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TRANSFUSION COMPLICATIONS

TRANSFUSION

Red blood cell alloimmunization in myelodysplastic syndromes: Associations with sex, DAT-positivity, and increased transfusion needs

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Abstract

Background: Many patients with myelodysplastic syndromes (MDS) need repeated red blood cell transfusions which entails a risk of immunization and antibody formation. Associations between alloantibodies, autoantibodies and increased transfusion requirements have been reported, but their relationship remains unclear. In this study, we analyzed factors potentially associated with red blood cell alloimmunization, as well as changes in transfusion intensity and post-transfusion hemoglobin increments.

Methods: In a retrospective cohort study, we linked Swedish MDS patients diagnosed between 2003 and 2017 to transfusion and immunohematology data. Potentially associated factors were analyzed using Cox proportional hazards regression. The transfusion rate after detected alloimmunization was analyzed using a fixed effects Poisson regression. Post-transfusion hemoglobin increments before and after alloimmunization were compared using a mixed effects regression.

Results: Alloantibodies following MDS diagnosis were detected in 50 out of 429 patients (11.7%). Female sex and a positive direct antiglobulin test (DAT) were independently associated with alloimmunization, with hazard ratios of

Abbreviations: DAT, direct antiglobulin test; FE, fixed effect; IPSS-R, international prognostic scoring system revised; IRR, incidence rate ratio; IQR, interquartile range; MDS, myelodysplastic syndrome; MDS-EB, myelodysplastic syndromes with excess of blasts; MDS-MLD, myelodysplastic syndromes with multilineage dysplasia; MDS-RS, myelodysplastic syndrome with ring sideroblasts; MDS-SLD, myelodysplastic syndromes with single lineage dysplasia; MPN, myeloproliferative neoplasm; RBC, red blood cell; Rh, Rhesus; WHO, World Health Organization.

2.02 (95% confidence interval [CI] 1.08–3.78) and 9.72 (95% CI, 5.31–17.74), respectively. The transfusion rate following alloimmunization was increased with an incidence rate ratio of 1.33 (95% CI, 0.98–1.80) and the post-transfusion hemoglobin increment after alloimmunization was 1.40 g/L (95% CI, 0.52–2.28) lower per red blood cell unit ($p = .002$) compared to before alloimmunization, in multivariable analyses.

Discussion: Alloimmunization against blood group antigens was associated with sex, DAT-positivity, increased transfusion needs, and lower post-transfusion hemoglobin increments. These findings warrant further investigation to evaluate the clinical significance of up-front typing and prophylactic antigen matching in patients with MDS.

KEYWORDS

RBC transfusion

1 | INTRODUCTION

Myelodysplastic syndromes (MDS) constitute a heterogenous group of malignant hematopoietic stem cell disorders. The disease is characterized by ineffective hematopoiesis and dysplastic changes in the bone marrow, resulting in various degrees of unilineage or multilineage cytopenia.[1] Anemia is the most common cytopenia and the majority of the patients require at least one unit of red blood cells (RBCs) during the disease course.[2] RBC transfusions are essential in the supportive care of MDS patients but may also have unwanted effects,[3–5] including formation of alloantibodies against RBC blood group antigens.[6–8] For some disease entities that are associated with transfusion-dependency, such as the hemoglobinopathies, prophylactic antigen matching beyond ABO and RhD have reduced the alloimmunization rate[9–12] and is most often the standard of care in sickle cell anemia and thalassemia.[13–15] Similarly, a Canadian retrospective study observed significantly reduced rates of Kell and Rh immunization in MDS patients at institutions providing prophylactic antigen matched RBC units for RhCE and Kell compared to patients at institutions without such matching strategy.[16]

At each transfusion episode, the transfused patient is exposed to numerous of foreign RBC antigens. However, many patients receive large numbers of RBC transfusions without any evidence of alloimmunization. The overall risk of mounting an alloantibody response after RBC transfusion in recipients of any indication ranges between 2% and 9%[17,18] but higher rates between 12% and 27% have been reported for MDS patients.[6–8,19–21] Development of alloantibodies is of clinical importance, especially in patients with a chronic transfusion need, leading to a risk of delay in finding compatible units, increased laboratory work-load to identify the alloantibody, and an increased risk of hemolytic reactions.[13] Further, an association between alloantibodies and autoantibodies has been reported,[6,7] and there are also indications of increased transfusion requirements following alloimmunization.[7]

In this study, we identified Swedish patients with MDS and linked their clinical data with transfusion records and laboratory parameters with the aim to identify factors associated with alloimmunization and clinical consequences.

