

RESEARCH PAPER

RED CELL ALLOIMMUNIZATION AMONG HAEMATO-ONCOLOGIC PATIENTS IN A TEACHING HOSPITAL IN MALAYSIA

Noor Haslina Mohd Noor, Norahninzu Arifin, Mohd Nazri Hassan and Rapiaah Mustaffa

Transfusion Medicine Unit, Hospital Sains Malaysia, 16150 Kubang Kerian, Kelantan

Correspondence: Associate Prof. Noor Haslina Mohd Noor, Senior Lecturer/ Haematologist
Haematology Department, Health Campus, Universiti Sains Malaysia
Kubang Kerian, Kelantan, Malaysia. Tel: +60199390088

Email: drhaslina@kb.usm.my

 ORCID iD: <https://orcid.org/0000-0001-8357-1850>

Abstract

Background: The aim of the study was to estimate the prevalence and specificity of red blood cell alloantibody among haemato-oncologic patients and to correlate the association of antibody development with other factors such as age, gender, race, number of packed red blood cell (RBCs) transfused and diagnosis.

Methods: This prospective cross-sectional study was conducted in Transfusion Medicine Unit, HUSM. Clinical and serological data of 216 haemato-oncologic patients who received blood transfusions were collected and analysed. The blood samples were subjected to the standard immune-haematological procedure for RBC antibody screening and identification using reagents of Diamed-ID Gel microtyping system

Results: RBC alloimmunization rate among haemato-oncologic patients was 3.2%. Red cell antibodies were detected in seven patients. Four patients developed single antibody, while three develop multiple antibodies. However, we noted all seven patients were Malay with lymphoproliferative disease and majority were adult male (71.4%) patients.

Conclusions: The prevalence of RBC alloimmunization in post transfusion haemato-oncology patients was low despite of multiple transfusions and it is comparable with other diseases, which required multiple transfusions. Therefore, it is important to prioritize the extended antigen matching to patients who will be mostly benefitted.

Key words: red cell, alloimmunization, alloantibody, haemato-oncology, transfusion

Background

Alloimmunization to minor RBC antigens is a significant complication of transfusion therapy in haemato-oncology patients that leads to increased risk of transfusion reactions and limits the number of

compatible packed RBC available, results in restricting clinician's ability to safely transfuse packed RBCs in many situations. It has been recognized that alloimmunization is a significant complication and may compromise the therapeutic effect of transfusion.¹



This work is licensed under a Creative Commons Attribution 4.0 International License (CC BY)

Alloimmunization occurs when an incompatible antigen is introduced in an immunocompetent host and evokes an immune response. Variation in likelihood of acquiring new antibodies to RBC antigens is of clinical and economic consequence. Because of the large number of polymorphic antigens and the large number of epitopes on each antigen, every packed RBCs transfusion will introduce many foreign alloantigen.² Tormey and Stack's reported that the immunogenicity is dependent on few factors; number of antibodies with specificity, probability of exposure (patient being antigen negative and donor being antigen positive) and persistence of antibody³.

A retrospective study in 2006 reviewed 20 years of data on repeatedly transfused non haemato-oncology patients and suggested that those that were alloimmunized could benefit from a greater degree of phenotype/genotype matching.⁴

The differences in immunization risk and antibody specificity for various diseases are dependent on a number of factors. The genetic disparity between patient and donor RBC phenotypes is considered to be the main reason for the high immunization risk.⁵ A dysfunctioning of immune system, either hyper- or hyposensitive can result in an enhanced or reduced antibody production.⁵ The formation of RBC antibodies may be influenced by the patients' age at which the transfusions are given or when chronic transfusion therapy is started. Increasing number of alloimmunized patients were dependent on the number of transfusions, interval between transfusions events, the frequency of antibody testing (single versus serial) and specificity of the antibodies.⁵

To the best of our knowledge, this study of alloimmunization to RBC antigens in haemato-oncology patients was the first carried out in Kelantan population. It was done to estimate the prevalence of RBC

antibody in multiply transfused haemato-oncology patients and to look for the association factors contributing to its development.

