

## Editorial

# The role of red cell genotyping for transfusion management in thalassemia

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### Introduction

Thalassemia is the most common genetic disorder in Thailand and Southeast Asia. Approximately 30-40% of Thai population carries thalassemia genes<sup>1</sup>. Based on their disease severity and transfusion requirement, thalassemia could be clinically classified into transfusion-dependent thalassemia (TDT) and nontransfusion-dependent thalassemia (NTDT)<sup>2</sup>. The only curative treatment is hematopoietic stem cell transplantation and gene therapy.

Nowadays, chronic lifelong transfusion is needed in TDT group to increase the oxygen-carrying capacity by correcting anemia, prevent life-threatening organ damage, promote physical growth, improve quality of life, sufficiently suppress intra- and extramedullary hematopoiesis, and improve survival. While NTDT group may still require occasional transfusions in certain situation such as infection, surgery, or pregnancy.

Patients requiring multiple blood transfusion are at high risk of red cell alloimmunization, which leads to serologically incompatible blood transfusion and risk of hemolytic transfusion reaction. Additionally, the selection of appropriate red cell units for transfusion in patients with red cell alloantibodies could be labor-intensive and delaying. This review would emphasize on the pre-transfusion testing, the importance of partially phenotype-matched red cell units and the role of red cell genotyping for proper transfusion management in thalassemia.

### Pre-transfusion testing for thalassemia patients

The patient's ABO/RhD blood grouping and antibody screening must be performed before transfusion. To reduce the risk of red cell alloimmunization in chronically transfused thalassemia patients, extended red cell antigen typing using phenotyping and/or genotyping is recommended. Before the first blood transfusion, at least Rh C, c, E, e, and Mi<sup>a</sup> antigens should be serologically phenotyped although preferably a full red cell antigen typing including M, N, S, s, Le<sup>a</sup>, Le<sup>b</sup>, Jk<sup>a</sup>, Jk<sup>b</sup>, Fy<sup>a</sup>, Fy<sup>b</sup>, K, and Di<sup>a</sup> should be performed. Red cell genotyping is preferred in the patients who are already transfused because serology typing is not reliable after chronic transfusion.

A recent study in 68 Thai thalassemia patients showed that 96% (65/68) of chronically transfused patients had at least one inconclusive serological phenotype (a mixed field or weak reactivity) especially in MNS, Kidd, and Rh blood group systems<sup>3</sup>. Also, the discordant rate between serology typing and red cell genotyping was high in MNS, Duffy, and Kidd blood group systems. This led to the failure to obtain antigen-matched blood. Red cell genotyping revealed that C+c- (66%) and Mi(a+) (15%) antigen types were more common in Thai thalassemia patients compared with those of Western population as shown in Table 1.

The overall prevalence of red cell alloantibodies in Thai thalassemia patients was 29.8% and the top three red cell alloantibodies were anti-E, anti-Mi<sup>a</sup>, and anti-c<sup>4</sup>.

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**Table 1** Blood groups phenotypes of 68 analyzed patients by red cell genotyping method

Blood group	Predicted phenotypes patients (n = 68) N (%)		
Rh	C+c-: 45(66.2)	C-c+: 1(1.5)	C+c+: 22(23.5)
	E+e-: 1(1.5)	E-e+: 51(75)	E+e+: 16(23.5)
	CW-: 68(100)		
	V-: 68(100)		
	hrS+: 68(100)		
	VS-: 68(100)		
	hrB+: 67(98.5)	hrB-: 1(1.5)	
Kell	K-d+: 68(100)		
	Kp(a-b+): 68(100)		
	Js(a-b+): 68(100)		
Kidd	Jk(a+b-): 17(25)	Jk(a-b+): 10(14.7)	Jk(a+b+): 41(60.3)
Duffy	Fy(a+b-): 59(86.8)	Fy(a+b+): 8(11.7)	UN: 1(1.5)
MNS	M+N-: 28(41.2)	M-N+: 7(10.3)	M+N+: 33(48.5)
	S-s+: 63(92.6)	S+s+: 5(7.4)	
	U+: 68(100)		
	Mi <sup>a</sup> +: 10(14.7)	Mi <sup>a</sup> -: 58(85.3)	
Diego	Di(a-b+): 67(98.5)	Dia(a+b+): 1(1.5)	
Dombrock	Do(a+b-): 2(2.9)	Do(a+b+): 54(79.4)	Do(a+b+): 12(17.7)
	Hy+: 68(100)		
	Joa+: 68(100)		
Colton	Co(a+b-): 68(100)		
Cartwright	Yt(a+b-): 68(100)		
Lutheran	Lu(a-b+): 68(100)		

To avoid the development of red cell alloimmunization, all Thai thalassemia patients should be transfused with ABO, Rh (C, c, D, E, e) and Mi<sup>a</sup> compatible blood.

