for this phenomenon could be that younger individuals are exposed to more infectious pathogens, which could trigger the formation of HLA antibodies [30]. Unfortunately, infections were underreported in our cohort to answer this question.

HLA alloimmunization is also a risk factor for platelet refractoriness [11], which exposes patients to a higher risk for bleeding, but also challenges clinical management and requires significant resources of the providing transfusion centers. In our cohort interestingly HLA alloimmunization was not associated with more relevant bleeding events (WHO grade $\geq$ 2). However, bleeding is influenced by various factors (eg, comedication, comorbidities, age), and therefore the results should be interpreted cautiously. Our center transfuses HLA-matched platelet units in HLA-alloimmunized patients, possibly explaining the low bleeding rate in our cohort. This is a costly procedure [31] and cannot be followed in every center. Hence, strategies to prevent alloimmunization in AA patients still have a priority.

Besides the classical risk factors (transfusion, pregnancy) very severe disease (VSAA) turned out to be a significant risk factor for HLA alloimmunization in our AA cohort, irrespective of the number of previous transfusions. This might point toward a higher immunogenicity in this subgroup, corroborated by our finding that AA patients with known RBC alloimmunization also had significantly more HLA antibodies in bivariate analysis (Figure 1D). Holohan et al. [10] already described in 1981 a greater risk for HLA alloimmunization in AA than hematological neoplasia, and the cause for this association has not been elucidated so far. Acquired AA (93% of our patients) is thought to be an immune-mediated disease [32], and it is widely accepted that primarily cellular immunity—particularly cytotoxic T-cells—play a critical role [32], whereas the impact of humoral immunity in AA remains unclear. An increased incidence of autoantibodies of uncertain clinical relevance was documented by screening of serum samples of AA patients [33,34], and the authors hypothesized that the target autoantigens recognized by CD4 or CD8 T cells might produce a humoral response (ie, autoantibody production). However, abnormalities of B-cells and upregulation of antibody production has not been described as a key attribute of AA, leaving our finding of a higher rate of alloimmunization in AA compared to other malignant hematological diseases unanswered. Looking at other nonmalignant blood disorders, also high rates of HLA alloimmunization are reported in hemoglobinopathies, depending on previous transfusion numbers and leukocyte reduction. Friedman et al. [35] documented HLA antibodies in 85% of sickle cell disease patients with at least 50 non-leukocyte-reduced RBC transfusions, 48% of patients with fewer than 50 transfusions, and none of the 14 untransfused patients [35]. Nickel et al. [36] found in 29% of patients with sickle cell disease HLA antibodies receiving leukocyte reduced blood products. A similar rate of HLA alloimmunization (31%) is reported in patients with thalassemia [37]. Considering that all patients in our cohort have received leukocyte-reduced RBC transfusions, AA patients are possibly more affected by HLA alloimmunization than patients with hemoglobinopathy. Overall, both nonmalignant entities seem to show a high rate of HLA alloimmunization, which might point to the immune- and inflammation-mediated pathways in both diseases.

Evolving data from the immunogenetic field demonstrated that specific HLA variants (eg, HLA-A*33:03; HLA-A*68:01, HLA-B*40:02, HLA-B*14:02, HLA-DRB1*15:01) are associated with an increased risk of AA [9]. We could not demonstrate that these HLA variants correlated to the HLA antibody specificities in our cohort; therefore, we cannot conclude that specific alleles are more frequently targeted by antibody formation.

Finally, several limitations of the study merit consideration. First, the relatively small number of patients and the retrospective design might have predisposed to selection bias and residual confounding. We could not assess transfusion number and pregnancy rate in the historical cohort because this data was not available. In 30% of patients HLA antibody testing was performed during or after treatment. Therefore test results could have been influenced by therapeutic agents, especially ATG and intravenous immunoglobulins, which are known to contain anti-human HLA antibodies and can partly be detected by anti-human IgG [30]. False-positive results can also occur because of exposure to neo-epitopes and unspecific binding of serum matrix components [38].

CONCLUSION

HLA alloimmunization remains frequent in patients with AA tested by current techniques but has significantly decreased in recent decades. Pregnancy and disease severity are the main risk factors for HLA alloimmunization in AA, whereby the number of transfusions seems to be less important recently. Overall, treatment and patient outcome are not affected by HLA alloimmunization, but given increases in mismatched HCT, HLA antibodies might play an important role as DSA in the future. Larger-scale prospective studies are needed to identify additional risk factors and underlying causes of HLA alloimmunization in AA, ultimately preventing HLA alloimmunization in AA patients at greatest risk.

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2021.07.017.

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