Red cell autoimmunization and alloimmunization in myelodysplastic syndromes: prevalence, characteristic and significance

Rakchha Chhetri,[1,2] Li Yan A. Wee,[1,2] Romi Sinha,[3] Monika M. Kutyna,[2,4] Anh Pham,[5] Helen Stathopoulos,[5] Lakshmi Nath,[6] Shriram V. Nath,[6,7] Nicholas Wickham,[8] Tim Hughes,[1,2,4] Deepak Singhal,[1,2,4] David J. Roxby[5,9] and Devendra K. Hiwase[1,2,4]

[1]Haematology Department, Royal Adelaide Hospital, Central Adelaide Local Health Network; [2]Precision Medicine, South Australian Health and Medical Research Institute; [3]Blood, Organ and Tissue Programs, Public Health and Clinical Systems, Department of Health and Wellbeing; [4]School of Medicine, The University of Adelaide; [5]Transfusion Medicine, SA Pathology; [6]Haematology, Clinpath Laboratories; [7]Adelaide Haematology Centre, Ashford Specialist Centre; [8]Adelaide Cancer Centre, Ashford Specialist Centre and [9]Haematology and Genetic Pathology, Flinders University, Bedford Park, Adelaide, South Australia, Australia

Correspondence: DEVENDRA K. HIWASE - devendra.hiwase@sa.gov.au.
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Supplementary Section

Methods

Pre-transfusion EDTA blood samples were submitted to the transfusion laboratory before each transfusion for ABO/RhD typing and antibody screening. Antibody screening was performed using a 3-cell screen panel and the indirect antiglobulin technique (IAT) using the DiaMed Microtyping System. A positive antibody screen was further investigated by testing patient plasma against an extensively phenotyped 11-cell panel and including an auto-control (using patient's RBCs and plasma).

In samples with a positive auto control, a direct antiglobulin test (DAT; DiaMed Microtyping System) was performed to test for the presence of RBC bound IgG or complement (C3d) or both. Elution studies were performed, using acid glycine, on IgG DAT positive samples while C3d samples were not subjected to elution testing. Eluates were tested against an 11-cell panel by IAT using the DiaMed Microtyping System. Elution results were reported as non-reactive, non-specific reactivity (reactive with all panel cells with no identified specificity) or reactive with antibody specificity.

RBC autoimmunization was assessed in 927 MDS patients who were followed for at least three months. We also compared the incidence of RBC autoantibodies in RBC transfused (n=794) and non RBC-transfused MDS patients (n=126). As we identified close association of RBC autoimmunization with RBC-alloimmunization, we characterized RBC alloantibodies in all RBC transfused patients (n=794; Fig S1-Level 1 exclusion criteria). Most of the MDS patients are older and some require RBC transfusions before the MDS diagnosis for clinical indication unrelated to MDS. Hence, we closely assessed clinical reasons for RBC transfusions prior to the MDS diagnosis. All RBCs transfused to alleviate persistent or progressive anemia within three months and after the MDS diagnosis were considered MDS-related (n=749; Figure S1). While RBCs transfused >3 months before MDS diagnosis for MDS-unrelated causes such as gastrointestinal bleeding, surgery, pregnancy or trauma was considered MDS-unrelated (Figure S1-Level 2 exclusion criteria).

To assess the impact of autoimmunization on RBC transfusion requirements, we compared the total number and intensity of RBC transfusions (average number of RBC units transfused/month) during pre- and post-alloimmunization period in

patients with and without autoantibodies. As the disease course of MDS is dynamic, various disease and/or treatment related factors could influence RBC transfusion requirements. To minimize the influence of these factors, we excluded 36 alloimmunized patients for various reasons detailed in Figure S1 (Level 3 exclusion criteria). RBC transfusion requirements analysis during pre- and post-alloimmunization period was based on 50 eligible alloimmunized patients (Fig S1). Of these 50 patients, DAT results could not be retrieved for one patient, and was excluded from transfusion intensity analysis of autoantibody positive and negative patients.

Statistical analysis

Categorical variables were summarized by frequency analysis, and comparison between groups was done using Fisher's exact test or the Chi square test. Numerical variables were summarized by mean (±SD) and comparison of numerical variables between the groups using non-parametric tests such as the Mann-Whitney or Kruskall Wallis ANOVA tests.

