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*Document Version*
Publisher's PDF, also known as Version of record

*Publication date:*
2022

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# A clinical effect of disease-modifying treatment on alloimmunisation in transfused patients with myelodysplastic syndromes: data from a population-based study

Johanne Rozema[1,2]*, Christiaan L. Slim[3]*, Nic J.G.M. Veeger[4,5], Robby E. Kibbelaar[6], Harry de Wit[3], Eric N. van Roon[1,2], Mels Hoogendoorn[7]

*Johanne Rozema and Christiaan L. Slim contributed equally to this manuscript as co-first Author

[1]Unit of Pharmacotherapy, Epidemiology and Economics, Department of Pharmacy, University of Groningen, Groningen, the Netherlands;
[2]Department of Clinical Pharmacy and Pharmacology, Medical Centre Leeuwarden, Leeuwarden, the Netherlands;
[3]Certe Medical Diagnostics and Advice, Medical Centre Leeuwarden, Leeuwarden, the Netherlands;
[4]Science Bureau, Medical Centre Leeuwarden, Leeuwarden, the Netherlands;
[5]Department of Epidemiology, University of Groningen, University Medical Centre Groningen, Groningen, the Netherlands;
[6]Pathologie Friesland, Leeuwarden, the Netherlands;
[7]Department of Internal Medicine, Medical Centre Leeuwarden, Leeuwarden, the Netherlands

**Background** - Alloimmunisation against blood products is an adverse event, causing time-consuming compatibility testing. Current literature has not yet identified the influence of treatment on the risk of alloimmunisation in patients with myelodysplastic syndromes (MDS).

**Materials and methods** - An observational, population-based study, using the HemoBase registry, was performed including all transfused patients who were diagnosed with MDS between 2005 and 2017 in Friesland, a province in the Netherlands. Information about transfusion dates, types, and treatment regimens was collected from the health records. Blood products were matched for ABO and Rhesus D. The effect of disease-modifying treatment was estimated with incidence rates and a Cox time-dependent analysis.

**Results** - 233 patients were included in this study, with a median follow-up of 13.0 months. Alloimmunisation occurred in 21 patients (9.0%) and predominantly occurred early in follow-up. Three (5%) and 18 (11%) alloimmunisation events occurred in patients with and without disease-modifying treatment, respectively. The hazard ratio for alloimmunisation without treatment compared to during treatment was 2.7 (95% CI: 0.35-20.0), with incidence rates of 7.18 and 2.41 per 100 patient-years, respectively.

**Discussion** - In a non-selected real-world population of MDS patients receiving blood transfusions, the percentage of patients with alloimmunisation was below 10%. The results of this study support the hypothesis that disease-modifying treatment affects the ability of the immune system to mount an antibody response to non-self blood group antigens.

**Keywords:** *blood transfusion, myelodysplastic syndromes, population-based, immunisation.*

## INTRODUCTION

Ineffective haematopoiesis causes symptoms such as fatigue, frequent infections, or bruises and bleeds in patients with myelodysplastic syndromes (MDS). Low erythrocyte and/or platelet counts require blood transfusions and MDS patients often become transfusion-dependent[1-3]. Despite current techniques and protocols, various adverse

events can occur due to (frequent) blood transfusions, ranging from iron overload to immunisation[1,2].

The problem of immunisation (antibody formation) against blood group antigens is three-fold: it results in time-consuming red blood cell (RBC) compatibility testing, increased donor selection pressure on blood banks, and increased risk of acute haemolytic transfusion reactions in emergencies (when blood products cannot be matched to patient antibodies). Immunisation against blood group antigens is a serious condition that can lead to death[4-6]. Alloimmunisation (formation of antibodies against non-self blood group antigens) occurs in 2% of the general transfused population[4,6-9]. Dutch and British transfusion guidelines implement extensive matching for patient groups receiving regular transfusions, including those with MDS[10,11]. It is recommended for the complete Rhesus (Rh) system and the K-antigen (K) from the Kell blood group system, in addition to the standard blood group antigens ABO and RhD. The need for Rh/K matching is, however, a subject of debate. The reported alloimmunisation rate in MDS patients widely varies, from 14 to 59%[12,13]. Most studies included a small number of patients, or only a subgroup of MDS patients, so the true alloimmunisation rate and efficacy of Rh/K matching remains unclear[8,14-18].