2 | MATERIALS AND METHODS

2.1 | Data sources

The MDS Register/Biobank at the Karolinska University hospital, Stockholm, Sweden enrolls consecutive patients with MDS and MDS/myeloproliferative neoplasm (MPN) overlap disorders holds detailed disease and patient data and is continuously updated. Data on immunohematology and administered transfusions were retrieved from the transfusion database ProSang (CSAM e-Health Company, Oslo, Norway), which captured all of the blood transfusions given in the Stockholm area during the study period (for details on the pretransfusion RBC antibody screening and identification process, see Supporting Information). Hemoglobin measurements were obtained from the Laboratory Information System (Flexlab™; Tieto, Helsingfors, Finland) at the Clinical Chemistry Laboratory at the Karolinska University Hospital and from two private contractors in Stockholm (Aleris Medilab and Unilabs AB), providing laboratory data from September 1999 until May 2014.

2.2 | Study design and statistical analysis

Adult patients (≥18 years) diagnosed with MDS between January 1, 2003 and July 1, 2017 were identified in the local MDS Register/Biobank. Disease and patient parameters including age, sex, the Revised International Prognostic Scoring System (IPSS-R), the 2016 World Health Organization (WHO) classification and mutational status of recurrently mutated genes in MDS, were linked to RBC transfusions and information on alloantibodies and direct antiglobulin test (DAT) status, using national registration numbers. Considering that MDS patients might need RBC transfusion before the diagnosis is established, transfusions were considered MDS-related and included in the study if given less than 90 days before the date of diagnosis. Patients who had never received any RBC transfusion were excluded. Patients with alloantibodies detected earlier than 90 days before the date of diagnosis were excluded. Follow-up ended at the date of death, allogeneic stem cell transplantation, or October 1, 2017.

2.3 | Statistical analysis

Summary statistics for continuous variables were described as medians with interquartile ranges (IQRs). Explanatory variables and their association with alloimmunization were analyzed using univariable and multivariable Cox proportional hazards regression with 95% confidence intervals (CIs). The analyses were performed until the first alloimmunizing event. Considered covariates included sex, age (categorized as <65, 65–74, and >74), IPSS-R (categorized as lower-risk MDS if IPSS-R were very low, low, or intermediate, otherwise categorized as higher-risk MDS), WHO classification, RhD status, mutational status as binary variables comparing patients with the mutation to those without (categorized into mutations involved in chromatin modification, DNA methylation, splicing machinery, cohesin complex, signaling, transcriptional regulation, TP53, and others, Table S1 for details). Number of mutations (categorized as 0 or SF3B1 without TP53 mutation or complex karyotype, 1–2, or ≥3). DAT-positivity and number of RBC units were included as time varying covariates, using a binary variable to define time after the detection of a positive DAT, and number of transfused RBC units the last year prior to alloimmunization (categorized as 1–4, 5–8, or >8 units).

To investigate the effect of alloimmunization on the transfusion intensity, we set up a time-dependent fixed effects (FEs) Poisson regression model, starting follow-up at the first RBC transfusion. As the outcome of interest was number of RBC units per month, we estimated the incidence rate ratio (IRR) of transfusion intensity after alloimmunization. The FEs model included robust variance estimation to control for correlated observations. Time-dependent variables such as time from the first transfusion was included in the statistical model to acknowledge that disease progression might have an impact on transfusion burden as well as the detection of a positive DAT.

Time-to-event data were graphed using the Kaplan–Meier survival method. Competing risks regression was analyzed using cumulative incidence function based on Fine and Gray's proportional subhazards model.

The post-transfusion hemoglobin increment was estimated before and after immunization in a subgroup analysis. Patients who were diagnosed later than April 2014 were excluded due to incomplete hemoglobin data. We applied a mixed effects linear regression model, similar to the approach used in a study of storage time and post-transfusion hemoglobin increments in a MDS cohort.[22] The outcome variable, hemoglobin increment per RBC unit, was modeled by multiplying the covariates with the number of units at each transfusion episode. Most patients are transfused multiple times with repeated hemoglobin measurements, therefore, we included a random intercept, allowing the drop in hemoglobin concentration to vary with each subject. Because the analyses were based on observational data where the measurements of both pre-and post-transfusion hemoglobin were not standardized, the model included time from transfusion to subsequent hemoglobin measurement. Further, we modeled the hemoglobin increment for the full cohort and used a time-dependent exposure, acknowledging increasingly inefficient erythropoiesis during the course of the disease.

p-values below .05 were considered statistically significant. All statistical analyses were performed using Stata, version 13.1 (StataCorp). The conduct of this study was approved by the regional ethics review board in Stockholm, Sweden (2013/1448-31/1).

