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Red Blood Cell Alloimmunization in Korean Patients With Myelodysplastic Syndrome and Liver Cirrhosis

Hyun-Young Kim, M.D.[1], Eun-Jung Cho, M.T.[2], Sejong Chun, M.D.[3], Kyeong-Hee Kim, M.D.[4], and Duck Cho ⓘ, M.D.[2,5]

[1]Department of Laboratory Medicine, Gyeongsang National University Hospital, Gyeongsang National University School of Medicine, Jinju, Korea; [2]Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; [3]Department of Laboratory Medicine, Chonnam National University Hospital, Chonnam National University Medical School, Gwangju, Korea; [4]Department of Laboratory Medicine, Dong-A University College of Medicine, Busan, Korea; [5]Stem Cell & Regenerative Medicine Institute, Samsung Medical Center, Seoul, Korea

Red blood cell (RBC) alloimmunization varies across human populations and ethnic groups. We evaluated the characteristics of RBC alloimmunization and compared the risk of alloimmunization in Korean patients with myelodysplastic syndrome (MDS) and liver cirrhosis (LC), two representative diseases in which chronic transfusion is required. In total, 115 MDS patients and 202 LC patients transfused with RBCs between 2013 and 2015 were retrospectively included. Twenty patients (6.3%) were newly alloimmunized (five MDS patients, 4.3%; 15 LC patients, 7.4%). The median number of RBC units transfused in alloimmunized patients was nine (interquartile range, 4–15 units). As the number of transfused RBC units increased, the cumulative risk of alloimmunization was higher in LC than in MDS patients ($P=0.001$). The most common alloantibody detected in patients was anti-E (45%), followed by anti-c (17%), anti-e (10%), anti-C (7%), anti-Fy^b (7%), and anti-Jk^a (7%). The present data indicate the need for matching of extended RBC antigens (Rh, Duffy, and Kidd systems) for chronically transfused patients with MDS and LC in Korea.

Key Words: Red blood cell, Alloimmunization, Myelodysplastic syndrome, Liver cirrhosis, Korea

Red blood cell (RBC) alloantibodies are formed following exposure to RBC alloantigens through transfusion, pregnancy, or transplantation [1]. RBC alloimmunization increases the risk of a hemolytic transfusion reaction and makes it difficult to find compatible blood [2]. Alloimmunization is more common in patients who require chronic transfusions, those with sickle cell anemia and thalassemia, and those with hematologic malignancies, especially myelodysplastic syndrome (MDS) [3]. Several studies have sought to identify the risk factors for RBC alloimmunization, suggesting that alloimmunization is influenced by clinical condition, inflammatory status, genetic factors, gender, age, number of RBC units transfused, and immunogenicity of exposed antigens [4-6].

When assessing the risk of alloimmunization to RBCs, commonly occurring diseases in the countries of interest should be considered. MDS and liver cirrhosis (LC) are representative diseases requiring chronic transfusion in Koreans [7]. MDS, characterized by cytopenias associated with ineffective hematopoiesis, is usually treated with disease-modifying therapies and supportive care, such as transfusion [8]. In advanced liver diseases, such as LC, bleeding due to esophageal varices occurs in up to 25% of patients and can be further aggravated by multiple coagulation abnormalities associated with liver dysfunction [9]. However, there is no data on RBC alloimmunization in Korean patients with MDS and LC. Therefore, we evaluated the characteristics of RBC alloimmunization and compared the risk of alloimmunization in Korean MDS and LC patients.