Results

In our study, 12 patients developed anti-D, six following transfusion of RhD positive platelets and four following RBC transfusions. In the remaining two patients, there were no documented RBC or platelets transfusions in the participating institutions.

Of the eight patients with autoantibodies detected six months before or after alloantibody, two had chronic lymphocytic leukemia and the remaining six patients had no documented history of autoimmune hemolytic anemia.

Table S1: Demographic and clinical profile of 749 RBC-transfused MDS patients

Clinical Parameters	Alloimmunized patients n=86 (11.5%)	Non alloimmunized patients n=663 (88.5%)	P
Female: Male	33:53 (38%:62%)	232:431 (35%:65%)	0.55
Median age at diagnosis years (IQR range)	74 (68-80) years	72 (63-78) years	
WHO 2016 MDS Subtypes			
MDS-SLD	6 (7%)	39 (6%)	0.63
MDS-MLD	21 (24%)	136 (21%)	0.13
MDS-RS-SLD	4 (5%)	23 (3%)	0.53
MDS-RS-MLD	3 (3%)	26 (4%)	0.99
MDS-EB-1	15 (17%)	70 (11%)	0.06
MDS-EB-2	8 (9%)	81 (12%)	0.59
MDS with isolated del(5q)	5 (6%)	14 (2%)	0.05
Hypoplastic MDS	1 (1%)	12 (2%)	0.99
MDS-U	0	2 (<0.5%)	0.99
AML (<30% Blasts)	3 (3%)	46 (7%)	0.35
Atypical CML	0	4 (1%)	0.99
CMML	7 (8%)	76 (11%)	0.46
MDS/MPN-U	1 (1%)	17 (3%)	0.71
MDS/MPN-RS-T	0	9 (1%)	0.60
T-MN	12 (14%)	108 (16%)	0.64
IPSS-R Groups			
Very low	6 (7%)	84 (13%)	0.15
Low	33 (38%)	150 (23%)	**0.002**
Intermediate	19 (22%)	112 (17%)	0.22
High	10 (12%)	95 (14%)	0.62
Very high	10 (12%)	94 (14%)	0.62
Not applicable*	8 (9%)	92 (14%)	
Missing data**	0	36 (5%)	
Transfusion dependency status			
Transfusion dependent	66 (77%)	387 (58%)	**0.001**
Transfusion independent	17 (20%)	221 (33%)	0.009
Immature data/died within 4 months of diagnosis/ data missing***	3 (3%)	55 (8%)	
Treatment for MDS			
Supportive care	50 (58%)	361 (54%)	0.56
Disease-modifying therapy	35 (41%)	274 (41%)	0.99
Data missing	1 (1%)	28 (4%)	

Abbreviations: MDS-SLD: MDS with single lineage dysplasia; MDS-MLD: MDS with multilineage dysplasia; MDS-RS-SLD: MDS-SLD with ring sideroblasts; MDS-RS-MLD: MDS-MLD with ring sideroblasts;MDS-EB-1: MDS with excess blasts-1;MDS-EB-2: MDS with excess blasts-2;MDS-U:MDS Unclassifiable; AML: Acute Myeloid Leukemia; CMML: chronic myelomonocytic leukemia; MDS/MPN-U: myelodysplastic/myeloproliferative neoplasms, unclassifiable; MDS/MPN-RS-T: MDS/MPN-RS and thrombocytosis; T-MN: therapy-related myeloid neoplasms. *Revised International Prognostic Scoring System (IPSS-R) is not applicable for T-AML; proliferative CMMLs, atypical CML, MDS/MPN-U, MDS/MPN-RS-T.**IPSS-R could not be calculated due to missing blast % / blood counts / cytogenetics at diagnosis date.***RBC transfusion dependency could not be assessed in patients who had recently commenced transfusions (n=26) or died within 4 months of diagnosis (n=24) or transfusion history unknown (n=22).