3 | RESULTS

3.1 | Characteristics of the MDS cohort

The cohort of 429 patients had received 21,224 RBC units during a total follow-up of 1272 patient-years (Figure 1). Table 1 shows the clinical and diagnostic characteristics of the cohort stratified by alloimmunization status. The male proportion was 59.7% and the median age at diagnosis was 73 years (IQR, 66–79). IPSS-R score was available for 332 patients (89.0%) with primary MDS and was distributed as 11.7% very low, 31.9% low, 24.7% intermediate, 21.7% high, and 9.9% very high (data not shown). In contrast to the male predominance in the full MDS

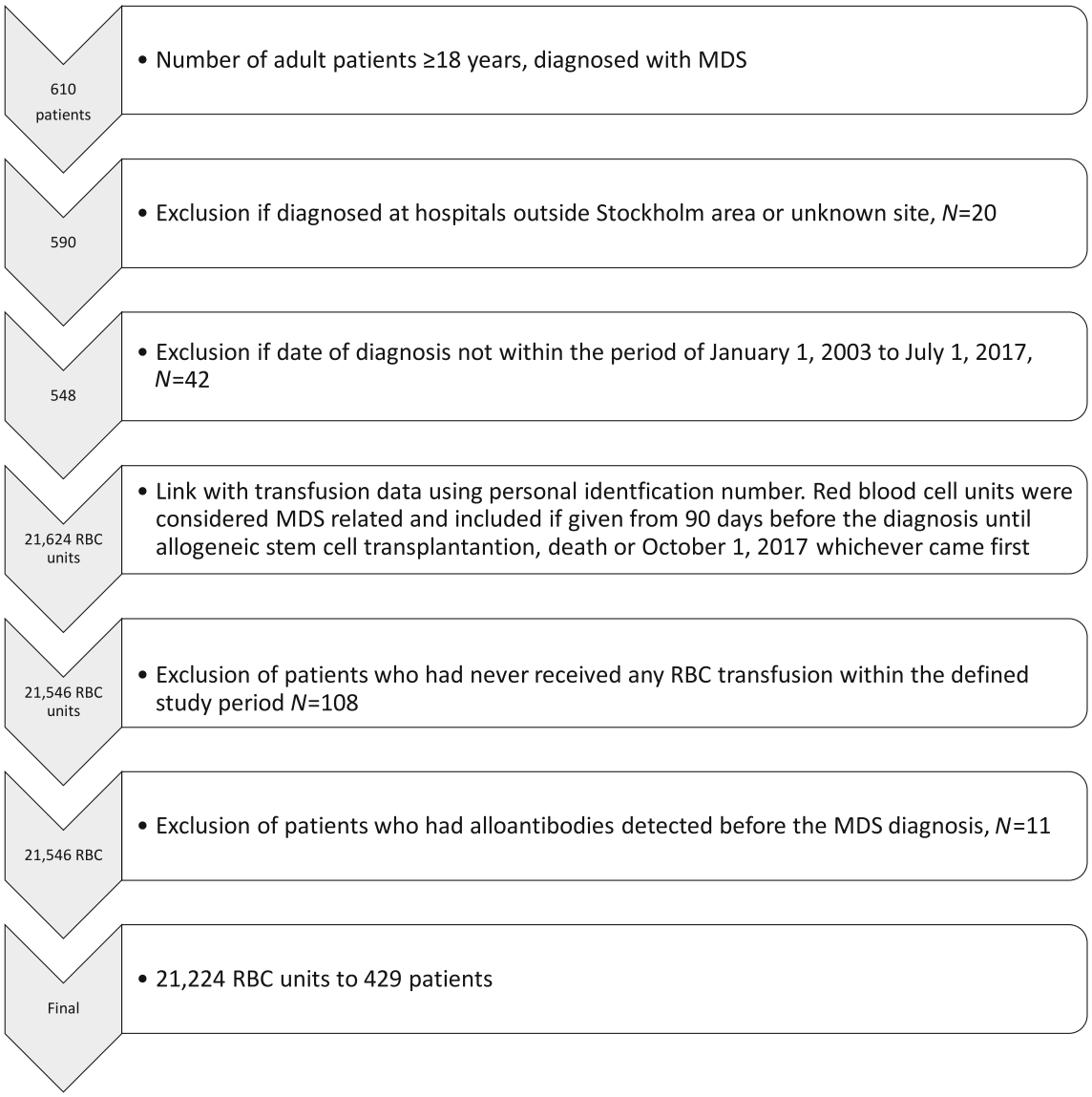

FIGURE 1 Flow-chart of included patients with myelodysplastic syndromes and red blood cell transfusions.

cohort, we observed a female predominance in the alloimmunized group. Patients with IPSS-R high, low, and intermediate had the highest alloimmunization rate (15.3%, 13.2%, and 12.2%). Studying the distribution over WHO classification, we observed a trend toward higher proportions of MDS-del5q, MDS-RS-MLD, MDS-SLD, and MDS-MLD, (21.9%, 18.6%, 18.2%, and 13.7%, respectively) in alloimmunized patients, and lower proportions of MDS-RS-SLD, MDS-EB-2, and MDS/MPN overlap disorders.

3.2 | Cumulative incidence of alloimmunization

The cumulative incidence of alloimmunization at the end of follow-up was estimated to 11.7% (95% CI, 8.9–15.1). At the first occurrence of alloantibody detection, the majority had single alloantibodies ($N = 42$, 84.0%), while eight patients had two ($N = 5$) or three ($N = 3$) alloantibodies detected. Five patients had additional alloantibodies detected after the first event (Table 2).

