

Clinical impacts of DNA-based typing and provision of antigen-matched red blood cell units for chronically transfused patients with thalassemia

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Blood transfusion, the main therapy for patients with severe thalassemia, is challenged by alloantibodies that can lead to hemolytic transfusion reactions. The use of prophylactic antigen-matched units is recommended, but serologic typing, before the first transfusion, is rarely performed and is not reliable after chronic transfusion. Patient DNA-based typing is a promising strategy, but clinical outcome data are lacking. The aim of this study was to determine the benefits of antigen-matched transfusion guided by DNA-based typing in terms of new alloantibody formation and increases in mean pre-transfusion hemoglobin (Hb) levels. We performed DNA-based typing on samples from 24 transfusion-dependent patients with thalassemia who had no serologic phenotyping performed before the first transfusion. These patients were then transfused with antigen-matched donor RBC units that were typed serologically. New alloantibody formation and mean pre-transfusion Hb levels were evaluated after implementing this extended common antigen-matching transfusion protocol. Sixty-three percent of the patients in this study were diagnosed as having beta-thalassemia Hb E. Alloantibodies were already present in 87.5 percent (21/24) of these patients, and most of these antibodies were multiple and/or unidentified. After the enrollment, there were 717 transfusion episodes comprising 1209 RBC units. The number of RBC units transfused to each patient ranged from 22 to 119 units. At the median duration of 25.5 months (range 10–34 months), no new alloantibodies were detected since the beginning of the protocol. Seventy-four transfusion episodes in six patients were cross-match-positive due to autoantibodies (patients 2, 4, 8, 9, and 14) or anti-Chido (patient 18) that had been identified before the study. There were no hemolytic transfusion reactions in this study. Five patients (patients 1, 2, 12, 15, and 20) showed increased mean pre-transfusion Hb levels (≥ 1 g/dL) and one patient (patient 16) had longer intervals between transfusions (compared with those before the protocol), suggesting longer RBC survival, although there was no statistical difference in the whole group. Our study highlights the benefits of DNA-based typing in chronically transfused patients with thalassemia who had no phenotyping data before the first transfusion. Patient DNA-based typing for antigen-matched transfusion is safe in thalassemia and allows us to obtain better-matched blood units for complicated patients. *Immunohematology* 2020;36:137–145.

Key Words: DNA-based typing, chronic transfusion, thalassemia, antigen-matched transfusion, red blood cell alloantibody

Chronic transfusion in patients with thalassemia is often complicated by red blood cell (RBC) alloantibodies to the lacking antigens on the patients' RBCs. The prevalence of alloantibodies ranges from 4.25 to 37 percent in patients with thalassemia.^{1–6} Clinically significant alloantibodies can shorten transfused erythrocyte survival due to hemolytic transfusion reactions. To reduce the alloantibody risk, RBC antigen serotyping for Rh (C, c, E, e) and MNS hybrid glycoporphins, especially for MNS7 (Mi^a), is recommended before the first transfusion by our national thalassemia guidelines. Subsequently, Rh- and Mi^a-matched blood units are transfused.⁷ However, phenotyping data before the first transfusion are unavailable at our institute, and most patients with thalassemia have been receiving blood regularly since childhood.

Serologic methods are the standard for determining ABO, D, and common blood group antigens. Nevertheless, this approach in chronically transfused patients with thalassemia is inaccurate because of the contaminating donor erythrocytes in patient circulation. DNA-based typing is superior in situations of recent transfusions.⁸ Moreover, it can guide the antigen-matched transfusion in individual patients. Furthermore, autoantibodies are frequently found in patients with thalassemia, causing crossmatch problems.⁹ The prevalence of autoantibodies is 0.47–28.2 percent by direct antiglobulin test and 57.6 percent by flow cytometry depending on study population and transfusion policy.^{10–13}

In this study, we implemented DNA-based typing for chronically transfused patients with thalassemia and provided antigen-matched RBC units accordingly. A better-matched unit should prolong the RBC survival *in vivo* and thus increase

the next pre-transfusion hemoglobin (Hb). Pre-transfusion Hb is an important goal to reach to ensure blood transfusion effectiveness. The aim of this study was to determine the clinical benefits of this strategy in terms of new alloantibody formation and increases in the mean pre-transfusion Hb that suggest the longer survival of transfused RBCs. We also assessed patients' clinical responses to crossmatch-positive, but antigen-matched, RBC transfusions and explored the predictors of Hb response in antigen-matched transfusions.

