Original article

Associated Factors of Red Blood Cell Alloimmunization among Solid Cancer Patients in Teaching Hospital in Malaysia

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Abstract:

Background: Alloimmunization is a known complication following repeated red blood cell transfusions. However, the associated factors of RBC alloimmunization are different among blood recipient groups. This study aimed to determine the associated factors of red blood cell alloimmunization among solid cancer patients in Hospital USM. ***Material and Methods:*** This cross-sectional study involved 322 adult solid cancer patients screened for red cell alloantibodies in our centre from October 2016 until February 2019. The clinical and transfusion data were obtained from the patient's medical record and laboratory information system (My Transfusi). The multiple logistic regression was used for analysis of potential associated factors of red blood cells alloimmunization, and *p*-value of less than 0.050 was considered statistically significant. ***Results:*** The patient's mean age was 52.0 years old, with the majority were Malays (91.0%) and females (61.2%). The most common cancers were breast (32.3%) followed by gastrointestinal (16.8%) and head and neck (14.3%). The presence of metastases (adjusted OR=13.53, p=0.017), numbers of packed red cell transfusions (adjusted OR=1.12, p= 0.015), and blood group A (adjusted OR=7.45, p=0.003) were the associated factors of alloimmunization. ***Conclusions:*** **S**olid cancer patients with metastases, received a higher number of packed red cell transfusions, and blood group A were at higher risk for alloimmunization. Strategies to provide phenotype RBC should focus on this type of patient to prevent red blood cells alloimmunization in solid cancer patients.

Keywords: Red blood cell; alloimmunization; solid cancer.

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Introduction:

Red blood cell (RBC) transfusion is one of the essential modalities in managing cancer patients with symptomatic anaemia or immediate correction of anaemia for pre-chemotherapy and pre-operative optimization. RBC alloimmunization is a known complication of repeated allogenic RBC transfusion. It is associated with transfusion delays if there is difficulty finding compatible blood and causesa haemolytic transfusion reaction if it is not detected during pre-transfusion testing.[1,2]

Only a few studies have reported RBC alloimmunization following repeated RBC transfusion in solid cancer patients. The prevalence was reported to be higher than in the haematological malignancy.[2,3] In Malaysia, all regularly transfused patients, such as thalassaemia patients, are required for RBC phenotyping for ABO, Rhesus (Rh), Kidd, Duffy (Fy) and MNS antigen at diagnosis or before the first transfusion as a strategy for prevention of RBC alloimmunization.[4]

Many factors are associated with RBC alloimmunization in repeated transfused patients involving the recipient and environmental factors. These factors includethe dose and immunogenicity of the RBC antigens, recipient's age and gender, pregnancy history, disease, immune status, state of inflammation and the number of transfusion units.[5,6] Solid cancers are reported to be one of the risk factors for RBC alloimmunization.[5] However, the information regarding the risk factors of RBC alloimmunization among solid cancer patients is still limited.

To our knowledge, no studies have yet on the Malaysian population concerning RBC alloimmunization among solid cancer patients in. The result might differ from the previous study, as the prevalence of RBC antigens and alloantibodies varies among the human population and ethnicities.[7] This study was conducted to determine the predictive factors of RBC alloimmunization among solid cancer in our centre. So, it would be useful to determine whether any clinical features capable of predicting RBC alloimmunization among solid cancer patients to justify the phenotype of RBC before blood transfusion.

Materials and methods:

Patients and study background

This is across-sectional study with a retrospective record review involving 322 adult oncology patients (age 18 years old and above) with solid cancers treated and screened for RBC alloantibody at Hospital Universiti Sains Malaysia (USM) from October 2016 until February 2019 [Table 1].

The clinical and transfusion data were collected from patient's record and the blood bank information system (*My Transfusi*) and included 1. demographic data include age, gender, race, pregnancy history, weight and height; 2. clinical data include type and stage of the disease, metastases status, performance status and treatment, and 3. laboratory data include ABO/RhD blood group, history and frequency of packed red cell (PC) transfusion.