3.3 | Time and number of transfusions until alloantibody detection

In half of the alloimmunized patients, the first alloantibody was detected within 6.8 (IQR, 1.4–16.3) months after their first MDS-related RBC transfusion (Figure 2A), corresponding to a median time of 12.0 (IQR, 2.6–25.7) months from diagnosis (Figure 2B). In patients with additional alloantibody finding, the new alloantibody/ies were detected 10.2 (IQR, 5.0–21.8) months after the first event. The median number of transfused RBC units until the first alloantibody detection, measured from the first

TABLE 1 Baseline characteristics of total patient cohort and by alloimmunization status.

	MDS cohort	Alloimmunized	Non-alloimmunized
Patients, *N* (% of total)	429 (100.0)	50 (11.7)	379 (88.3)
Sex			
Female	173 (40.3)	29 (16.8)	144 (83.2)
Male	256 (59.7)	21 (8.2)	235 (91.8)
Age at diagnosis, years	73 (66–79)	76 (69–78)	72 (65–79)
IPSS-R in primary MDS			
Very low	39 (11.5)	4 (10.3)	35 (89.7)
Low	106 (31.4)	14 (13.2)	92 (86.8)
Intermediate	82 (24.3)	10 (12.2)	72 (87.8)
High	72 (21.3)	11 (15.3)	61 (84.7)
Very high	33 (9.8)	0 (0.0)	33 (100.0)
2016 WHO classification			
MDS-SLD	11 (2.6)	2 (18.2)	9 (81.8)
MDS-MLD	73 (17.0)	10 (13.7)	63 (86.3)
MDS-RS-SLD	21 (4.9)	1 (4.8)	20 (95.2)
MDS-RS-MLD	43 (10.0)	8 (18.6)	35 (81.4)
del 5q	32 (7.5)	7 (21.9)	25 (78.1)
MDS-EB-1	79 (18.4)	9 (11.4)	70 (88.6)
MDS-EB-2	100 (23.3)	6 (6.0)	94 (94.0)
MDS/MPN overlap disorders	57 (13.3)	4 (7.0)	53 (93.0)
Mixed MDS-UNS/missing	13 (3.0)	3 (23.1)	10 (76.9)
Mutation involved in			
Histone modification	56 (13.1)	10 (17.9)	46 (82.1)
DNA methylation	105 (24.5)	13 (12.4)	92 (87.6)
Splicing machinery	135 (31.5)	17 (12.6)	118 (87.4)
Cohesin complex	15 (3.5)	5 (33.3)	10 (66.7)
Signaling	53 (12.4)	10 (18.9)	43 (81.1)
Transcriptional regulation	42 (9.8)	7 (16.7)	35 (83.3)
TP53	30 (7.0)	1 (3.3)	29 (96.7)
Other	15 (3.5)	2 (13.3)	13 (86.7)
Number of mutations			
0 or SF3B1 (no. TP 53 mutation or complex karyotype)	260 (60.6)	28 (10.8)	232 (89.2)
1–2	107 (24.9)	13 (12.1)	94 (87.9)
≥3	62 (14.5)	9 (14.5)	53 (85.5)
DAT ever positive during follow-up	73 (17.0)	31 (62.0)	42 (11.1)
DAT test performed during follow-up	194 (45.2)	37 (74.0)	157 (41.4)
Number of red cell transfusions per person	27 (10–62)	56 (24–107)	24 (9–54)
Duration of follow-up, years since MDS diagnosis	1.7 (0.7–4.0)	2.5 (1.6–4.2)	1.5 (0.6–4.0)
Duration of follow-up, years since first MDS-related transfusion	1.2 (0.5–2.8)	2 (1.1–3.2)	1 (0.5–2.7)

TABLE 2 Table of alloantibody specificity.

	Alloantibody specificity, first occurrence	Alloantibody specificity, second occurrence	Number of patients	(%)
Number of patients with ≥1 alloantibody during follow-up			50	100
One alloantibody			38	(76.0)
	Anti-K		17	(34.0)
	Anti-E		8	(16.0)
	Anti-C (w)		3	(6.0)
	Anti-Fy (a)		2	(4.0)
	Anti-Kp (a)		2	(4.0)
	Anti-Lu (a)		2	(4.0)
	Anti-C		1	(2.0)
	Anti-Jk (a)		1	(2.0)
	Anti-Yka		1	(2.0)
	Anti-c		1	(2.0)
Two alloantibodies			7	(14.0)
	Anti-E	Anti-E anti-K	1	(2.0)
	Anti-C	Anti-C anti-e	1	(2.0)
	Anti-C anti-C(w)		1	(2.0)
	Anti-E anti-C		1	(2.0)
	Anti-E anti-D		1	(2.0)
	Anti-E	Anti-E anti-c	1	(2.0)
	Anti-K anti-f		1	(2.0)
Three alloantibodies			*4*	(8.0)
	Anti-C anti-D anti-E		1	(2.0)
	Anti-E anti-K anti-Jk(a)		1	(2.0)
	Anti-c anti-Fy anti-K		1	(2.0)
	Anti-E	Anti-E anti-Lu(a) anti-Fy(a)	1	(2.0)
Four alloantibodies			*1*	(2.0)
	Anti-K anti-E	Anti-K anti-E anti-c anti-C(w)	1	(2.0)