Materials and Methods

Patients and Interventions

In our tertiary care hospital with many referred patients with thalassemia, RBC phenotype data before the first transfusion are unavailable for most patients. Therefore, we enrolled this group of patients who previously had crossmatch problems or history of unsatisfactory pre-transfusion Hb after chronic transfusion. Patients who did not receive regular transfusion ($n = 2$) were excluded from our analysis. Patient history, transfusion records, pre-transfusion Hb, and alloimmunization history were retrieved from hospital records. DNA-based typing was performed from 2014 to 2018. Determination of ABO, D, antibody screening, and crossmatching were performed before each transfusion using column agglutination technology (Ortho Clinical Diagnostics, Raritan, NJ). The study was approved by the institutional review board of the Faculty of Medicine, Chulalongkorn University, Thailand (Ethics Committee: 725/62).

Donor Phenotyping

Donor units were antigen matched using hemagglutination in gel cards (Bio-Rad; Cressier, Switzerland) for ABO, Rh (D, C, c, E, e), Mi^a , M, S, Jk^a , Jk^b , Fy^a , Fy^b , and Di^a , since they are the most common clinically significant alloantibodies in Thailand.^{10,14} Genotyping was not performed on the donor blood components. Typing for Rh (C, c, E, e), Jk^a , Jk^b , Fy^a , and Fy^b was performed according to the manufacturer's instructions (Bio-Rad). Anti- Mi^a and anti-M were produced by the Thai National Blood Centre, Thailand. S was assessed using the reagent antibody from CE-IMMUNDIAGNOSTIKA GmbH (Germany). Anti- Di^a was produced in-house from patient sera with anti- Di^a .

Recipient Genotyping Method

DNA-based typing for common blood group antigens was performed by the BLOODchip ID Core XT kit (Progenika

Biopharma, Derio, Spain) and the Luminex genotyping platform (Luminex, Austin, TX), which interrogates 29 polymorphisms to predict the expression of 37 antigens in 10 blood group systems. DNA samples were extracted from EDTA blood by spin-column separation (QIAmp; QIAGEN, Valencia, CA). DNA concentration was determined by measurement of optical density at 260 and 280 nm. Extracted DNA was kept at -20°C until testing. Samples were processed with one positive and one negative control in each run.

Outcome Measures

The primary outcome was to detect new alloantibodies after transfusion of antigen-matched units, since RBC alloimmunization is associated with a risk of hemolytic transfusion reaction. According to the treatment guideline for chronically transfused patients with thalassemia, a pre-transfusion Hb level between 9 and 10.5 g/dL is recommended to promote growth, improve physical activities, and suppress abnormal bone marrow expansion.¹⁵ Therefore, the pre-transfusion Hb response to antigen-matched transfusion was assessed. Marked improvement was defined as an increase of mean pre-transfusion Hb of at least 1 g/dL from 6 months from the patient's enrollment to the last follow-up visit compared with that of the 12-month period before enrollment.

Crossmatch problems could delay blood transfusion for some patients with thalassemia having an autoantibody or antibody to a high-prevalence antigen even though antigen-matched units for all clinically significant common antibodies were issued. Therefore, the secondary outcome was to assess the response to crossmatch-positive but antigen-matched RBC units. Also, finding antigen-matched units for all clinically significant common antibodies was both time and labor consuming. We attempted to identify the predictors of patient responses to antigen-matched RBC units, such as history of three or more antibodies and patient age.

Patients received regular transfusions every 1–4 weeks. Laboratory tests including complete blood count (CBC) and ferritin were performed every 3 months. Therefore, pre-transfusion Hb was not available at every time point. The safety outcome was the incidence of hemolytic transfusion reactions. Definition of hemolytic transfusion reaction was based on the National Healthcare Safety Network Biovigilance Component Hemovigilance Module Surveillance Protocol by the U.S. Centers for Disease Control and Prevention.¹⁶ According to our hospital hemovigilance policy, all transfusion reactions were reported to the Transfusion Medicine Unit.

Statistical Analysis

Continuous variables were summarized descriptively by means, medians, ranges, and standard deviations (SDs) when appropriate. The Wilcoxon signed-rank test was used to compare the baseline pre-transfusion Hb and mean pre-transfusion Hb levels from 6 months after the enrollment. The Mann-Whitney *U* test and Wilcoxon signed-rank test were used to compare patient ages between those patients with and without pre-transfusion Hb response to antigen-matched transfusion.