Performance status was assessed using Eastern Cooperative Oncology Group (ECOG) performance status scale ranging from 0 to 5.[8] Higher ECOG score indicates a lower performance status of the patient. Meanwhile, the type and staging of solid cancer were based on TNM classification system. For this study purpose, the stages were categorized into early (stage I and II) and the advanced stages (stage III and IV).[9]

Antibody screening and identification

Antibody screening was performed using a three-screening cell panel at 37°C by saline indirect antiglobulin test (ID-Diacell I, II, III Asia). Those samples with positive antibody screening were further analyzed with antibody identification test using another eleven-cell panel, using low ionic strength solution and enzyme method (papain). The entire tests were performed using a commercialized cell panel by microcolumn gel agglutination method from Diamed-ID micro typing system (DiaMed-ID®; Bio-Rad Laboratories, DiaMed GmbH, Cressier FR Switzerland).

Statistical analysis

The data were analyzed using IBM Statistical Package for the Social Sciences software (SPSS) Statistic version 26.0 for Windows. The descriptive results are expressed as mean and standard deviation (SD) for numerical and the categorical variables were described in frequency and percentage (%). Simple (SLR) and multiple logistic regression (MLR) were used to analyze the potential independent factors of RBC alloimmunization as it able to control all the potential confounders in the data analysis. The variables that have *p*-value of less than 0.250 in the SLR were included in the MLR analysis. The *p*-value of less than 0.050 was considered statistically significant.

Ethical Clearance:

This study was approved by Human Research Ethics Committee USM, with the protocol number: USM/JEPeM/17120684.

Results:

The patients' mean age was 52.0 (SD±13.7) years which majority were Malays (91.0%). More than half of the patients were females (61.2%). The three most common solid cancers were breast (32.3%) followed by gastrointestinal (16.8%) and head and neck (14.3%) cancer [Table 1]. There were 17 (5.3%) patients with RBC alloimmunization among 322 solid cancer patients. The majority of alloimmunized solid cancer patients were female (n=12), Malay (n=17), at an advanced stage (n=15) and with metastases (n=13), had a history of PC transfusion (n=14) and history of chemotherapy (n=13) [Table2].

In univariate analysis, we found a significant association between ECOG score (p=0.001), presence of metastases (p=0.018), number of PC transfusion (p=0.005) and A blood group (p=0.026) with RBC alloimmunization. Alloimmunized solid cancer patients have a significantly higher mean of ECOG score, 2.4 (±1.1) and mean of PC transfused, 9.5 (±9.0) compared to non-alloimmunized patients. In multivariate analysis, the presence of metastases (adjusted odds ratio (OR) =13.53, p=0.017), number of PC transfusions (adjusted OR=1.12, p = 0.015) and A blood group (adjusted OR=7.45, p= 0.003)were found to be independent factors for RBC alloimmunization among solid cancer patients [Table 2].

Table 1: Type of solid cancer patients among subjects (n=322)

Type of cancer	Frequency, n	Percentage, %
Breast ca	**104**	32.3
Gastrointestinal	**54**	16.8
Colorectal ca	46	
Gastric ca	3	
Pancreatic ca	2	
Gall bladder ca	1	
Oesophageal ca	2	
Head and neck	**46**	14.3
Nasopharyngeal ca	30	
Thyroid ca	4	
Tongue ca	6	
Nasal ca	2	
Parotid gland ca	4	
Musculoskeletal ca	**22**	6.8
Gynaecological	**29**	9.0
Cervical ca	21	
Ovarian ca	4	
Endometrial ca	4	
Lung ca	**20**	6.2
Urogenital	**24**	7.5
Bladder ca	8	
Testicular ca	10	
Prostate ca	6	
Others	**23**	7.1

ca=cancer

Discussion:

This study reported that 5.3% of solid cancer patientshave RBC alloimmunization, within the reported ranges of <1% to 11% by previous studies withsimilar groups of subjects.[2,3] However, the reported rate of RBC alloimmunization in the other groups of multi-transfused patients varied, ranging from 1-2% and even much higher, up to 30% in thalassaemia patients.[1,7,10] The observable range can be attributed to many factors, including the demography of the patient's cohort, number of PC transfusion exposure, local transfusion policy and the type of disease and comorbidities.