Ideally, patient or disease-related factors that identify patients at risk of alloimmunisation should be known. Unfortunately, current studies have not yet fully identified the risk factors and dynamics of alloimmunisation in individual patients. Characteristics such as age, gender and MDS subtype may be risk factors, but transfused units (RBCs and platelets) and their non-ABO/RhD antigen compatibility may also be factors for the risk of alloimmunisation in MDS patients.

Current treatments for MDS include hypomethylating agents (HMAs), immunomodulating agents (IMIDs) and chemotherapy. These disease-modifying treatments (DMTs) may decrease MDS patients' susceptibility to alloimmunisation and could potentially lead to lower alloimmunisation rates[4,5,19-22]. In addition to studying MDS treatment as a factor per se, it would be worthwhile to include temporal dynamics (e.g., exposed and non-exposed periods) in the analysis of alloimmunisation throughout the disease. A time-dependent analysis provides a more accurate picture of the effect of DMT.

This study addresses these issues and aims to determine the alloimmunisation rate in MDS patients, identify risk factors for alloimmunisation, study alloimmunisation dynamics over time in a population-based setting, and analyse whether DMT can abrogate or decrease alloimmunisation in these patients.

## MATERIALS AND METHODS

This was an observational and population-based study. All MDS patients from Friesland, a province in the Netherlands, were identified using the HemoBase registry[23]. The bone marrow specimens of all MDS patients diagnosed between 2005 and 2017 were subjected to blind review according to the 2016 World Health Organization (WHO) classification[24] by an expert panel comprising a haematologist, a haematopathologist and bone marrow cytologists. Information on transfusion dates, types and treatment regimens was collected from health records and laboratory systems. Patients were followed up from their first transfusion to March 2019. Information on transfused blood products was available from 1998. The observational study did not require an ethical review, and it was approved without consent in accordance with Dutch regulations.

Blood products were matched for ABO and RhD, and transfused using the "type and screen" system: electronic matching of blood group phenotype between patient and donor. One hospital cross-matched blood products for ABO, full Rh and K. After a positive screen, antibody identification and Rh/K phenotyping was performed and subsequent blood products were cross-matched for antibody specificity and ABO/Rh/K[10]. Blood group typing, antibody screening, antibody identification and Rh/K phenotyping was performed using the DiaMed ID-System (Bio-Rad Laboratories, Cressier, Switzerland). The Fisher Exact test (p<0.05) was used for antibody identification[25]. Antibody screening was valid for 72 hours.

Follow-up was defined as the time between the first and last transfusion, or first transfusion and primary alloimmunisation event. DMT was defined as HMA, IMID (lenalidomide) or chemotherapy. Patients who underwent allogeneic stem cell transplantation (SCT) prior to their first documented transfusion were excluded. Patients who underwent SCT after this were censored from the date of transplantation.

Only units prior to alloimmunisation were used to calculate transfused RBC and platelet units in alloimmunised patients. A cumulative frequency distribution was generated to study the dynamics of alloimmunisation. Differences in alloimmunisation within gender, MDS subtype and the revised International Prognostic Scoring System (IPSS-R) score were studied using a Fisher's Exact test or Pearson $\chi^2$ test. A Mann-Whitney U test was used for age and number of transfusions (RBC and platelets). The effect of DMT as a temporary risk factor for alloimmunisation was estimated from incidence rates, with the individual observation time limited to a maximum of 30 months. A Cox time-dependent analysis was performed, taking into consideration the actual exposure to DMT. In this analysis, exposure to treatment was determined for each individual patient on each day during the first 30 months of follow-up. Exposure was defined as the time from the start of DMT to 91 days after termination of DMT, to include a continuing treatment effect and wash-out period. Statistical analyses were performed using IBM SPSS version 24 and SAS version 9.4.