We retrospectively reviewed medical records of MDS and LC

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patients transfused with RBCs at Samsung Medical Center, Seoul, Korea. This study was approved by the Institutional Review Board of Samsung Medical Center. Among patients transfused with RBCs between January 2013 and December 2015, a list of patients diagnosed as having MDS or LC was obtained from the electronic medical records. Patients displaying alloantibodies before the first RBC transfusion at the center or with no follow-up antibody screening test (AST) results were excluded. Initial AST was carried out with RBC panels from the Galileo NEO (Immucor Gamma, Norcross, GA, USA) or from QWALYS 3 (Diagast, Loos, France). When an antibody was detected, the AST was reiterated by a LISS/Coombs gel assay using ID-DiaCell I, II (Bio-Rad, Cressier-sur-Morat, Switzerland), and then, antibody specificity was identified using the ID-DiaPanel (Bio-Rad). If antibodies of undetermined specificity were found, they were additionally identified by the tube technique using the RESOLVE Panel A (Ortho-Clinical Diagnostics, Raritan, NJ, USA). Alloimmunization was defined as the positive conversion of AST after RBC transfusion in patients with initial negative AST. Follow-up duration of AST was defined as the period between the initial negative AST and follow-up positive AST in alloimmunized patients, and the period between the initial and last negative AST in non-alloimmunized patients. The number of transfused RBC units was recorded during the follow-up of AST.

Data were summarized as median with interquartile range (IQR) or as numbers and percentages. Patient characteristics were compared according to alloimmunization status, using the Mann–Whitney, chi-square, or Fisher's exact test. RBC alloimmunization rates were compared between the MDS and LC patients using the chi-square test. Cumulative risk of alloimmunization was estimated using a Kaplan–Meier plot with alloimmunization as the event and the cumulative number of transfused RBC units as the time variable. Alloimmunization risk was compared using the log-rank test. All statistical analyses were performed using IBM SPSS Statistics version 21 (IBM Corp., Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

Among the 133 MDS patients and 254 LC patients, 18 MDS and 52 LC patients were excluded for the following reasons: three MDS and nine LC patients were positive for initial AST, and 15 MDS and 43 LC patients did not undergo follow-up AST after RBC transfusion. Finally, 115 MDS patients and 202 LC patients were included (median age: 63 years; IQR: 54–72 years). The median number of transfused RBC units and the median duration of follow-up in AST were eight units (IQR: 3–24 units) and 102 days (IQR: 12–434 days), respectively. Patients received pre-storage leukocyte-reduced RBCs obtained from the Korea Red Cross blood center, leukocyte-depleted RBCs prepared in Samsung Medical Center, or general RBCs as clinically ordered. None of the patients had received antigen-matched RBC units before the RBC transfusion in this study.

There were 20 newly alloimmunized patients among the 317 patients (6.3%) (Table 1). The median number of RBC units transfused in alloimmunized patients was nine, which was not significantly different from that in non-alloimmunized patients (eight units; $P = 0.929$).

With regard to disease, alloantibodies were observed in five (4.3%) and 15 (7.4%) MDS and LC patients, respectively ($P = 0.278$), and the median transfused RBC units (MDS vs. LC; 12 vs. 8 units; $P = 0.405$) did not differ. However, as the number of transfused RBC units increased, the cumulative risk of alloimmunization was higher in LC than in MDS patients (log-rank $P = 0.001$) (Fig. 1).

Of the 20 patients with alloantibodies, 10 (50%) had a single antibody and eight (40%) had multiple antibodies (Table 2). Six-

Table 1. Characteristics of MDS and LC patients

Characteristics	MDS			LC			Total		
	Non-alloimmunized (N=110)	Alloimmunized (N=5)	P	Non-alloimmunized (N=187)	Alloimmunized (N=15)	P	Non-alloimmunized (N=297)	Alloimmunized (N=20)	P
Age (yr)	66 (54–74)	69 (58–81)	0.463	61 (54–69)	62 (48–71)	0.712	63 (54–72)	63 (50–71)	0.987
Male, N (%)	72 (65.5)	4 (80.0)	0.661	103 (55.1)	6 (40.0)	0.260	175 (58.9)	10 (50.0)	0.433
Transfused RBC units (N)*	26 (14–48)	12 (6–23)	0.068	4 (2–10)	8 (4–14)	0.055	8 (3–26)	9 (4–15)	0.929
F/U duration of AST (day)†	277 (95–602)	910 (39–2,064)	0.355	35 (4–235)	748 (24–1,817)	<0.001	90 (10–395)	829 (33–1,862)	0.001