Solid cancer patients were shown to have a higher risk of RBC alloimmunization since they were postulated most likely at the chronic inflammatory activation state.[5] Our study further explores a few selected clinical and laboratory factors that can predict RBC alloimmunization in this group of patients. Our results showed that the presence of metastases, a higher number of PC transfusions and A blood group are the associated factors of RBC alloimmunization in solid cancer patients.

We found that solid cancer patients with metastases have 13.53 times more odds of developing RBC alloimmunization than those without metastases. The acute inflammatory state has been reported to cause a higher risk for RBC alloimmunization.[11] The presence of metastases implies a more advanced disease state, and it is associated with a more systemic inflammatory response in cancer patients, which puts them at higher risk for RBC alloimmunization.[11-14] A study reported conflicting results with our results, where they found that the presence of metastases was not associated with RBC alloimmunization in solid cancer patients.[8] However, since this study only involved a small sample, their result can still be debated.

Table 2: Risk factors of RBC alloimmunization among solid cancer patients by multivariate analysis (n=322)

Variables	Alloimmunization, n (%)		Crude OR (95% CI)	p[1]	Adjusted OR (95% CI)	p[2]
	Yes (n=17)	No (n=305)				
Age (years) [a]	51.0 (10.4)	52.5 (13.9)	0.99 (0.96, 1.03)	0.665	-	-
ECOG score [a]	2.4 (1.1)	1.3 (1.1)	**2.16 (1.39, 3.36)**	**0.001**	1.35 (0.73, 2.50)	0.345
Number of unit PC transfused [b]	9.5 (9.0)	4.8 (5.1)	**1.10 (1.03, 1.17)**	**0.005**	**1.12 (1.02, 1.22)**	**0.015**
Gender						
Male	5 (29.4/)	120 (39.3)	1.00			
Female	12(70.6)	185(60.7)	1.69 (0.59, 4.88)	0.327	-	-
Race						
Malay	17 (100.0)	276 (90.5)	1.00			
Non-Malay	0 (0.0)	29 (9.5)	0.00 (0.00, -)	0.998	-	-
History of PC transfusion						
No	3 (17.6)	61 (20.0)	1.00			
Yes	14 (82.4)	244 (80.0)	1.25 (0.35, 4.47)	0.726	-	-
History of pregnancy [c, d]						
Yes	11 (100.0)	133 (85.8)	1.00			
No	0 (0.0)	22 (14.2)	0.00 (0.00, -)	0.998	-	-
Diagnosis						
Breast ca	4 (23.5)	100 (32.8)	1.00	-		
Gastrointestinal ca	2 (11.8)	52 (17.1)	0.96 (0.17, 5.42)	0.965	-	-
Gynaecology ca	3 (17.6)	26 (8.5)	2.89 (0.61, 13.70)	0.183	-	-
Head & neck ca	2 (11.8)	44 (14.4)	1.14 (0.20, 6.44)	0.885	-	-
Lung cancer	1 (5.9)	19 (6.2)	1.32 (0.14, 12.43)	0.881	-	-
Musculoskeletal ca	1 (5.9)	21 (6.9)	1.19 (0.13, 11.20)	0.879	-	-
Urogenital ca	1 (5.9)	23 (7.5)	1.09 (0.12, 10.19)	0.942	-	-
Others	3 (17.6)	20 (6.6)	3.75 (0.78, 18.06)	0.099	-	-
Stage[d]						
Early (I/II)	0 (0.0)	52 (20.1)	1.00			
Advanced (III/IV)	15 (100.0)	207 (79.9)	0.00 (0.00, -)	0.997	-	-
Metastases [d]						
No	2 (11.8)	135 (44.3)	1.00			
Yes	13 (76.5)	143 (46.9)	**6.14 (1.36, 27.70)**	**0.018**	**13.53 (1.61, 113.96)**	**0.017**
Blood group						
Non-A	8 (47.1)	223/ (73.1)	1.00			
A	9 (52.9)	82 (26.9)	**3.06 (1.14, 8.20)**	**0.026**	**7.45 (1.94, 28.64)**	**0.003**
Chemotherapy [d]						
Yes	13 (81.2)	255 (89.5)	1.00			
No	3 (18.8)	30 (10.5)	1.96 (0.53, 7.28)	0.314	-	-
Radiotherapy [d]						
Yes	7 (43.8)	136 (47.6)	1.00			
No	9 (56.2)	150 (52.4)	1.17 (0.42, 3.22)	0.767	-	-