## RESULTS

Of the 292 MDS patients identified, 239 received transfusions and were eligible for this study. Six patients were excluded. Two female patients were excluded because alloimmunisation was related to previous pregnancies and had occurred several years before the first transfusion. Three patients were excluded because they received an allogenic SCT prior to their first documented transfusion. The follow-up of two patients was censored from the date of transplantation. One male patient was excluded because the precise date of alloimmunisation was unknown.

The median follow-up per patient was 13.0 months (95% CI: 9.8-16.0). Median age was 76.2 years (range: 27.5-92.0), and 70% of the study population were male. Supportive care was provided to 67% of the patients, and DMT was initiated in 32% (**Table I**). HMA (67.6%, n=50) was the most common type of DMT in these patients, followed by chemotherapy (41.9%, n=31).

### Dynamics of alloimmunisation

Patients with alloimmunisation received a median of seven RBC units (range: 2-228) prior to alloimmunisation (total=32, range: 6-231). Patients without alloimmunisation

**Table I** - *Clinical characteristics of the study population*

| PATIENTS' CHARACTERISTICS | With antibody n=21 | | Without antibody n=212 |
|---|---|---|---|
| Male | 10 (47.6) | | 152 (71.7) |
| Median age (range) at diagnosis | 79.5 y (57.5-86.9) | | 75.9 y (27.5-92.0) |
| **MDS subtype** | | | |
| SLD | 3 (14.3) | | 29 (13.7) |
| MLD | 8 (38.1) | | 25 (11.8) |
| RS-SLD | 3 (14.3) | | 33 (15.6) |
| RS-MLD | 1 (4.8) | | 21 (9.9) |
| Del (5q) | 0 (0) | | 5 (2.4) |
| EB-1 | 4 (19.0) | | 41 (19.3) |
| EB-2 | 1 (4.8) | | 32 (15.1) |
| U | 0 (0) | | 5 (2.4) |
| Not specified | 1 (4.8) | | 21 (9.9) |
| **IPSS-R** | | | |
| Very low | 3 (14.3) | | 14 (6.6) |
| Low | 3 (14.3) | | 61 (28.8) |
| Intermediate | 7 (33.3) | | 26 (12.3) |
| High | 0 (0) | | 18 (8.5) |
| Very high | 1 (4.8) | | 13 (6.1) |
| Unknown | 7 (33.3) | | 80 (37.7) |
| **TRANSFUSIONS AND TREATMENT** | With antibody n=21 | | Without antibody n=212 |
| **RBCs** | *Total RBCs* | *RBCs before AI* | *Total RBCs* |
| Median (n. units [range]) | 32 (6-231) | 7 (2-228) | 19 (2-322) |
| Mean (n. units) | 56 | 27 | 32 |
| ≥40 units | 8 (38.1) | 3 (14.3) | 54 (25.5) |
| **PLTs** | *Total PLTs* | *PLTs before AI* | *Total PLTs* |
| Median (n. units [range]) | 0 (0-45) | 0 (0-39) | 0 (0-41) |
| Mean (n. units) | 3 | 2 | 3 |
| **Treatment** | | | |
| BSC | 18 (85.7) | | 138 (65.1) |
| DMT | 3 (14.3) | | 71 (33.5) |
| IMID* | 0 (0) | | 8 (3.8) |
| HMA* | 2 (9.5) | | 48 (22.6) |
| Chemotherapy* | 2 (9.5) | | 29 (13.7) |
| Unknown | 0 (0) | | 3 (1.4) |
| Transplantation# | 0 (0) | | 2 (0.9) |