Statistical analysis of the relationship between predictors and the secondary outcome of the patients used bivariate comparisons of categorical variables by two-tailed χ^2 tests. A *p* value <0.05 was considered statistically significant.

Results

Patient Characteristics

Twenty-four transfusion-dependent patients with thalassemia were initially enrolled; two were later excluded because of lack of transfusion. Nineteen (86.4%, 19/22) were female. The median age was 24 years (range 2–58 years). Thirteen (59.1%, 13/22) patients were diagnosed with beta-thalassemia Hb E. Eight patients (36.4%, 8/22) were homozygous for beta-thalassemia, and one patient (4.5%, 1/22) had Hb H Constant Spring. Twelve patients had three or more alloantibodies, five had two alloantibodies, four had a single alloantibody, and three had no alloantibody. The three most common antibodies were anti-Mi^a, anti-E, and anti-c (Table 1). All patients had no previous history of hemolytic transfusion reactions or hyperhemolytic transfusion events.

Outcomes of Providing Phenotype-Matched Transfusions According to Patient Genotype

DEVELOPMENT OF NEW ALLOANTIBODIES

After the enrollment, there were 717 transfusion episodes comprising 1209 antigen-matched units. Blood transfusion to each patient ranged from 22 to 119 units. During the median follow-up of 25.5 months (range 10–34 months), no new alloantibodies were detected.

INCREASE IN PRE-TRANSFUSION HEMOGLOBIN

Among the 24 patients, 2 patients (patients 22 and 24) had not received regular transfusions before the enrollment and were excluded. For the remaining 22 patients, there was no statistically significant difference between the pre-transfusion

Hb level before date of enrollment and the level after 6 months of antigen-matched transfusions from date of enrollment until the last visit (*p* = 0.455). The mean pre-transfusion Hb level before enrollment versus the level after 6 months of antigen-matched transfusions from enrollment was 7.2 ± 1.8 versus 7.4 ± 1.6 g/dL, which was calculated from the total of 166 and 300 CBC results, respectively. The mean pre-transfusion Hb levels for individual patients are shown in Figure 1.

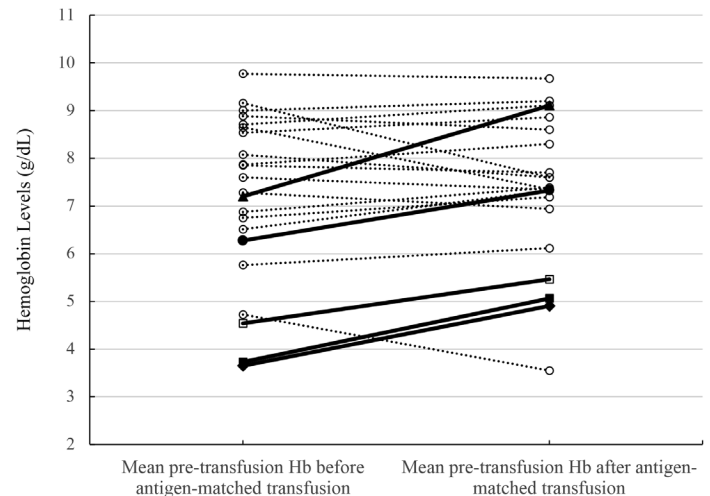


Fig. 1 Mean pre-transfusion hemoglobin (Hb) levels before and after antigen-matched transfusion. Hb levels in g/dL for 22 regularly transfused thalassemia patients are shown. The mean pre-transfusion Hb level before enrollment versus the level after 6 months of antigen-matched transfusions from enrollment was 7.2 ± 1.8 versus 7.4 ± 1.6 g/dL. Seventeen patients with no improvement in mean pre-transfusion Hb are represented by dashed lines. The five patients who showed significant improvement in the mean pre-transfusion Hb levels (≥ 1.0 g/dL) are represented by solid lines.

Interestingly, five patients (patients 1, 2, 12, 15, and 20) showed marked improvement in mean pre-transfusion Hb levels (≥ 1.0 g/dL) compared with levels before the enrollment. Of these patients, three patients had three or more alloantibodies, one patient had two alloantibodies, and one patient had one alloantibody.

Patient 1, with beta-thalassemia Hb E, had multiple antibodies. Her average baseline pre-transfusion Hb was 4.5 g/dL. She received antigen-matched transfusions for 26 months. Six months after her enrollment, her mean pre-transfusion Hb was 5.5 g/dL.