Methodology

Study design and population

This cross sectional study was conducted over a one- year period at our institution. Ethical approval was obtained from the Ethics Committee of university hospital. Written consent from patients were also obtained. 216 haemato-oncologic patients receiving packed RBCs transfusions were included in this study. Data were obtained from the patient's clinical records and blood bank information system. Clinical transfusion records of the patients who fulfilled the inclusion and exclusion criteria were reviewed for the demographic data.

Patients were excluded from study if they had RBC antibodies that were present before the exposure to blood transfusion, received platelet transfusions and intravenous immunoglobulin. All patients were routinely transfused with the blood that was matched for only ABO and Rhesus D (RhD).

Laboratory methods

Using standard blood bank methods, serum was analyzed prior to each transfusion for detection of new antibody to RBC antigen. All the pre-transfusion sera were also tested to determine their phenotype and genotype for ABO and Rhesus blood group.

Prior to every transfusion, plasma was tested for the presence of alloantibodies by using commercial three-cell panel (Diacell, Bio-Rad, Switzerland). All alloantibody screening positive samples were evaluated to identify the antibody specificity. Detection of antibody specificity was

performed using a commercial 11-cell identification panel (Diapanel, Bio-Rad, Switzerland). Autocontrol was performed in each case to identify autoantibodies. It was done by incubating patient's cell with patient's plasma at 37°C for 15 minutes and then centrifuging for 10 minutes on gel card containing poly specific antihuman globulin. All the tests were performed using the gel card method by Diamed ID (Switzerland), as per manufacturer's guidelines. Elution and adsorption methods were employed in patients with suspected autoantibodies.

In cases where the RBC antibody identification did not show any specificity, additional method was used using CSL Abtectcell™ III screening cell and CSL Phenocell™ antibody identification (Australia). All pretransfusion sera were also tested to determine their phenotype for the following blood group systems: ABO; Rh (D, C, E, c, and e); Kell (K, k), Kidd (Kpa, Kpb) and Duffy (Fya, Fyb).

Statistical analysis

Descriptive statistics and Fisher's exact test were performed and a p-value of less than 0.05 was considered significant. The results were analyzed using the SPSS version 11.5 (SPSS Inc, Chicago, IL, US).

Results

A total of 216 haemato-oncology patients (119 males and 97 females, age ranges 1-86 years and majority were adults) who received packed RBCs were included in the study. Majority were Malay with other races include Chinese, Indian, Orang Asli and Siamese.

Total number of packed RBCs receives by 216 patients were 909 units with the number of units transfused per patients ranged from one to 21. Majority of patients received packed RBCs transfusion of less than ten units. The mean of packed RBCs transfused was about 4.21. Patients usually received packed RBCs transfusion during the diagnostic workup and during early course of chemotherapy because of anaemia due to bone marrow suppression either secondary to disease itself or because of the chemotherapy.

Antibody screening was positive in seven patients (3.2%). All alloimmunized patients were Malay and only one patient was from paediatric age group (2-year-old). All of patients were diagnosed with lymphoproliferative diseases which three of them had Acute Lymphocytic Leukaemia (ALL), three had Non-Hodgkin Lymphoma (NHL) and one patient with Multiple Myeloma (MM). The specificity of the alloantibodies found in seven patients was against Rh, Lewis, MNS and Duffy blood group system. Four patients developed single antibody, which was anti-M, anti-E and anti-S. One patient had double antibodies, anti-E and anti-Lea, one patient developed triple antibodies, anti-E, -Lea and -Mia and one patient had multiple antibodies, anti-N, -S, -Lea and -Fya and -Fyb.

The number of packed RBCs received by all alloimmunized patients was between two units to eight units. Most of them developed antibody after two units of packed cell transfusion. Data of patients with alloantibody were shown in Table 1.0.

Table 1: Data of patients with alloantibody/ies

Patient	1	2	3	4	5	6	7
Gender	Male	Male	Male	Female	Male	Male	Female
Race	Malay	Malay	Malay	Malay	Malay	Malay	Malay
Age	18	55	44	61	60	2	14
Diagnosis	ALL	NHL	MM	NHL	NHL	ALL	ALL
ABO Blood Group	B	B	A	A	O	A	B
Rhesus	Pos	Pos	Pos	Pos	Pos	Pos	Pos
Number of packed cell transfused	8	2	3	6	6	2	2
Antibody phenotype	E + Le ^a	N, S, Le ^a , Fy ^a , Fy ^b	S	M	E + Le ^a + Mia	M	E