Values are reported as number (%) of patients, unless stated otherwise. MDS: myelodysplastic syndromes; SLD: single lineage dysplasia; MLD: multi lineage dysplasia; RS: ring sideroblasts; EB: excess blasts; U: unclassifiable; IPSS-R: Revised International Prognostic Scoring System; RBCs: red blood cells; PLTs: platelets; AI: alloimmunisation; BSC: best supportive care; DMT: disease-modifying treatment; y: years. *The combined total can be >100%, because patients can receive multiple consecutive treatments. #This only includes transplantations during the observation period.

received a median of 19 RBC units (range: 2-322) (**Table I**). **Figure 1** shows the cumulative frequency distribution of 7,426 transfused RBC units in the study population. Alloimmunisation occurred in 21 patients (9.0%), predominantly soon after the first transfusion: 67% of alloimmunisation events took place in the first year, and 86% of the patients had formed alloantibodies within the first three years. Eleven alloimmunisation events (52%) occurred before the 8th RBC transfusion and within the first four months (**Figure 1**). Sixteen patients (76%) were alloimmunised before the 20th RBC unit, whereas only two (10%) presented with alloantibodies after receiving over 100 RBC units. Anti-E and anti-K were the most common alloantibodies, followed by anti-Wra (**Table II**).

### Risk factors for alloimmunisation

Age, MDS subtype, IPSS-R, number of RBC and platelet units were not found to be associated with alloimmunisation, but there was an association with gender. The incidence of alloimmunisation was higher (p=0.044) in female (16%, 95% CI: 8.70-25.83) *vs* male patients (6%, 95% CI: 3.26-11.12). Female patients received significantly fewer RBC units than male patients (median 11 [range: 2-322] and 22 [range: 2-228], respectively; p=0.003). No other significant differences were found.

### Alloimmunisation and disease-modifying treatments

DMT was given to 74 patients (32%), of which 63 (27%) after their first transfusion (**Table III**). Patients who received DMT at any point during follow-up (n=63) had more RBC transfusions than patients not receiving DMT (median 34 [range: 3-154] and 11 [range: 0-322] units respectively; p<0.01). Three alloimmunisation events (5%) occurred in patients with DMT and 18 (11%) in patients without DMT (**Table III** and **Figure 2**). Of the three patients who had an event in the DMT group, one had already terminated treatment five months before alloimmunisation occurred.

### Cox time-dependent analysis

In addition to an estimate of the absolute risk (incidence rates), a Cox time-dependent exposure analysis provided the relative risk of alloimmunisation in patients without DMT *vs* those who received DMT. Four patients were excluded due to incomplete data on the start and/or termination of treatment. This analysis showed a hazard ratio of 2.7 (95% CI: 0.35-20.0; p=0.34) for alloimmunisation without DMT *vs* during DMT. The corresponding incidence rates were 7.18 per 100 patient-years (95% CI: 4.10-11.66) for patients not receiving DMT and 2.41 per 100 patient-years (95% CI: 0.06-13.43) during DMT. The number of RBC units was not found to be a confounding factor.

One of the five hospitals performed screening according to the Dutch recommendations, and cross-matched MDS
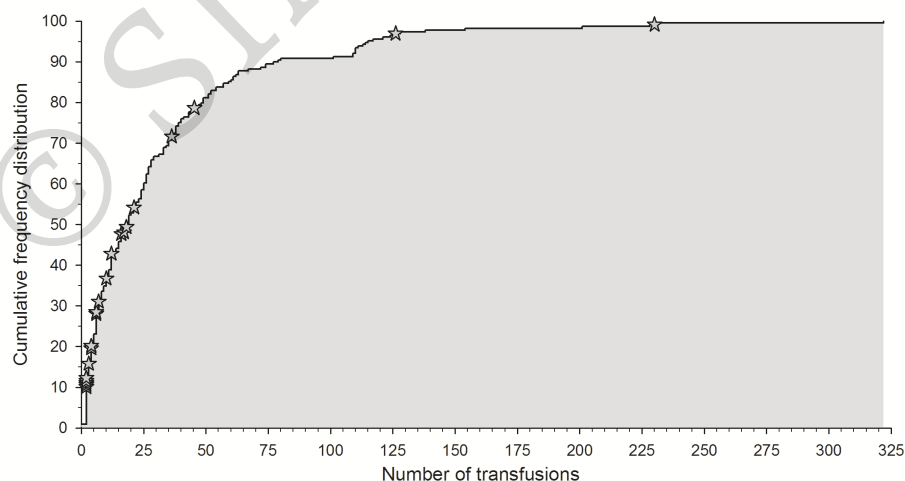