Patient 2, with homozygous beta-thalassemia, had multiple antibodies and did not respond well to transfusion. Three years previously, he underwent a splenectomy in an attempt to maintain better pre-transfusion Hb. Nevertheless, his pre-transfusion Hb was unimproved after splenectomy. His

Table 1. Clinical characteristics of 24 patients with thalassemia

Patient number	Age, years	Gender	Type of thalassemia	Splenectomy	Complications/comorbidities	Antibody specificity	Predicted phenotype*	Mean pre-transfusion Hb levels before antigen-matched transfusion (g/dL)	Mean pre-transfusion Hb levels after antigen-matched transfusion (g/dL)
1	29	F	Beta-thalassemia Hb E	No	Iron overload	Le ^a , Le ^b , E, Mi ^a , Fy ^b , Di ^a	E-, K-, Fy(b-), S-, Mi(a-), Di(a-)	4.5	5.5
2	12	M	Homozygous beta-thalassemia	Yes (3 years before the study)	Iron overload, chronic portal, and splenic vein thrombosis	Mi ^a , Le ^b , Jk ^b , unidentified	E-, K-, Fy(b-), Jk(b-), Mi(a-), Di(a-)	7.2	9.1
3	3	F	Homozygous beta-thalassemia	No	Iron overload	E, c, Mi ^a , S	E-, c-, K-, Fy(b-), N-, S-, Mi(a-), Di(a-)	8.9	8.6
4	36	F	Beta-thalassemia Hb E	No	Iron overload	E, c, Mi ^a , S, Jk ^b	E-, c-, K-, Jk(b-), Fy(b-), N-, S-, Mi(a-), Di(a-)	6.8	7.2
5	40	F	Homozygous beta-thalassemia	No	Iron overload	M, c, K, Jk ^b , Mi ^a	E-, c-, K-, Jk(b-), M-, S-, Mi(a-), Di(a-)	7.3	6.9
6	39	F	Beta-thalassemia Hb E	Yes (23 years before the study)	Iron overload	Le ^a , Le ^b , S, unidentified	K-, Fy(b-), N-, S-, Di(a-)	6.9	7.4
7	14	M	Beta-thalassemia Hb E	No	Iron overload	E, c, Mi ^a , Di ^a	E-, c-, K-, Fy(b-), N-, S-, Mi(a-), Di(a-)	7.9	7.7
8	20	F	Homozygous beta-thalassemia	Yes (16 years before the study)	Iron overload, transaminitis	E, c, S, Chido	E-, c-, K-, N-, S-, Di(a-)	8.7	7.4
9	27	M	Homozygous beta-thalassemia	Yes (23 years before the study)	Iron overload	E, c, Le ^a , Fy ^b	E-, c-, K-, Fy(b-), S-, Mi(a-), Di(a-)	4.7	3.6
10	42	F	Beta-thalassemia Hb E	No	Iron overload	Jk ^a , Mi ^a , unidentified	C-, K-, Fy(b-), Jk(a-), M-, S-, Mi(a-), Di(a-)	5.8	6.1
11	53	F	Hb H Constant Spring	Yes (20 years before the study)	Iron overload, pulmonary hypertension, chronic deep vein thrombosis, and pulmonary embolism	E, c, Mi ^a	E-, c-, K-, S-, Mi(a-), Di(a-)	9.8	9.7
12	44	M	Homozygous beta-thalassemia	Yes (during the study)	Iron overload, extramedullary hematopoiesis at spine causing neurodeficiency	E, c, Mi ^a	E-, c-, K-, Fy(b-), Mi(a-), Di(a-)	6.3	7.3
13	58	F	Beta-thalassemia Hb E	Yes (13 years before the study)	Iron overload	E, auto-C	E-, c-, K-, Jk(b-), Fy(b-), S-, Mi(a-), Di(a-)	8.1	7.6
14	20	F	Homozygous beta-thalassemia	Yes (6 years before the study)	Iron overload, pulmonary hypertension	Mi ^a , S	E-, c-, K-, Jk(b-), S-, Mi(a-)	6.5	7.3
15	20	F	Beta-thalassemia Hb E	No	Iron overload, epilepsy	E, c	E-, c-, K-, S-, Mi(a-), Di(a-)	3.7	5.1
16	2	F	Homozygous beta-thalassemia	No	None	Jk ^a , unidentified	K-, Jk(a-), N-, S-, Mi(a-), Di(a-)	9.0	9.2