MDS-related RBC transfusion, was 12 (IQR, 6–26) (Figure 2C). We modeled the cumulative incidence of alloimmunization in the presence of death as competing risk with sex as a covariate and observed twice the subhazard (2.14, 95% CI, 1.22–3.75) for females compared to males ($p = .008$) (Figure 2D). Neither the number of RBC units ($p = .659$), nor the time ($p = .847$) until a first alloantibody detection differed between the sexes.

3.4 | Alloantibody specificity and direct antiglobulin test

All patients with multiple alloantibodies had at least one alloantibody within the Kell or the Rh blood group systems. Half of the single formed alloantibodies belonged to the Kell ($N = 19$, 50%) blood group system, followed by the Rh ($N = 13$, 34.2%) and to a lesser extent Duffy, Lutheran and Kidd blood group systems (Table 2). In the clinical setting, DAT is tested for if clinical parameters are suggestive of hemolysis or part of antibody identification. We observed that less than half of the patients had been tested for DAT during the study period ($N = 194$) (Table 1). In line with DAT being tested for during antibody investigation, we observed both a higher proportion of patients that had been tested for, and were DAT positive in the alloimmunized group (Table 1). The majority were positive for IgG ($N = 65$, 89%), followed by positivity for both IgG and C3d ($N = 5$, 6.9%), and C3d only ($N = 3$, 4.1%). Among 31 patients where data included both alloantibodies and DAT-positivity during follow-up, 16 patients were subjects of both alloantibodies and