**Figure 1 - Cumulative frequency distribution of number of received red blood cell units and the occurrence of alloantibodies in myelodysplastic syndromes patients**
The stars represent an alloimmunisation event (n=21). Note that transfusions given after the alloimmunisation event were disregarded.

**Table II** - *Detailed information of myelodysplastic syndromes (MDS) patients with alloantibodies*

| Patient n. | Gender | MDS subtype | Phenotype | Antibody | Date of first transfusion | Date of positive screening | Total n. of RBCs received | N. RBCs prior to AI | Total n. of PLTs received | N. PLTs prior to AI | Therapy |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Male | MLD | ddee | Anti-E | Feb 2003 | Sep 2014 | 231 | 228 | 2 | 2 | BSC |
| 2 | Male | SLD | CcDee, K-neg | Anti-E, anti-K | Mar 2007 | May 2007 | 36 | 5 | 0 | 0 | BSC |
| 3 | Female | n.o.s. | ccDEe, K-neg | Anti-K | Apr 2011 | Jun 2011 | 9 | 4 | 0 | 0 | BSC |
| 4 | Female | MLD | CcDee, K-neg | Anti-E, anti-K, anti-Jk[b] | Sep 2007 | Nov 2007 | 9 | 2 | 0 | 0 | BSC |
| 5 | Male | MLD | Dee | Anti-E | Dec 2009 | Jan 2010 | 9 | 7 | 1 | 1 | BSC |
| 6 | Female | EB-2 | D, Jk[a]-neg | Anti-Jk[a] | Jan 2010 | Mar 2010 | 28 | 2 | 0 | 0 | BSC |
| 7 | Female | EB-1 | ccddee, K, Wr[a]-neg | Anti-E, anti-C, anti-Wr[a] | Mar 2014 | Jul 2014 | 12 | 2 | 0 | 0 | BSC |
| 8 | Female | EB-1 | Dee, K-neg, Wr[a]-neg | Anti-E, anti-K, anti-Wr[a] | Dec 1997 | May 2008 | 127 | 15 | 0 | 0 | BSC |
| 9 | Female | SLD | D | Unknown * | May 2016 | Jun 2016 | 6 | 6 | 2 | 2 | BSC |
| 10 | Female | RS-MLD | ddee | Anti-E | Jul 2007 | Jul 2007 | 21 | 2 | 1 | 1 | BSC |
| 11 | Male | MLD | CCDee, K-neg, Wr[a]-neg | Anti-K, anti-c, anti-Wr[a] | Jun 2010 | Dec 2010 | 72 | 16 | 7 | 1 | BSC |
| 12 | Male | MLD | CcDee, Wr[a]-neg | Anti-Wr[a] | Jul 2009 | Oct 2011 | 184 | 125 | 0 | 0 | BSC |
| 13 | Female | SLD | ccDEE, K-neg, Wr[a]-neg, Lua-neg | Anti-K, anti-Lu[a], anti-Wr[a] | Mar 2011 | Dec 2013 | 44 | 12 | 45 | 39 | DMT |
| 14 | Male | MLD | Dee, K-neg | Anti-E, anti-K | Aug 2008 | May 2009 | 32 | 21 | 0 | 0 | BSC |
| 15 | Male | EB-1 | K-neg, Wr[a]-neg | Anti-K, anti-Wr[a] | May 2013 | Mar 2019 | 17 | 17 | 0 | 0 | DMT |
| 16 | Male | MLD | D, M-neg | Anti-M | May 2009 | Jun 2009 | 20 | 3 | 2 | 2 | DMT |
| 17 | Male | RS-SLD | CcDee, K-neg | anti-E, anti-K, anti-Kp[a], anti-Wr[a] | May 2015 | Feb 2017 | 82 | 45 | 1 | 1 | BSC |
| 18 | Female | MLD | ccddee; Jk[a] neg, S-neg, Fy[a]-neg, Wr[a]-neg; Jk[b]-pos, Fy[b]-pos, s-pos | Anti-E, anti-D, anti-C, anti-Wr[a] | Apr 2013 | Nov 2014 | 152 | 36 | 1 | 0 | BSC |
| 19 | Male | RS-SLD | CcDee, K-neg; Kp[a]-neg, Wr[a]-neg | Anti-E, anti-K, anti-Kp[a], anti-Wr[a] | Apr 2014 | Jul 2014 | 39 | 4 | 0 | 0 | BSC |
| 20 | Female | RS-SLD | D, K-neg | Anti-K | Sep 2014 | Dec 2014 | 42 | 9 | 0 | 0 | BSC |
| 21 | Female | EB-1 | d | Unknown * | May 2017 | Oct 2017 | 6 | 2 | 0 | 0 | BSC |