Discussion

This prospective study of alloimmunization to RBC antigens in 216 haemato-oncologic patients was the first carried out in the Kelantan population. In the present study, the overall alloimmunization rate was 3.2%, which was low when compared with other studies done in patients with myelodysplastic syndrome or chronic myelomonocytic leukaemia (MDS/CMML) and myeloid neoplasms patients, which was 15% and 10.8% respectively.^{6,7} However, in similar studies in Western India and Sub-Saharan Africa reported the alloimmunization rate was slightly lower, 6.1% and 6.7% respectively.⁸

We observed the alloantibodies were from Rh, Lewis MNS and Duffy blood group system. The most prevalent antibodies in our study were against anti-E, anti-Le^a,

anti M and anti-S. Similar findings were reported in oncology patients in India where the alloantibodies identified were from Rh, Duffy and Lewis blood group system with the commonest alloantibody being anti- Le^a that was identified in 5/12 of patients⁹. A study on RBC alloimmunization among MDS/CMML patients observed antibodies formation involves the Rh system and Kell.⁶ In a previous study among patients with myeloid neoplasms, the most frequent antibody specificity was anti-E, followed by anti-Wra, -Lua, -D, -C and -Jka.⁷ Philip J et al reported that 72.7% antibody in multi-transfused patients belonged to Rh blood group system with the following subspecificities: anti-D, anti-E and anti-c.⁸

The factors for alloimmunization are complex and involve many contributing elements such as RBC antigenic differences between the blood donor and

the recipient, recipient's immune status and immunomodulatory effect of the allogeneic blood transfusions. A study on RBC phenotyping among blood donors by National Blood Centre of Malaysia reported that the Rh type and Lewis group system had significant different distributions among the donors. The RhD positive genotype cDE/cDE (R2R2) was rare and the cDE/CDE (R2Rz) was found in only two Malay donors. It was also reported that the Lewis and Duffy system were common and comparable with the previous findings in Asian populations. A study of the Lewis system showed that the expression of the Le^{a-b+} was more than 50% in all the study groups. Fy^{a+b-} was the most common phenotype in the Duffy system. The MN phenotype was common among Malays and Chinese which were 44% and 43.1% respectively.¹⁰

Genetic differences between individuals influence inter-individual variation in alloimmunization rates. When donors and recipients are more closely matched for non-ABO antigens, alloimmunization rates have been noted to dramatically decrease.¹¹ On the other hand, donor populations that have substantial variation in the expression of non-ABO antigens in comparison to recipients establishes greater numbers of antigenic mismatches and higher number of alloantibodies will be induced.¹¹

A low rate of alloimmunization may be expected when there is homogeneity of RBC antigens between the blood donors and recipients. The homogeneity between the patient and blood donor population may be the reason of low rate of alloimmunization in our study.

In the current study, all alloimmunized patients received between two units to eight units of packed RBCs and we observed that most of our patients developed antibodies after the second transfusion. Similar findings were reported

by Dhar S et al where antibody formation was first occurred after a median of six packed RBCs had been transfused to oncology patients.⁹ Philip et al reported higher alloimmunization rates in patients receiving more than the median of 46 units, compared to who had received less than the median of 46 units (81.6 vs 1.6%).⁸ However study by Zalpuri S et al showed no significant difference in the risk of alloimmunization in intensively transfused patients and non-intensively transfused patients.¹²

We did not find any association of gender with rate of alloimmunization. Majority of alloimmunized patients in the present study were male (5/7). A study among multiply transfused patients in Western India reported that females (8 of 11 [72.7%]) appeared to have a greater risk of RBC alloimmunization compared with males.¹³ Verduin EP et al reported a relative 80% higher risk in women older than 45 years of age and that elder women beyond childbearing age have a higher risk of post transfusion antibody formation compared to men.¹⁴ Few other studies on multiply transfused patients also reported that women were more often prone to develop RBC antibodies compared to males.¹⁵ This was probably due to more exposure to immunizing events through pregnancy and/or transfusion.¹⁴ Zalpuri reported immunization rate was comparable for male and female over the age of 45 years but lower alloimmunization frequency among women age < 45 years of age.¹⁶