Data are shown as median (interquartile range) or number (percentage).
*In non-alloimmunized patients, N of RBC units transfused between the initial negative AST and the last negative AST; in alloimmunized patients, N of RBC units transfused between the initial negative AST and the F/U positive AST; †In non-alloimmunized patients, the period between the initial negative AST and the last negative AST; in alloimmunized patients, the period between the initial negative AST and the F/U positive AST.
Abbreviations: RBC, red blood cell; F/U, follow-up; AST, antibody screening test; MDS, myelodysplastic syndrome; LC, liver cirrhosis.
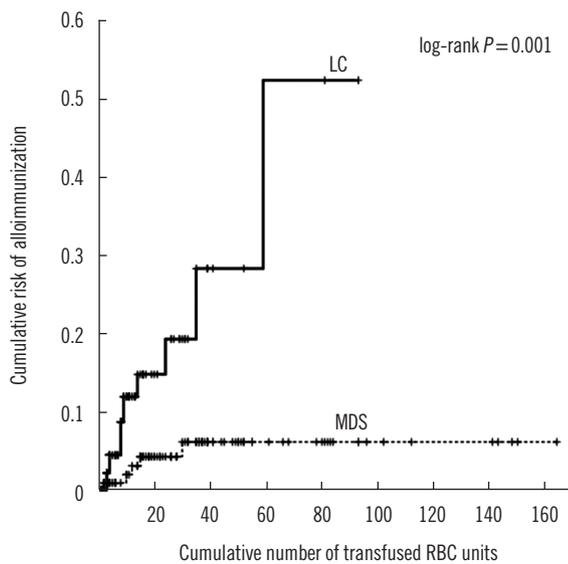
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Fig. 1. Kaplan–Meier plot for cumulative risk of alloimmunization in MDS and LC patients.
Abbreviations: MDS, myelodysplastic syndrome; LC, liver cirrhosis; RBC, red blood cell.

teen patients (80%) had at least one alloantibody of the Rh system. The most common alloantibody was anti-E (45%), followed by anti-c (17%), anti-e (10%), anti-C (7%), anti-Fy^b (7%), and anti-Jk^a (7%) (Supplemental Data Table S1). All of them were clinically significant alloantibodies that could cause hemolytic transfusion reactions [10].

Alloantibody specificity varies according to ethnicity. While anti-E and anti-K are the most common alloantibodies in individuals of European descent, anti-K is rare in East Asians, likely because of the low prevalence of K antigen (0–1.4%) in East Asians [11, 12] in comparison to individuals of European descent (9%) [10].

RBC alloimmunization has been reported in 0.5–1.4% of Korean patients in the initial AST [13-15], and one study reported that 0.6% of patients showed positive conversion of AST from an initial negative AST [13]. In our study, the overall RBC alloimmunization rate was 6.3%, which means that patients with MDS and LC requiring chronic transfusions have higher RBC alloimmunization rates than other transfused patient populations.

Table 2. Characteristics of alloimmunized patients and identified RBC alloantibodies

No. case	Diagnosis	Age (yr)	Gender	Identified RBC alloantibodies	Transfused RBC units (N)*	F/U duration of AST (day)†
M1	MDS	74	M	Anti-E	30	910
M30	MDS	69	F	Anti-E, Anti-Jk^a	12	49
M31	MDS	87	M	Anti-E, Anti-c	10	2,076
M50	MDS	57	M	Anti-E, Anti-c, Anti-Fy^b	15	2,051
M124	MDS	59	M	Anti-E	2	28
L15	LC	36	M	Anti-E	24	2,069
L39	LC	67	F	Anti-e	8	67
L71	LC	48	M	Anti-C, Anti-e	3	2,491
L72	LC	71	F	Anti-E	4	6
L76	LC	49	F	Anti-E	3	1,877
L112	LC	75	F	UnID	8	24
L124	LC	62	F	Anti-E, Anti-c	4	1,817
L126	LC	48	M	Anti-C, Anti-e	59	1,514
L136	LC	69	F	Anti-E, Anti-c	3	748
L139	LC	82	F	Anti-Fy^b	9	1,715
L143	LC	64	M	Anti-E, Anti-c	35	1,031
L156	LC	60	M	UnID	14	297
L209	LC	43	F	Anti-E	4	6
L218	LC	71	F	Anti-E	8	396
L252	LC	51	M	Anti-Jk^a	9	13

*N of RBC units transfused between the initial negative AST and the follow-up positive AST; †The period between the initial negative AST and the follow-up positive AST.
Abbreviations: RBC, red blood cell; F/U, follow-up; AST, antibody screening test; MDS, myelodysplastic syndrome; M, male; F, female; LC, liver cirrhosis; UnID, unidentified.