SUPPLEMENTARY FIGURES

Fig. S1

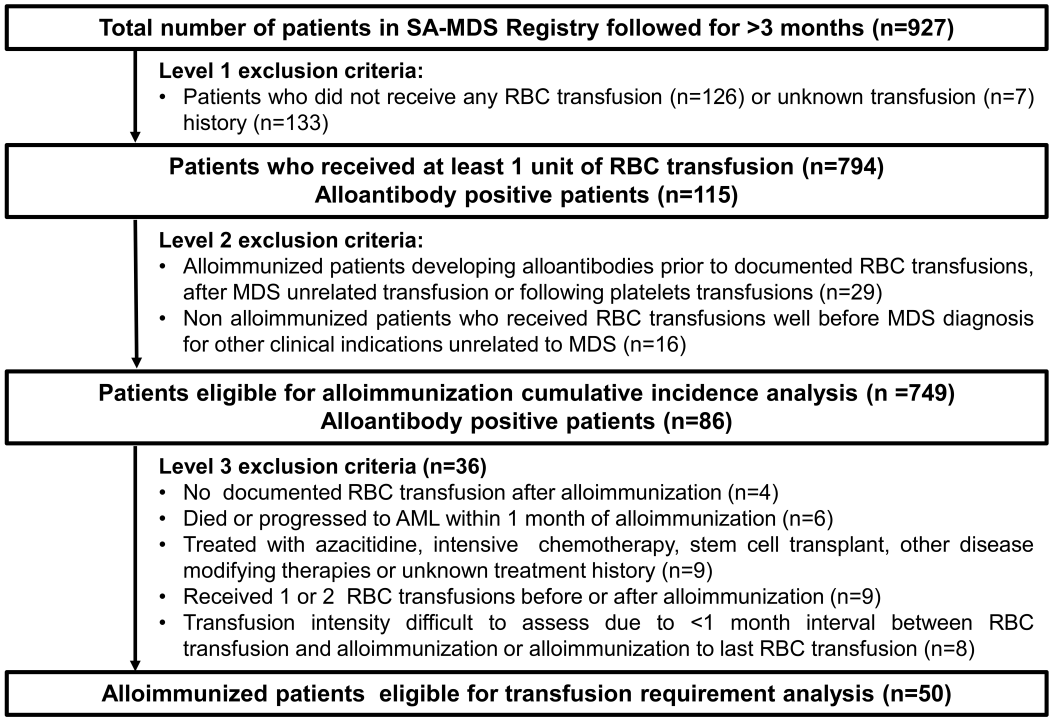

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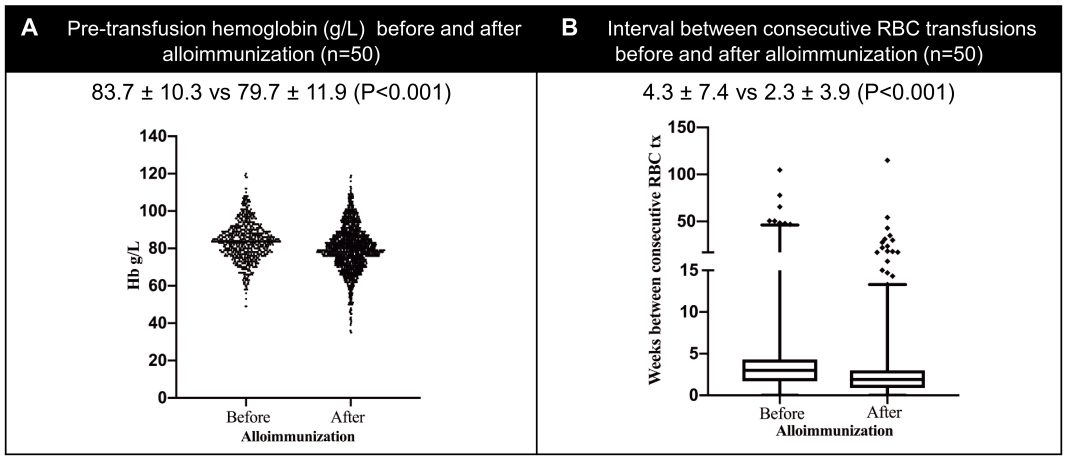
Figure S1. Flow chart depicting patient selection

Fig. S2



C Comparison of pre-transfusion hemoglobin (g/L) and interval between consecutive RBC transfusions during pre- and post-alloimmunization period