In the present study, we could not see any association between risks of alloimmunization with age. Only one patient from paediatric age group developed red cell alloantibody. Higgins JM et al observed that age was not a factor to cause patients to be a responder.² However, another study showed aging may also affect RBC alloimmunization rates. It has been reported that age > 77

years is associated with a decreased rate of blood group antigen alloimmunization.¹⁷ Payal C. Desai reported that RBC antibody formation was significantly associated with increased patient age (median age: 31 years).¹⁸

All of our alloimmunized patients were diagnosed with lymphoproliferative diseases. Arora K et al reported the rate of alloimmunization was lower in patients who had hematologic malignancies (7%) compared with those who had solid cancers (22.6%) or myelodysplastic syndrome (23%). Lower rates of alloimmunization in haematologic malignancies may be caused by lymphocyte impairment of the disease.¹⁹ Dorothea et al reported risks associated with red cell alloimmunization are significantly reduced in patients treated for acute leukaemia, mature lymphomas and recipients of an autologous or allogeneic HSCT.²⁰ Diminished immune responses most likely reflect the intensity of treatment-associated immunosuppression.⁶ A study on alloimmunization rate according to disease entity and treatment regimen has shown a two-fold higher alloimmunization rates for patients treated with immunomodulating therapies compared to patients not receiving immunomodulating therapies.⁷ However, the study by Higgins JM et al reported disease is not a contributing factor for development of RBC antibody.² The underlying disease state may potentially predispose an individual to responder status, perhaps via associated inflammation or genetic polymorphisms.¹¹

Red cell alloimmunization rates among patients with a haemato-oncologic disease is comparable with that of patients with other diseases requiring multiple transfusions. Limiting RBC phenotyping to the most immunogenic groups and appropriately matching donors and receivers might be considered interim standards to mitigate alloimmunization

and cost effective in resource-limited circumstances. We recommended a practical, cost-effective, and feasible approach where RBC antigen typing should be performed before first transfusion in oncology patients and to issue Rh phenotype specific blood in order to limit alloimmunization.

Acknowledgements

We would like to thank the staff of the transfusion Medicine Unit, for their technical help in this study.

Conflict of Interest

There is no conflict of interest.

References

1. Nance S. Management of alloimmunized patients. *ISBT Science Series* 2010,5 (n1), 274-278.
2. Higgins J. M., Sloan S. R. Stochastic modeling of human RBC alloimmunization: evidence for a distinct population of immunologic responders. *Blood* 2008,112 (6), 2546-2553. <https://doi.org/10.1182/blood-2008-03-146415>
3. Tormey C. A., Stack G. IMMUNOHEMATOLOGY: The characterization and classification of concurrent blood group antibodies. *Transfusion* 2009,49 (12), 2709-2718. <https://doi.org/10.1111/j.1537-2995.2009.02337.x>
4. Schonewille H., Van De Watering L. M. G., Brand A. Additional red blood cell alloantibodies after blood transfusions in a nonhematologic alloimmunized patient cohort: is it time to take precautionary measures? *Transfusion* 2006,46 (4), 630-

635. <https://doi.org/10.1111/j.1537-2995.2006.00764.x>

5. Schonewille, H.; Van De Watering, L. M. G.; Loomans, D. S. E.; Brand, A. Red blood cell alloantibodies after transfusion: factors influencing incidence and specificity. *Transfusion* 2006,46 (2), 250-256. <https://doi.org/10.1111/j.1537-2995.2006.00708.x>

6. Sanz C., Nomdedeu M., Belkaid M., Martinez I., Nomdedeu B., Pereira A. Red blood cell alloimmunization in transfused patients with myelodysplastic syndrome or chronic myelomonocytic leukemia. *Transfusion* 2013,53 (4), 710-5. <https://doi.org/10.1111/j.1537-2995.2012.03819.x>

7. Leisch M., Weiss L., Lindlbauer N., Jungbauer C., Egle A., Rohde E., Greil R., Grabmer C., Pleyer L. Red blood cell alloimmunization in 184 patients with myeloid neoplasms treated with azacitidine - A retrospective single center experience. *Leukemia research* 2017,59, 12-19. <https://doi.org/10.1016/j.leukres.2017.05.006>