In our study, the RBC alloimmunization rate in MDS patients was 4.3%. RBC alloimmunization rates in MDS patients from various Western countries and Australia ranged from 11% to 59% [8, 16, 17]. This variation may reflect the differences in patient numbers, study design, treatment, and ethnicity. Few studies have examined RBC alloimmunization in liver disease. Bajpai et al. [18] reported a 5.2% alloimmunization rate in 842 multitransfused liver disease patients in India, similar to that found in our study for LC patients.

In this study, the cumulative risk of alloimmunization according to the increase in transfused RBC units was significantly higher in LC than in MDS patients. Sanz et al. [16] reported a 12.4% cumulative risk of alloimmunization after transfusion of 25 RBC units in MDS and chronic myelomonocytic leukemia patients, and the risk plateaued at 19.5% after transfusion of 130 RBC units. Ortiz et al. [19] reported an 8.3% risk of alloimmunization after transfusion of 25 RBC units in MDS patients and demonstrated a lower rate of alloimmunization in patients treated with azacitidine than in untreated patients, suggesting that immunosuppression by azacitidine leads to the development of immunological tolerance. The immunosuppression effect of the treatment as well as high phenotypic homogeneity of the Korean population are potential causes of the relatively low alloimmunization rate and cumulative risk in the MDS patients in the current study.

In our study, more than half (60%) of the alloimmunized patients developed alloantibodies before transfusion of 10 RBC units. This finding was similar to the finding by Sins et al. [20] that half of the alloimmunized patients with sickle cell disease developed alloantibodies before transfusion of eight RBC units. Although the number of transfused RBC units is a risk factor for RBC alloimmunization, our results suggest that alloimmunization can develop after a limited number of RBC transfusions in more patients than expected. With respect to the time interval between transfusion and alloantibody detection, alloantibodies were detected within 14 days in three patients (15%) and after 14 days in 17 patients (85%), similar to previously reported rates of 17% and 83% within and after 14 days after transfusion, respectively ($P=0.829$) [1]. Although the direct antiglobulin test was not conducted for three patients with early alloantibody formation within 14 days after transfusion, there was no evidence of hemolytic transfusion reaction in these patients. In two female patients, early alloantibody formation was considered a booster immune response on the basis of their pregnancy history. For a male patient with anti-Jk[a], we could not determine whether early alloimmunization was a primary immune response or a booster response.

We did not investigate the general RBC alloimmunization rate in all patients who received RBC transfusions at Samsung Medical Center during the study period, and further large-scale studies are needed.

In conclusion, Korean MDS and LC patients requiring chronic transfusions had higher RBC alloimmunization rates than other transfused patient populations, and the cumulative risk of alloimmunization according to the increase in transfused RBC units was significantly higher in LC than in MDS patients. Moreover, the developed RBC alloantibodies were from the non-matching of Rh, Duffy, and Kidd systems. Therefore, extended RBC antigen matching of Rh, Duffy, and Kidd blood groups should be a priority for Korean MDS and LC patients requiring chronic transfusions.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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Supplemental Data Table S1. RBC alloantibodies identified in 20 MDS and LC patients

Identified RBC alloantibodies	MDS		LC		Total	
	N	%	N	%	N	%
Anti-E	5	55.6	8	40.0	13	44.8
Anti-c	2	22.2	3	15.0	5	17.2
Anti-e	0	0	3	15.0	3	10.3
Anti-C	0	0	2	10.0	2	6.9
Anti-Fy[b]	1	11.1	1	5.0	2	6.9
Anti-Jk[a]	1	11.1	1	5.0	2	6.9
UnID	0	0	2	10.0	2	6.9
Total	9	100	20	100.0	29	100

Abbreviations: MDS, myelodysplastic syndrome; LC, liver cirrhosis; RBC, red blood cell; UnID, unidentified.