Increased Incidence of Red Blood Cell Alloantibodies in Myelodysplastic Syndrome

Uri Rozovski MD[1]*, Ofira Ben-Tal MD[2,3]*, Ilya Kirgner MD[2,3], Moshe Mittelman MD[4] and Mara Hareuveni PHD[3]

[1]Division of Hematology, Davidoff Cancer Center, Rabin Medical Center, Petah Tikva, Israel
[2]Department of Hematology and Bone Marrow Transplantation, [3]Transfusion Medicine Unit, and [4]Department of Medicine, Tel Aviv Sourasky Medical Center, affiliated with Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

ABSTRACT: **Background:** Approximately 80% of patients with myelodysplastic syndromes (MDS) receive multiple red blood cells (RBC), often multiple transfusions, and are therefore prone to develop alloantibodies against RBC. Because of increasing evidence for the role of immune dysregulation in the pathobiology of MDS, we hypothesized that in patients with MDS there is an increase in alloantibody formation beyond that expected from multiple transfusions.

Objectives: To determine the prevalence rates of alloantibodies in patients with MDS who are transfusion dependent and compare them to rates of non-MDS patients matched for number of RBC units they received.

Methods: The blood bank database was screened to identify non-MDS patients matched for age and number of units transfused. Logistic regression analysis was applied to determine factors affecting alloantibody formation.

Results: Of 60 patients with MDS, 18 (30%) developed alloantibodies against RBC. Transfusion-dependent MDS and non-MDS patients (N=56 each), matched for number of RBC units and age, were compared. Fifteen MDS patients (27%) but only 12 non-MDS patients (12%) developed alloantibodies (P = 0.057). The relative risk for developing antibodies in MDS patients was 2.14, and MDS was the strongest predictor for formation of alloantibodies during transfusion therapy (odds ratio 3.66, confidence interval 1.4–9.3).

Conclusions: Patients with MDS are at increased risk to develop RBC alloantibodies, partly because these patients receive multiple RBC transfusions. Whether matching for RH and KEL would lead to lower rates of RBC alloantibodies remains to be determined.

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KEY WORDS: myelodysplastic syndrome (MDS), alloantibodies, transfusion therapy, immune dysregulation, multiple transfusions

In memory of the second author, Dr. Ofira Ben-Tal

Myelodysplastic syndromes (MDS) are a group of clonal hematopoietic disorders characterized by ineffective hematopoiesis. Patients typically present with peripheral cytopenia in one or more hematopoietic lineages despite hypercellular bone marrow [1]. Fifteen hundred new cases of MDS are expected annually in the United States, about 80% of whom will require blood transfusion at some point during the course of their illness [2,3].

Multiple red blood cell (RBC) transfusions predispose patients to develop RBC alloantibodies against RBC antigens. Among 186 heavily transfused patients, 140 with hematological disorders, 22 (11.8%) developed such antibodies [4]. Similarly, among 272 patients with transfusion-dependent MDS or chronic myelomonocytic leukemia (CMML), 42 (15%) developed 81 types of RBC alloantibodies and 7 types of autoantibodies. Three additional patients developed only autoantibodies [5].

Whether multiple RBC transfusions is the only risk factor for the development of antibodies in patients with MDS is not known. Considering the increasing evidence that immune dysregulation plays a role in the pathobiology of MDS [6], we hypothesized that in addition to multiple transfusions, having MDS is an independent risk factor that contributes to RBC alloantibodies formation. To test this we compared the incidence of alloantibodies in a cohort of transfusion-dependent MDS patients to that of non-MDS recipients matched for number of transfusions and age.

PATIENTS AND METHODS

This was a retrospective study conducted at a single institute, Tel Aviv Sourasky Medical Center, Israel, a 1400 bed tertiary medical academic facility. The study was approved by the local institutional review board (approval number #70004948). MDS was diagnosed and classified according to World Health Organization (WHO) 2008 criteria [7]. We included all consecutive MDS patients treated with blood transfusion at our institution during the 45 month study period. MDS patients

*The first two authors contributed equally to this study

who underwent allogeneic bone marrow transplantation were excluded.

The control, non-MDS patients, was retrieved directly from the blood bank database. Patients were matched for age and the number of RBC units transfused. Diagnoses were taken from the hospital ICD-9 discharge diagnosis list.

ANTIBODY SCREENING AND DETERMINATION

Antibody detection and identification was performed by gel agglutination (DiaMed, Switzerland), with the LISS/indirect antiglobulin test (IAT) and the one-stage enzyme method (DiaMed, Switzerland).