8. Philip J., Biswas A. K., Hiregoudar S, Kushwaha N. Red blood cell alloimmunization in multitransfused patients in a tertiary care center in Western India. *Laboratory medicine* 2014,45 (4), 324-30. <https://doi.org/10.1309/LMUCV97YUWQKAHU4>

9. Dhar S., Basu S. Red cell alloimmunisation in oncology patients: A study from eastern India. *Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis* 2015,52 (3), 345-9. <https://doi.org/10.1016/j.transci.2015.02.019>

10. Musa R. H., Ahmed S. A., Hashim H., Ayob Y., Asidin N. H., Choo P. Y., Al-Joudi F. S. Red cell phenotyping of blood from donors at the National blood center of Malaysia. *Asian journal of transfusion science* 2012,6 (1), 3. <https://doi.org/10.4103/0973-6247.95042>

11. Gehrie E. A., Tormey C. A. The Influence of Clinical and Biological Factors on Transfusion-Associated Non-ABO Antigen Alloimmunization: Responders, Hyper-Responders, and Non-Responders. *Transfusion medicine and hemotherapy : offizielles Organ der Deutschen Gesellschaft fur Transfusionsmedizin und Immunhamatologie* 2014,41 (6), 420-9. <https://doi.org/10.1159/000369109>

12. Zalpuri S. M. R., Schonewille H, de Vooght KM, le Cessie S, van der Bom JG, Zwaginga JJ. Intensive red blood cell transfusions and risk of alloimmunization. *Transfusion* 2014,54 (2), 278-284. <https://doi.org/10.1111/trf.12312>

13. Ngoma A. M., Mutombo P. B., Ikeda K., Nollet K. E., Natukunda B., Ohto H. Red blood cell alloimmunization in transfused patients in sub-Saharan Africa: A systematic review and meta-analysis. *Transfusion and apheresis science : official journal of the World Apheresis Association : official journal of the European Society for Haemapheresis* 2016,54 (2), 296-302. <https://doi.org/10.1016/j.transci.2015.10.017>

14. Verduin EP, B. A., Middelburg RA, Schonewille H. Female sex of older patients is an independent risk factor for red blood cell alloimmunization after transfusion. *Transfusion*. 2015,Jun (55(6 Pt 2)), 1478-85. <https://doi.org/10.1111/trf.13111>

15. Verduin E. P, Brand A., Schonewille, H. Is Female Sex a Risk Factor for Red Blood Cell Alloimmunization After Transfusion? A Systematic Review. *Transfusion medicine reviews* 2012,26 (4), 342-53.

<https://doi.org/10.1016/j.tmr.2011.12.001>

<https://doi.org/10.3324/haematol.2016.152074>

16. Zalpuri S., Zwaginga J., le Cessie S., Elshuis J., Schonewille H., van der Bom J. Red blood cell alloimmunization and number of red blood cell transfusions *Vox sanguinis* 2012,102 (2), 144-149.

<https://doi.org/10.1111/j.1423-0410.2011.01517.x>

17. Gonzalez-Porras J. R., Graciani, I. F., Perez-Simon J. A., Martin-Sanchez, J., Encinas, C., Conde, M. P., Nieto, M. J., Corral, M. Prospective evaluation of a transfusion policy of D+ red blood cells into D- patients. *Transfusion* 2008,48 (7), 1318-24.

<https://doi.org/10.1111/j.1537-2995.2008.01700.x>

18. Desai P.C., Deal D. A., Pfaff E.R., Qaqish B., Hebden L.M., Park Y.A., Ataga K.I. . Alloimmunization is associated with older age of transfused red blood cells in sickle cell disease. *Am J Hematol.* 2015 90 (8), 691-695.

<https://doi.org/10.1002/ajh.24051>

19. Arora K., Kelley J., Sui D., Ning J., Martinez F., Lichtiger B., Tholpady A. Cancer type predicts alloimmunization following RhD-incompatible RBC transfusions. *Transfusion* 2017,57 (4), 952-958.

<https://doi.org/10.1111/trf.13999>

20. Evers D, Zwanninga J., Tijmensen J, Middelburg RA, de Haas M, de Vooght KM, van de Kerkhof D, Visser O, Péquériaux NC, Hudig F, van der Bom JG. Treatments for hematologic malignancies in contrast to those for solid cancers are associated with reduced red cell alloimmunization. *Haematologica.* Jan;: 2017,102 (1), 52-59.