MLD: multi lineage dysplasia; SLD: single lineage dysplasia; n.o.s.: MDS not otherwise specified; EB-1/2: excess blasts 1/2; RS-SLD: ring sideroblasts with single lineage dysplasia; RS-MLD: ring sideroblasts with multi lineage dysplasia; RBC: red blood cells; AI: alloimmunisation; BSC: best supportive care; DMT: disease-modifying treatment. *Not tested: case 9 died shortly after alloimmunisation and further analyses were not performed; there was no additional information available for case 21.

**Table III** - *Absolute risk of alloimmunisation in myelodysplastic syndromes (MDS) patients*

|  | N (%) |
|---|---|
| **All transfused MDS patients** | |
| Total N. | 233 (100) |
| N. with event | 21 (9.0) |
| Incidence rate per 100 years (95%CI) | 4.59 (2.92-6.90) |
| **DMT use during follow-up** | |
| Total N. | 63 (27.0) |
| N. with event | 3 (4.8) |
| Incidence rate per 100 years (95%CI) | 2.70 (0.69-7.36) |
| **No DMT use during follow-up** | |
| Total N. | 170 (73.0) |
| N. with event | 18 (10.6) |
| Incidence rate per 100 years (95%CI) | 5.19 (3.18-8.05) |

Values are reported as number (%) of patients, unless stated otherwise. DMT: disease-modifying treatment; CI: confidence interval. For a comparison of the incidence rates, refer to the relative risk derived from the Cox time-dependent analysis.
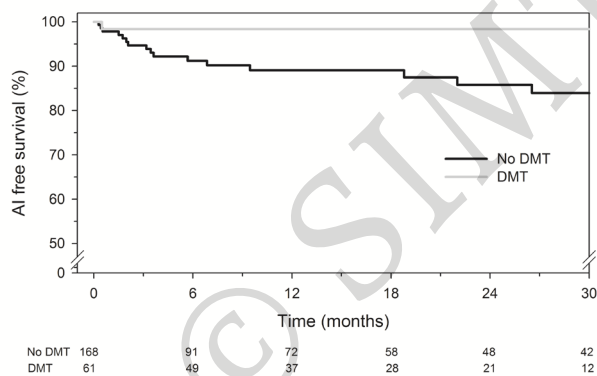


**Figure 2 - Kaplan-Meier curve of alloimmunisation events in myelodysplastic syndromes patients, divided by use of disease-modifying treatment**
AI: alloimmunisation, DMT: disease-modifying treatment.

patients for ABO, full Rh and K. One alloimmunisation event occurred in 42 patients (2.4%). The other hospitals reported highly varying alloimmunisation rates: 5.0% (6 out of 121), 15.0% (3 out of 20), 20.0% (1 out of 5). One hospital reported an alloimmunisation rate of 22.2% (10 out of 45), despite having the same policy as the other three. There was no significant difference in median RBC units between hospitals.