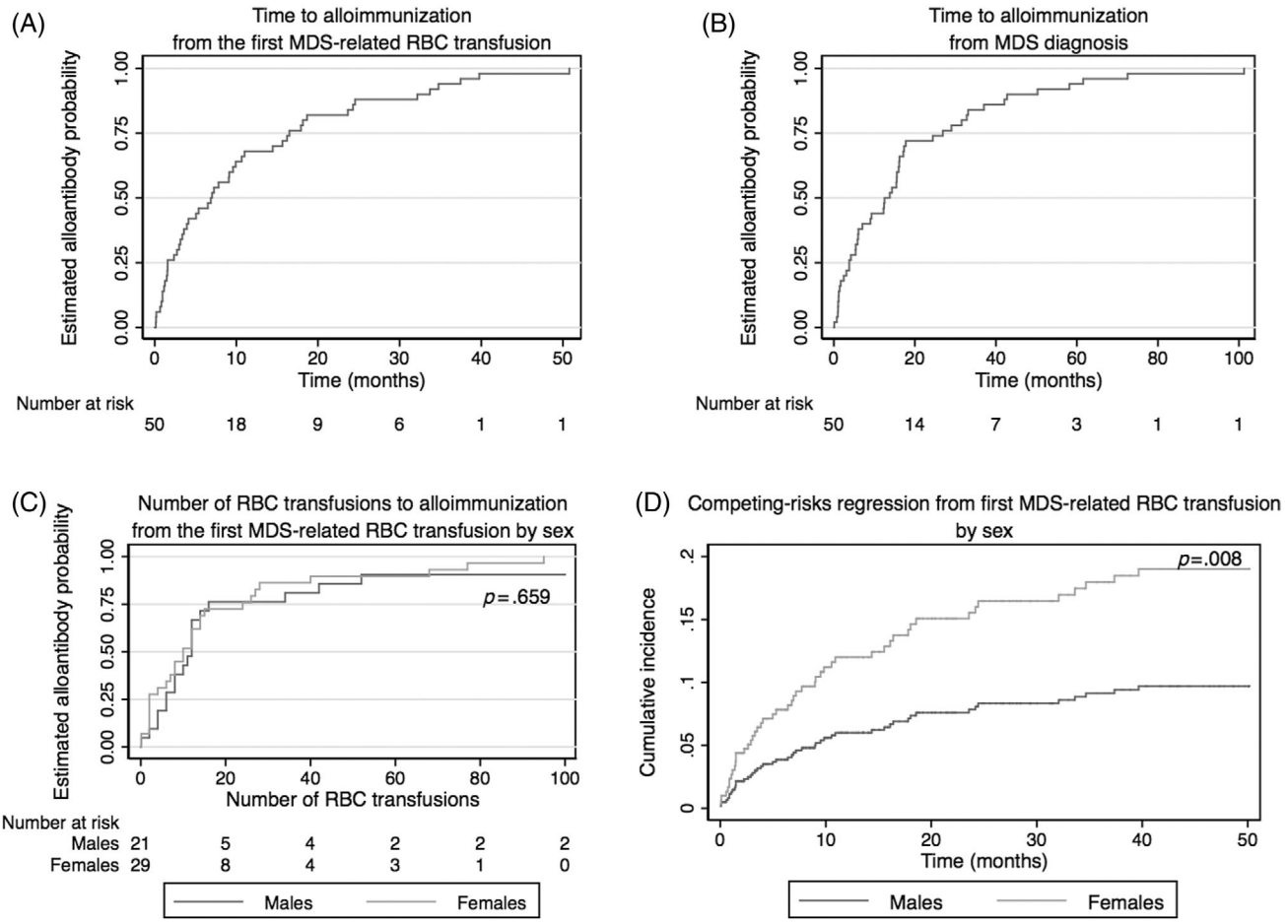

FIGURE 2 Estimated alloantibody probability over time and number of red blood cell (RBC) transfusions. Figures showing time from the first MDS-related red blood cell (RBC) transfusion until alloimmunization (A) and time from MDS diagnosis until alloimmunization (B). (C) Number of RBC transfusions until alloimmunization by sex and (D) the cumulative incidence of alloimmunization stratified by sex.

DAT-positivity detected on the same date. Seven patients had DAT-positivity as the first registered laboratory finding with a median interval of 103 days (IQR, 19–837) between DAT-positivity and detected alloantibodies. In the eight patients with DAT-positivity after alloantibody detection, the corresponding median interval was 44 days (IQR, 19–644). In 23 patients, DAT was registered before starting transfusion therapy.

3.5 | Factors associated with alloimmunization

Possible factors associated with alloimmunization were evaluated using univariable Cox regression for each considered covariate. Significant estimates were observed: in female sex with hazard ratio (HR) of 1.85 (95% CI, 1.06–3.26) compared to male sex; more than 8 RBC transfusions the past year HR 2.99 (95% CI, 1.44–6.24), compared to patients receiving 1–4 RBC units ($N = 29$ and $N = 15$, respectively; cohesin complex mutations HR 3.95 (95% CI, 1.56–10.00) compared to patients without, and DAT-positivity with HR of 9.11 (95% CI, 5.21–15.90) compared to patients without a positive test. Interestingly, the only molecular finding being associated with alloimmunization was cohesin complex mutations, and no other disease related variable such as WHO classification or IPSS-R were associated with alloimmunization. In the multivariable analysis which included both diagnostic parameters and time varying covariates, we found that female sex and a positive DAT status were significantly and independently associated with alloimmunization (Table 3).

3.6 | Changes of the transfusion burden after alloimmunization

Our study aimed to investigate the clinical implications of alloimmunization, specifically with regard to any

TABLE 3 Univariable and multivariable Cox regression of factors and their association with alloimmunization.

***N* = 429**		**Univariable HR (95% CI)**	***p*-value**	**Multivariable[a] HR (95% CI)**	***p*-value**
Sex					
Male	256	1.0 (ref)		1.0 (ref)	
Female	173	1.85 (1.06–3.26)	0.032	2.02 (1.08–3.78)	0.028
Age at diagnosis, years					
<65	96	0.76 (0.31–1.87)	0.551	0.74 (0.28–1.94)	0.535
65–74	141	1.0 (ref)		1.0 (ref)	
>74	192	1.34 (0.72–2.52)	0.357	1.4 (0.71–2.75)	0.333
IPSS-R					
Higher-risk	105	1.0 (ref)		1.0 (ref)	
Lower-risk	227	0.77 (0.38–1.57)	0.613	1.30 (0.70–2.42)	0.407
2016 WHO classification					
MDS without ring sideroblasts (SLD + MLD)	84	1.0 (ref)			
MDS with ring sideroblasts	64	0.74 (0.31–1.75)	0.486	n/a	n/a
5Q-	32	1.10 (0.43–2.81)	0.846	n/a	n/a
MDS-EB-1, 2	179	0.71 (0.33–1.53)	0.383	n/a	n/a
MDS-MPN	57	0.48 (0.15–1.48)	0.201	n/a	n/a
MDS-UNS or missing	13	2.00 (0.56–7.08)	0.185	n/a	n/a
Bone marrow blast (%)					
<5	217	1.0 (ref)			
≥5%	205	0.72 (0.38–1.34)	0.300	n/a	n/a
RhD status					
Negative	49	1.0 (ref)		1.0 (ref)	
Positive	361	1.18 (0.47–2.98)	0.731	1.73 (0.65–4.62)	0.273
Splicing factor mutation					
No	294	1.0 (ref)			
Yes	135	0.90 (0.50–1.62)	0.736	n/a	n/a
Signaling factor mutation					
No	376	1.0 (ref)			
Yes	53	1.79 (0.89–3.57)	0.101	n/a	n/a
Cohesin complex mutation					
No	414	1.0 (ref)			
Yes	15	3.95 (1.56–10.00)	0.004	n/a	n/a
Epigenetic factor mutation					
No	296	1.0 (ref)			
Yes	133	1.02 (0.57–1.82)	0.953	n/a	n/a
Transcription factor mutation					
No	387	1.0 (ref)			
Yes	42	1.75 (0.78–3.90)	0.172	n/a	n/a
TP53 mutation					
No	399	1.0 (ref)			
Yes	30	0.32 (0.04–2.32)	0.260	n/a	n/a