RBC AND RANDOM DONOR PLATELET (RDP) PRODUCTS

All cellular products were leukodepleted throughout the entire study period. When platelets were indicated, six units of random pooled platelets (RDP) were transfused, with ABO compatible with the patient's ABO blood group whenever available. No female patient was at childbearing age and therefore no anti-D prophylaxis was warranted.

STATISTICAL ANALYSIS

Clinical and categorical variables are presented as percentages (N). Differences between continuous variables were tested using analysis of variance (ANOVA). Fisher's exact test and the chi-square test were used for comparison of categorical variables, and the Mann-Whitney non-parametric test was employed to compare medians.

To compare alloantibody formation among MDS patients and non-MDS control blood recipients, an algorithm was devised to randomly match blood recipients of similar age and with similar number of RBC units transfused to each MDS patient. For MDS patients who received more than 70 RBC units we used less stringent criteria and the algorithm was revised to find non-MDS individuals who received more than 70 RBC units and matched exactly for age only.

To determine factors that influence alloantibody formation against RBC antigens, logistic regression was applied with age, gender, disease status, number of RBC units, transfusion duration and number of type and screen (T&S) tests as the predictive values. With this model the Exp(ß) was used to calculate the odds ratio (OR) and the 95% confidence interval (CI). SPSS v16 (SPSS Inc., Chicago, IL) was used for all data analyses.

RESULTS

Between June 2006 and March 2010 (45 months) 60 patients who received RBC transfusions met the diagnostic criteria for MDS by the WHO 2008 criteria [7]. Transfusion history data pertaining to these 60 patients were collected for all patients from time of diagnosis to last day of follow-up.

Thirty-seven MDS patients (61%) were male and the median age at the time of diagnosis was 76 years (range 59–96). Overall, patients received a median of 24 RBC units (range 1–170) and 18 patients (30%) also required platelet transfusions. These patients received a median of 48 platelet units (range 6–438).

Eighteen patients (30%) developed RBC alloantibodies. The first antibody was detected at a median of 9 months (range 0.3–77) and after a median cumulative number of 11 RBC units (range 1–127) [Table 1]. In 15 patients (83%) more than one antibody was detected during the follow-up period, and in 4 patients multiple antibodies were already detected at the first type and screen (T&S) test. When only a single alloantibody was detected at the first T&S test it was typically either anti-K (N=7) or anti-E (N=5) antibodies. In two cases after heavy exposure (median 77 RBC units), low prevalence alloantibodies (Kpa or Lua) were the first alloantibodies detected.

To compare the incidence of alloantibodies among MDS and non-MDS blood recipients we randomly selected non-MDS blood recipients from the blood bank database matched for age and number of RBC units, using the algorithm mentioned above. From approximately 35,000 non-MDS blood recipients during the 88 months of follow-up we were able to match non-MDS controls for 56/60 patients. Therefore, we compared 56 MDS patients with 56 blood recipients, matched for age and number of RBC units; in each group 66% were male. The control group was classified according to the primary indication for blood transfusion to five groups (chronic renal failure, bleeding during surgery or trauma, solid tumors, hematological malignancies, other causes).

Fifteen patients with MDS (27%) developed RBC alloantibodies, but only 7 (12%) in the non-MDS matched group ($P = 0.057$). The relative risk of patients with MDS diagnosis compared to non-MDS transfusion recipients was 2.14. A logistic regression model revealed that having MDS was the

Table 1. Transfusion history of the study population

	N (%)	Cumulative RBC Median (range)	Duration of transfusion Median (range)	Alloantibody detection N (%)	Cummulative RBC before the first antibody Median (range)	Months to first antibody Median (range)
Total cohort	60 (100)	24 (1–170)	18 (0.2–88)	18 (30)	11 (1–127)	9 (0.3–77)
RA	25 (40)	45 (2–170)	19 (0.2–88)	8 (20)	12 (1–127)	11 (0.3–77)
RARS	4 (7)	24 (7–130)	49 (18–68)	1 (25)	23 (4–30)	21 (4–39)
RCMD	16 (28)	18 (1–113)	9 (0.2–83)	7 (44)	12 (4–23)	10 (2–35)
RAEB	13 (22)	29 (4–88)	12 (1–88)	2 (9)	8 (6–10)	3 (2–3)
5q-	3 (2)	9 (8–10)	32 (16–47)	0		

RBC = red blood cells, RA = refractory anemia, RARS = refractory anemia with ring sideroblasts, RCMD = refractory cytopenia with multilineage dysplasia, RAEB = refractory anemia with excess blasts

Table 2. Comparison of MDS (N=56) and non-MDS (N=56) blood recipients matched for age and number of RBC units