## DISCUSSION

In this population-based study, comprising a complete 13-year cohort of MDS patients receiving over 7,000 blood transfusions, alloimmunisation was observed in 9.0%. Events predominantly occurred at the beginning of follow-up. No clear risk factors for alloimmunisation were identified. Our data further suggest that DMT exposure may protect MDS patients from alloimmunisation, as absence of DMT was associated with a 2.7-fold increase in risk of alloimmunisation.

The alloimmunisation rates in MDS patients in the literature vary from 14 to 59%[12,13]. The trend is for studies with a relatively large sample size to report lower rates: recent studies with increased sample sizes illustrate rates of 15% (n=272), 11% (n=695), and 13% (n=749)[20,26,27]. Our results are in line with these studies and suggest that the rate in MDS patients is lower than previously estimated.

Every transfusion is a potential alloimmunisation event. Paradoxically, our cumulative frequency distribution showed that the risk of alloimmunisation did not increase with higher numbers of RBC units. Patients who received their first RBC units without an event were less likely to form alloantibodies during the course of their disease: most patients either alloimmunised shortly after their first transfusion, or not at all. Singhal *et al.*, who also studied an MDS population with DMT, similarly showed that 73% of alloimmunised patients developed antibodies within 20 RBC units[20]. These findings do not support the hypothesis that cumulative transfused RBCs is a strong predictor of alloimmunisation, as several studies suggest, although translation to clinical use remains problematic[9,15,21,26]. This raises intriguing questions regarding the possibility of extended matching for a select group of patients. Ideally, a distinction could be made between patients with an early alloimmunisation event and patients without alloimmunisation. However, baseline characteristics (apart from gender) and the number of transfusions were not found to be related to alloimmunisation.

Patients had lower alloimmunisation rates during DMT exposure, indicating that DMT may have a protective effect on the formation of alloantibodies. The Cox

time-dependent exposure analysis, addressing the potential changes in exposure status (DMT initiation and termination), confirmed this finding and provided a more accurate estimate of the relative risk while patients were receiving transfusions. We observed a non-significant difference in incidences of alloimmunisation in patients with and without DMT (2.41 and 7.18 per 100 patient-years, respectively).

Similar findings on immunomodulating treatment effects have been reported, although not studied in depth for MDS patients[4,5,21,22]. Two studies reported that HMAs are associated with lower alloimmunisation rates[4,21]. The cytotoxic effects of chemotherapeutic agents are also associated with immunosuppression[4]. The alloimmunisation mechanism is generally T-cell mediated[4,5,28]. Chemotherapy and HMAs frequently induce bone marrow suppression, compromising the immune system and impairing T-cell response[4]. Our findings support the hypothesis that DMT impedes the immune system's ability to form antibodies to non-self blood group antigens. In theory, alloimmunisation should be more common in patients receiving immune-activating therapies. Indeed, recent studies of patients receiving such therapies, e.g., interferons and checkpoint inhibitors, reported higher alloimmunisation rates[22]. This further underscores the influence of DMT on alloimmunisation. Again, translation to clinical use is still difficult, and more research is needed to quantify the effect of immunomodulating drugs on alloimmunisation and to examine the effect of response to immunomodulating drugs on immunity and alloimmunisation rates. Other effects of immunosuppression, e.g., increased infections, were beyond the scope of this study.

Current Dutch guidelines recommend extended matching for MDS patients, based mainly on the study by Lin *et al*.[29], which reported a reduction in the alloimmunisation rate from 23 to 11% when blood products were matched for full Rh and K[10]. Even though the alloimmunisation rate before preventive matching was relatively high (23%) compared to recent reports and this study, our data show a similar tendency as one hospital cross-matched blood products for MDS patients according to current guidelines (i.e., for ABO, full Rh and K) and had the lowest alloimmunisation rate (2.4 *vs* 5.0-22.2% in the other hospitals)[7,20,26,27]. It remains unclear, however, whether the difference is due solely to

matching procedures or if there are other reasons (e.g., patient differences between study samples). Indeed, most alloantibodies in our study would have been prevented if extended matching had been applied (33%), but it would also result in time-consuming RBC compatibility testing, increased costs, and increased donor selection pressure on blood banks. These effects might not outweigh the gains of extended matching. There is no known "number needed to treat" to aid in this dilemma and cost-effectivity analyses may be warranted, supported by data on specific risk factors for alloimmunisation, such as DMT.