(Continues)

TABLE 3 (Continued)

N = 429		Univariable HR (95% CI)	p-value	Multivariable[a] HR (95% CI)	p-value
Number of mutations					
0 or SF3B1 (no. TP53 mutation or complex	260	1.0 (ref)		1.0 (ref)	
1–2	107	1.29 (0.66–2.49)	0.453	1.41 (0.64–3.08)	0.391
≥3	62	1.54 (0.72–3.26)	0.264	1.66 (0.74–3.73)	0.219
Number of RBC transfusions the past year (time-dep.) in					
1–4	15	1.0 (ref)			
5–8	6	1.45 (0.54–3.95)	0.456	n/a	n/a
>8	29	2.99 (1.44–6.24)	0.003	n/a	n/a
DAT status (time-dep.)					
Negative	27	1.0 (ref)		1.0 (ref)	
Positive	23	9.11 (5.21–15.90)	<0.001	9.72 (5.31–17.74)	<0.001

[a]Adjusted for sex, age, IPSS-R, RhD status, number of mutations, and DAT-status.

TABLE 4 Transfusion rate before and after immunization.

	Univariable model			Multivariable model[a]		
	Number of patients	Transfusions/ person-month	Incidence rate ratio (95% CI)	Likelihood ratio p-value	Incidence rate ratio (95% CI)	Likelihood ratio p-value
Alloimmunization status						
Before detected alloantibodies	429	17963/9645 = 1.9	1.0 (ref)		1.0 (ref)	
After detected alloantibodies	50	3261/1039 = 3.1	1.53 (1.17–2.01)	0.002	1.33 (0.98–1.80)	0.066
Direct antiglobulin test status						
Before positive test	429	17391/9201 = 1.9	1.0 (ref)		1.0 (ref)	
After positive test	73	3833/1483 = 2.6	1.57 (1.16–2.12)	0.003	1.38 (1.00–1.90)	0.048
Alloimmunization status						
Before detected alloantibodies	50	1028/538 = 1.9	1.0 (ref)		1.0 (ref)	
After detected alloantibodies	50	3261/1039 = 3.1	1.57 (1.17–2.11)	0.003	1.32 (0.90–1.91)	0.156
Direct antiglobulin test status						
Before positive test	50	2109/937 = 2.3	1.0 (ref)		1.0 (ref)	
After positive test	31	2180/640 = 3.4	1.75 (1.15–2.69)	0.009	1.49 (0.90–2.48)	0.124

[a]Adjusted for sex and DAT-status.

changes in transfusion requirements. Descriptive data revealed that individuals who were confirmed to have alloimmunization had a significantly higher burden of RBC transfusions, with a median of 47 (IQR 17–89) units per person compared to 24 (IQR 9–54) units in the non-alloimmunized group ($p = .015$) (Table 1). To evaluate if this difference was due to a higher transfusion intensity following alloimmunization, we included all patients and analyzed the transfusion rate before and after alloimmunization using a FEs Poisson regression. In total, the 50 patients who had detected alloantibodies received 3261 units of RBC transfusions following the first alloantibody detection, corresponding to an increase of the transfusion burden from 1.9 to 3.1 RBC units/person-month (Table 4). In the univariable analysis of transfusion intensity after registered alloimmunization,

TABLE 5 Estimated difference of the post-transfusion hemoglobin increment per RBC unit following alloimmunization.

	Estimated difference (g/L) per RBC unit, (95% CI)	*p*-value	Estimated difference (g/L) per RBC unit, (95% CI)[a]	*p*-value
Before alloimmunization	0 (ref)		0 (ref)	
After alloimmunization	−0.85 (0.00–(−1.71))	0.051	−1.40 (−0.52–(−2.28))	0.002

[a]Adjusted for sex and DAT+.

we estimated the IRR to 1.53 (95% CI, 1.17–2.01), and after a positive DAT test 1.57 (95% CI, 1.16–2.12). Combining both events in a multivariable analysis also including sex, we estimated the IRR after alloimmunization to 1.33 (95% CI, 0.98–1.80) and 1.38 (95% CI, 1.00–1.90) after a positive DAT (Table 4). In an additional analysis, we included only alloimmunized patients ($N = 50$) and observed similar estimates as in the full cohort, but with loss of statistical significance in the multivariable model (Table 4).