	Alloantibody detection	Cumulative RBC	Duration of treatment (months)	No. of T&S tests
	N (%)	Median (range)	Median (range)	Median (range)
MDS patients (N=56)	15 (27)	22 (1–170)	26 (3–101)	26 (3–101)
Non-MDS patients (N=56)	7 (12)	21 (1–190)	13 (2–11)	13 (2–110)
Malignancy (N=24)	5 (21)	40 (7–189)	17 (3–69)	25 (6–76)
Solid (N=13)	4 (31)	24 (7–189)	14 (3–69)	13 (8–69)
Hematological (N=11)	1 (9)	60 (7–132)	20 (3–64)	39 (6–76)
Surgical bleeding (N=15)	0 (0)	7 (1–74)	3 (1–140)	6 (3–21)
Non-surgical bleeding (N=6)	0 (0)	15 (4–190)	8.5 (0.5–78)	9 (5–110)
CRF (N=6)	1 (17)	29 (1–130)	34 (6–74)	34 (3–73)
Other (N=5)	1 (20)	15 (4–190)	20 (0.5–51)	15 (3–32)

MDS = myelodysplastic syndromes, RBC = red blood cells, CRF = chronic renal failure

strongest risk factor to predict alloantibody formation: OR = 3.66 (95%CI 1.4–9.3). Duration of transfusion therapy and the number of RBC units transfused had only minor and clinically irrelevant impact: OR = 1.03 (CI95% 1.00–1.04) for the duration of transfusion therapy and OR = 1.02 (CI95% 1.00–1.03) for the number of RBC units transfused.

DISCUSSION

Our study found that 30% of patients (18/60) with MDS treated with blood transfusions at a large tertiary center developed alloantibodies against RBC. This lies within the range of 15% [5], 20% [8,9], 31% [4] to 59% [10] reported in the literature.

The main contribution of this retrospective analysis is the comparison between MDS and other transfusion-dependent patients. This is important because it is still unclear whether the high rate of RBC alloantibodies during the course of transfusion treatments is due solely to the fact that patients are receiving multiple transfusions or whether having MDS is an independent risk factor. We show here that 27% (15/56) of transfusion-dependent MDS patients produced RBC alloantibodies, compared with only 12% (7/56) matched for age and transfusion load controls. Moreover, in a multivariate model, having MDS and not the number of RBC transfused was the strongest predictor for development of alloantibodies during the course of treatment (OR = 3.66) for the diagnosis of MDS compared to non-MDS indication for blood transfusion. Increased propensity for alloantibody formation is also evident from the finding of multiple alloantibodies among 83% of patients with MDS, which was significantly higher than the 22%–33% reported in other hematological malignancies [11].

The control group, which was randomly selected from a pool of 35,000 transfusion recipients, reflects a variety of indications for multiple blood transfusions including various malignancies (both solid and hematological), bleeding (surgical or non-surgical), and chronic renal failure. The incidence of alloantibodies among patients with solid tumors and MDS was comparable. However, across all other indications blood recipients had a lower tendency to form alloantibodies. It is interesting that only one of nine patients with hematological malignancies had alloantibodies, probably because patients receiving high dose chemotherapy – particularly patients with acute leukemia – exhibit a lesser antibody response than do other transfusion recipients [12,13]. The control group also differed (although not significantly) from the patient group in shorter duration of RBC therapy and fewer T&S tests performed. However, the lower incidence of alloantibodies was evident both in patients with chronic renal failure who had longer exposure and those with surgical bleeding with the shortest duration of RBC therapy. Moreover, in a multivariate analysis the duration of transfusion and the number of T&S tests had only minor predictive power.

The higher incidence of alloantibodies in MDS blood recipients is yet another aspect of the immune dysregulation that characterizes this disorder [6,14]. Immune dysregulation is the rationale for exploring the use of immunomodulatory agents, particularly lenalidomide, in patients with low risk MDS [15,16]. Whether lenalidomide treatment which may induce, albeit rarely, autoantibodies against RBC 17,18] also increases the formation of alloantibodies during transfusion therapy is unknown.

CONCLUSIONS

One-third of patients with MDS who receive blood transfusions will develop alloantibodies, often more than one. Since alloantibodies are detected at higher frequency in MDS than in non-MDS patients prospective studies are warranted to investigate whether using RBCs matched for RH (D, C, c, e) and KEL (K) system reduces alloantibody formation in patients with MDS.

Correspondence
Dr. U. Rozovski
Division of Hematology, Davidoff Cancer Center, Rabin Medical Center, Petah Tikva, Israel
email: rozovski.uri@gmail.com

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