Our study population was unique in several respects, containing detailed patient and transfusion data and including all types of MDS patients in a real-life setting. Secondly, the laboratory systems contained information about every transfused blood product since 1998. This enabled all blood products received in the period before patients were diagnosed with MDS to be evaluated. Thirdly, this analysis of MDS patients who did not receive full Rh/K matched blood products a priori, despite the recommendations of the Dutch transfusion guidelines, was only possible due to the policy that the hospital laboratories were blinded for the MDS diagnosis and thus preventive matching was not performed[10].

The incidence of alloimmunisation was higher in female than in male patients. Pregnancy is a factor known to elicit alloimmunisation[6,20,30,31]. Further detailed analysis was not feasible due to incomplete data on pregnancies.

We did not include platelet transfusions in our time-dependent analyses, because they were far fewer than RBC units, and the risk of antibody response to platelet transfusions is significantly lower than for RBC[13,20,22,27,30]. There was no significant difference in the number of platelet transfusions between patients with and without alloantibodies in this study. Allogenic SCT can also provoke alloimmunisation events. Because the role of allogenic SCT in inducing alloimmunisation is not clear, we excluded/censored patients who received an allogenic SCT[4,19,21,32]. This study was performed in a mainly Caucasian population. The frequencies of blood group antigens vary among different ethnicities[7,32,33]. These results should be confirmed in other communities before extrapolating to populations of different ethnicity.

Due to the retrospective nature of this study, the IPSS-R could not be determined for 37% of the population. This

is a known obstacle in population-based studies, as the clinician's choice is paramount and not all haematologists were convinced that additional cytogenetic analysis could alter the choice of treatment[34]. Given the retrospective design of our study, the causality of the relationship between DMT use and lower alloimmunisation rates still needs to be determined. Because of the low incidence of alloimmunisation events, it was not possible to study multiple episodes of exposure over a long follow-up period in the Cox time-dependent analysis or the effect of each type of treatment. Larger prospective studies are, therefore, needed to confirm the protective effect of DMT on alloimmunisation. Further research into potential risk factors, e.g., type of immunotherapy, status of the immune system and inflammation, and gender, is warranted.

## CONCLUSIONS

In a non-selected real-world population of MDS patients receiving blood transfusions, the percentage of patients with alloimmunisation was below 10%. Alloimmunisation predominantly occurred early in follow-up and the risk did not increase with RBC units. Like other recent studies, our study does not support the high alloimmunisation rates in MDS patients reported previously. Although not conclusive, a clinically relevant protective effect of DMT on alloimmunisation in MDS patients treated in a real-life setting was found. In this respect, our study supports the hypothesis that DMT affects the ability of the immune system to mount an antibody response to non-self blood group antigens.

## ACKNOWLEDGEMENTS

The Authors would like to thank Petra Meestringa (Nij Smellinghe Hospital, Drachten, the Netherlands) for support in data collection, Jisk Koopmans (Certe, Leeuwarden, the Netherlands) for revision of bone marrow cytology specimens, and the Frisian hospitals for their co-operation.

## AUTHORSHIP CONTRIBUTIONS

JR and CS contributed equally to this work.
JR and CS collected the data, analysed the data and wrote the manuscript. CS and HW designed the project. JR and NV analysed the data. RK, MH, ER and HW provided input

on data analysis and edited the manuscript. All Authors contributed to the critical revision and final approval of the manuscript.

*The Authors declare no conflicts of interest.*

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