Most blood banks perform antibody screening and an indirect antiglobulin test crossmatch before each transfusion. In blood banks that strictly adhere to regulations of computer systems, sample labeling, and other critical issues, an electronic crossmatch may be used in thalassemia patients without history of red cell alloimmunization and not pregnant<sup>5</sup>. Importantly, new clinically significant antibodies must be identified and red cell units lacking the corresponding antigens are selected when using either approach.

The length of time between the sample acquisition and antibody screening and the transfusion of red cell

unit for chronically transfused thalassemia patient is usually 72 hours but may be as long as one week in regularly transfused patients with full Rh and Mi<sup>a</sup> antigen matching<sup>6</sup>. However, the interval between samples for antibody screening and transfusion should not be longer than 72 hours in irregularly transfused or started transfusion later in life because of the higher risk of red cell alloimmunization<sup>5</sup>.

#### Blood component specification and preparation

Chronically transfused thalassemia patients are at greatest risk for transfusion-transmitted infections (TTI). To minimize the risk of TTI, WHO recommends, at a minimum, donation screening for human immunodeficiency viruses (HIV), hepatitis B virus (HBV), hepatitis C

virus (HCV), and syphilis<sup>5</sup>. In Thailand, standard blood donor screening includes syphilis, HBsAg, anti-HCV, HIV Ag/Ab, and nucleic acid amplification testing (NAT) for HBV, HCV and HIV.

According to the guidelines for the management of transfusion-dependent  $\beta$ -thalassemia (TDT) 5<sup>th</sup> edition, leukocyte depleted red cell unit (reduction to  $1 \times 10^6$ /L or less leukocytes per unit) is considered the critical threshold to eliminate adverse reactions such as febrile non-hemolytic transfusion reactions (FNHTR) and HLA sensitization<sup>5</sup>. Pre-storage filtration of whole blood is preferred because it offers high efficiency filtration compared with pretransfusion, laboratory filtration and bedside filtration. Due to the budget constraint, leukocyte poor packed red cell unit (reduction to  $1.2 \times 10^6$ /L or less leukocytes per unit) is also allowed to transfuse for thalassemia patients in Thailand<sup>7</sup>. However, if the patient developed transfusion reaction such as FNHTR, leukocyte depleted red cell units are considered over leukocyte poor packed red cell units.

Thai guidelines for the management of thalassemia recommends using fresh red cell unit that is less than 2 weeks old for thalassemia patients<sup>7</sup>. Due to the blood shortage situation, many patients were transfused with red cells greater than 2 weeks old without reports of transfusion reaction. Red cell genotyping of donors and patients can increase the likelihood of better matched red cell transfusion with lower rates of red cell alloimmunization<sup>5</sup>. A better matched unit is prioritized over the age of the red cell unit. In resource-limited country with unavailable phenotype-matched red cell transfusion, the red cell units less than 2 weeks old should be used for TDT patients.

#### **The clinical impacts of antigen-matched transfusion guided by red cell genotyping in thalassemia patients**

The critical role of red cell genotyping in blood management of Thai thalassemia has been shown in a study from King Chulalongkorn Memorial Hospital<sup>8</sup>. Blood samples from 24 TDT patients without serologic phenotyping, red cell genotyping were performed before

the first transfusion. These patients were then transfused with extended antigen-matched red cell units that were typed serologically for Rh (C, c, E, e), Mi<sup>a</sup>, M, S, Jk<sup>a</sup>, Jk<sup>b</sup>, Fy<sup>a</sup>, Fy<sup>b</sup>, and Di<sup>a</sup> antigens. No new red cell alloantibodies and hemolytic transfusion reactions were detected since the enrollment (median duration 25.5 months). Of 20 patients, five patients showed increased mean pre-transfusion hemoglobin levels ( $\geq 1$  g/dL) and one patient had longer intervals of transfusions. This study suggests the benefit of red cell genotyping in transfusion management of regularly transfused thalassemia patients in terms of reducing rate of red cell alloimmunization and providing better-matched red cell units for complicated thalassemia patients.

According to our studies, thalassemia patients without serology typing before the first transfusion would be red cell genotyped to identify the accurate red cell antigens. Those thalassemia patients with multiple red cell alloantibodies at King Chulalongkorn Memorial Hospital would be transfused with extended antigen-matched red cell units. On the other hand, ABO, Rh (C, c, D, E, e), and Mi<sup>a</sup> antigens would be matched for non-complicated thalassemia patients.

#### **Conclusion**

Red cell genotyping enabled the actual determination of red cell antigens in chronically transfused thalassemia patients. Blood banks could use this information to find the proper individual antigen-matched red cell units which result in minimized red cell alloimmunization.

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