[a]= mean (±SD); [b]= among patient with history of PC transfusion; [c]= among female patients; [d]=presence of missing data; ECOG=Eastern Cooperative Oncology Group; PC=packed red cell; ca=cancer; [1]=simple logistic regression; [2]=multiple logistic regression, Backward LR method was applied. No multicollinearity and no interaction were found

We also include other variables which associated with the inflammatory state, including performance status (ECOG score), disease stage and treatment regime with chemotherapy and radiotherapy. Patients with low performance status and at the advanced stage were considered to have a higher inflammatory response.[8] On the other hand, patients who received chemotherapy and/or radiotherapy were considered to have a less inflammatory response due to immunosuppression.[15] However, we found that these variables were not an independent risk factor for RBC alloimmunization although univariate analysis showed the association of ECOG score with RBC alloimmunization. A patient with a high ECOG score is only associated with RBC alloimmunization if the other risk factors coexist in a particular patient. These findings also could be explained by the higher number of missing data in those variables and involved a small sample of alloimmunized patients.

We found that the number of PC transfused among transfused patients was an independent factor for RBC alloimmunization rather than the history of transfusion itself. The average number of PC transfusions in alloimmunized patients was 9.5 units compared to 4.8 units in non-alloimmunized patients. Our result shows that one-unit increase of PC transfusion among transfused patients has 1.12 times the odds of developing RBC alloimmunization. It has been shown that an increased number of transfusion translating into more antigenic exposure impose a greater risk for RBC alloimmunization.[2,16]As treatment protocols for solid cancer improve and patient's survival is prolonged, the patients may require more PC transfusion and subsequently putting the patient at risk to develop alloantibodies,.[2,16]

Another finding that has not been published earlier is the association of the ABO blood group with RBC alloimmunization. We found that blood group A is an independent factor for RBC alloimmunization in solid cancer patients. Blood group A patients have 7.45 times the oddsof developing RBC alloimmunization compared to non-A patients. We could not find previous studies that had demonstrated a significant association between ABO blood groups and the risk of RBC alloimmunization. Thus, a larger scale of study is warranted to support this finding. Whether it is because of the limited sample size or actual non-association remained undetermined.

It was reported that the risk of RBC alloimmunization differs between solid cancer and haematological malignancy, the former having a higher risk.[5] Arora et al. reported that cancer type (solid cancer and myelodysplastic syndrome) predicts RhD alloimmunization following RhD incompatible RBC transfusion.[17] The significant difference between different type of solid cancers remains not well reported. We attempted to find an association between specific cancer and RBC alloimmunization risk; however, no association was found. Whether this is because of the limited sample size or actual non-association remained undetermined.

We found that although most alloimmunized patients were female and at a younger age, our results showed no significant association between gender and age with the risk of RBC alloimmunization. Regarding gender, a few studies have shown female as an independent risk factor for RBC alloimmunization among the different study populations.[5,18-19] However, a systematic review involving 30 articles which fulfilled the inclusion criteria, could not confirm this association.[19]

Our study has limitations where many missing data in certain variables might reduce the study's statistical power and the subject's representativeness and cause bias in the estimating of the variables. The main weakness is that this study involved a relatively small number of alloimmunized than non-alloimmunized patients due to the limited number of cases available within the study period. Thus, the results must be cautiously inferred since they might not represent the reference population. This study concludes that the presence of metastases, who received a higher number of PC transfusions and blood group A are the predictors for RBC alloimmunization in solid cancer patients. Strategies to provide phenotype-matched PC should focus on the patients with blood group A, received multiple PC transfusion and advanced disease with metastases to prevent RBC alloimmunization. However, further studies involving a larger sample size are needed to confirm these associations and investigate their possible underlying mechanism.

Conflict of interest:

All the authors declare that there was no conflict of interest.

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Authors' Contribution

Type Of Contribution	Contributors
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Study design	Mohd Nazri Hassan Noor Haslina Mohd Noor
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