Patient groups	Pre-transfusion hemoglobin during pre- and post-alloimmunization period	Interval (weeks) between consecutive RBC tx during pre- and post-alloimmunization period
Autoab^{positive} Cohort (n=32)	84.3 ± 11.6 vs 79.9 ± 11.6 P<0.001	3.6 ± 5.7 vs 2.2 ± 4.2 P<0.001
Autoab^{negative} Cohort (n=17)	79.8 ± 8.7 vs 79.6 ± 14.8 P=0.88	6.4 ± 11.0 vs 2.8 ± 2.5 P<0.001

Figure S2. Compared to pre-alloimmunization period, pre-RBC transfusion Hb levels were significantly lower despite increased RBC transfusions frequency during post-alloimmunization period. (A) Pre-transfusion hemoglobin level was significantly lower in post-alloimmunization period compared to pre-alloimmunization period. (B) Interval between consecutive RBC transfusions was significantly shorter following alloimmunization period compared to pre-alloimmunization period. (C) Comparing pre-transfusion hemoglobin and interval between consecutive RBC transfusions in autoantibody positive and autoantibody negative cohort.

Fig. S3

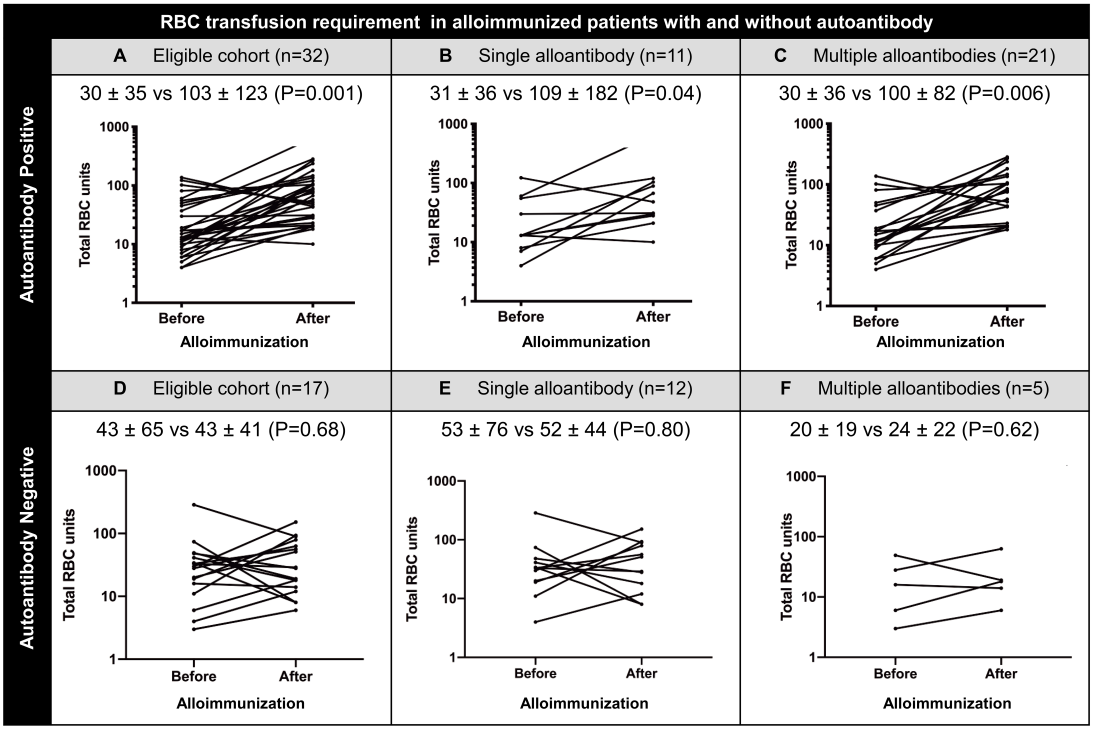


Figure S3. Autoimmunization is associated with significant increase in RBC transfusion requirement in alloimmunized patients: Total RBC transfusion requirement was higher in alloimmunized patients developing autoantibodies in (A) all eligible, (B) single alloantibody (C) and multiple alloantibodies patients. However, RBC transfusion requirement did not change significantly in alloimmunized patients without autoantibodies in (D) all eligible (E) single alloantibody (F) and multiple alloantibodies patients.