3.7 | Changes of the post-transfusion hemoglobin increment after alloimmunization

To evaluate whether the increased transfusion intensity was also reflected by a smaller hemoglobin increase after immunization compared to before immunization, we estimated the difference between "post-transfusion hemoglobin increment," before and after alloimmunization. Hemoglobin values were available for 211 patients (49.2%) of these, 28 patients were had detected alloantibodies during follow-up. In total, this resulted in 3215 RBC transfusion episodes with pre- and post-transfusion hemoglobin measurements for analysis. We estimated that the post-transfusion hemoglobin increment after alloimmunization was 0.85 g/L lower (95% CI, 0.00–1.71) per RBC unit compared to before alloimmunization, possibly contributing to an increased transfusion need over time. Recognizing the sex bias and the possible confounding of autoantibodies, we adjusted for sex and DAT+, which resulted in a slight attenuation of the post-transfusion hemoglobin increment following the first detection of alloimmunization, 1.40 g/L lower (95% CI, 0.52–2.28) (Table 5).

4 | DISCUSSION

In this study of alloimmunization and associated clinical consequences in a Swedish cohort of 429 patients with MDS, we evaluated the cumulative incidence of alloimmunization, factors potentially associated with alloimmunization and the hypothesized increased transfusion intensity following immunization. Published papers have reported a higher incidence of alloimmunization in the MDS population compared to the incidence in the overall recipient of RBC transfusions.[6–8,19,20,23] We included patients that had at least one registered transfusion episode and observed that the risk of alloimmunization was 11.7% which is close to the lower range in other published papers who reported risks between 12% and 27%. This may be due to a shorter follow-up or a lower transfusion burden at inclusion in this study.[6–8,19,20] The majority of the patients had at least one alloantibody within the Kell or Rh blood group systems ($N = 44$, 88%), which is consistent with previous studies.[7,16,18]

Females had a higher alloimmunization rate during follow-up, which has also been observed in a large observational study of alloimmunization in RBC transfusion recipients overall.[18] Almost one fifth of all women with MDS developed alloimmunization during follow-up and we could validate that female sex was independently associated with alloimmunization.[24] We did not have information on previous pregnancy, but tried to evaluate indirect signs of evanescent primary immunization and hypothesized that primary immunization was associated with a more rapid finding of alloantibodies after MDS-associated blood transfusions. Here, we did not observe any sex difference with regard to the time from the first RBC transfusion or number of RBC transfusions until the first detected alloantibody, the first mentioned could otherwise have supported pregnancy as a possible risk factor for a previous sensitization.[25]

We hypothesized that patients with lower-risk MDS would be more prone to develop alloimmunization given their better overall survival and presumed higher exposure of RBC transfusions over the disease course but when categorizing patients into lower and higher-risk MDS, we found similar hazard ratios of alloimmunization. Having an acquired mutation involved in the cohesin complex was the only disease characteristics associated with immunization. This was also the smallest group consisting of only five patients. MDS-specific treatment was not included in this study, but other groups have found an association between lower immunization rates and treatment with hypomethylating agents and other disease modifying therapies.[7,26] The probability of a positive DAT was 42% (95% CI, 31.7–54.0) in this study

given alloimmunization, and several other studies have confirmed increased incidence of autoantibodies in alloimmunized patients with MDS.[7,23]

Theoretically, we would not expect an increase of the transfusion need after alloimmunization alone, since alloimmunized patients are pheno- or genotyped and RBC units are matched for by the specific alloantibody and sometimes by further matching. Irrespectively, we observed an increased transfusion intensity up to 57% after detected alloantibodies. When including time-dependent DAT-positivity, the statistical significance was lost for alloimmunization. These results could argue for a positive DAT, not the alloimmunization as such, being the primary reason for increasing transfusion needs, as a consequence to increased hemolysis. When we estimated the difference between the "post-transfusion hemoglobin increment" before and after alloimmunization, we found lower post-transfusion hemoglobin increase after alloimmunization, possibly indicating increased destruction of RBCs. This finding is interesting but should be interpreted with care given the small estimates and borderline significance. Results might also be confounded by unmeasured differences between those who were and were not immunized.

Strengths of this study include the well-characterized cohort of MDS patients, where baseline characteristics were largely in line with national and international published data.[27,28] Transfusions given in the county of Stockholm were considered complete due to the computerized local transfusion database. However, despite starting with a fairly large cohort of patients, data on alloimmunization were modest which is why we could not study the transfusion intensity and post-transfusion hemoglobin increment after alloimmunization in subgroups with different types and combinations of immunizations. Further limitations are based on the heterogeneity of the data, mainly with regard to DAT which is not performed in all patients and not regularly, but instead on the attending physicians call or at antibody identification which might introduce bias. In addition, a positive DAT might be due to a recently detected alloantibody which must be taken into consideration when interpreting the results. A prospective study could better evaluate the relationship between alloantibodies and DAT-positivity as well as the impact of DAT-positivity and subclinical hemolysis.

To conclude, in this study female sex was associated with alloimmunization, but this finding is not sufficiently predictive in the clinical setting to help distinguish which patients are at greatest of alloimmunization. However, considering that alloimmunization is associated with clinical consequences such as DAT-positivity and increased transfusion needs, a strategy of typing and upfront matching at least for Rhesus and Kell which constitute more than 80% of the alloantibodies, could be of clinical importance in patients with MDS.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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