Table 1. Clinical characteristics of 24 patients with thalassemia (continued)

Patient number	Age, years	Gender	Type of thalassemia	Splenectomy	Complications/comorbidities	Antibody specificity	Predicted phenotype*	Mean pre-transfusion Hb levels before antigen-matched transfusion (g/dL)	Mean pre-transfusion Hb levels after antigen-matched transfusion (g/dL)
17	19	F	Beta-thalassemia Hb E	No	Iron overload	E, unidentified	E-, c-, K-, Jk(b-), Fy(b-), M-, S-, Mi(a-), Di(a-)	7.9	8.3
18	7	F	Beta-thalassemia Hb E	No	Iron overload	Chido	E-, c-, K-, Jk(b-), Fy(b-), S-, Mi(a-), Di(a-)	8.7	9.1
19	11	F	Beta-thalassemia Hb E	No	Iron overload, ventricular septal defect/atrial septal defect	Mi ^a	E-, K-, N-, S-, Mi(a-), Di(a-)	7.6	7.3
20	35	M	Beta-thalassemia Hb E	Yes (22 years before the study)	Iron overload	E	E-, c-, K-, Fy(b-), S-, Mi(a-), Di(a-)	3.7	4.9
21	28	F	Beta-thalassemia Hb E	Yes (23 years before the study)	Iron overload	Nonspecific cold	E-, K-, Fy(b-), N-, S-, Mi(a-), Di(a-)	8.5	8.9
22	6	F	Homozygous beta-thalassemia	No	Iron overload	No alloantibody	E-, c-, K-, Jk(a-), N-, S-, Mi(a-), Di(a-)	Patient did not receive regular transfusion before enrollment; excluded from study.	
23	17	F	Beta-thalassemia Hb E	No	Iron overload	No alloantibody	E-, c-, K-, Fy(b-), N-, S-, Mi(a-), Di(a-)	9.2	7.6
24	27	F	Beta-thalassemia Hb E	No	Iron overload	No alloantibody	E-, c-, K-, Jk(b-), N-, S-, Mi(a-), Di(a-)	Patient did not receive regular transfusion before enrollment; excluded from study.	

*Red blood cell phenotype predicted by genotyping method. Hb = hemoglobin.

baseline pre-transfusion Hb after splenectomy was 7.2 g/dL. He received antigen-matched transfusions for 28 months. Six months after the enrollment, his mean pre-transfusion Hb was 9.1 g/dL.

Patient 12, with homozygous beta-thalassemia, had anti-E, anti-c, and anti-Mi^a. His average baseline pre-transfusion Hb was 6.3 g/dL. He received antigen-matched transfusions for 14 months. Six months after his enrollment, his mean pre-transfusion Hb was 7.3 g/dL. Note that he underwent a splenectomy 8 months after the enrollment, however. Seven months after splenectomy, his Hb was 9.0 g/dL, and he has not received a blood transfusion since then. Thus, the improvement of his pre-transfusion Hb could be a result of the splenectomy.

Patient 15, with beta-thalassemia Hb E, had anti-E and anti-c. Her average baseline pre-transfusion Hb was 3.7 g/dL. She received antigen-matched transfusions for 34 months. Six months after this enrollment, her mean pre-transfusion Hb was 5.1 g/dL.

Patient 20, with beta-thalassemia Hb E, had anti-E. Antigen-negative RBC units for E, c, and Mi^a were given, but

his pre-transfusion Hb was unimproved. His baseline pre-transfusion Hb was 3.7 g/dL. He received antigen-matched transfusions for 28 months. Six months after this enrollment, his mean pre-transfusion Hb was 4.9 g/dL.

MEAN TIMING BETWEEN RBC TRANSFUSIONS

The mean timing between transfusions before and after antigen-matched transfusion was not statistically different ($p = 0.426$). The patients with Hb responses tended to show longer intervals between transfusions, although there was one patient (patient 16) who had prolonged timing between transfusions with similar pre-transfusion Hb level (Fig. 2). This homozygous beta-thalassemia patient had anti-Jk^a and an unidentified antibody. Her average baseline pre-transfusion Hb was 9.0 g/dL. She received antigen-matched transfusions for 31 months. Six months after this enrollment, her mean pre-transfusion Hb was 9.2 g/dL. However, the timing between transfusions was improved from 14 days before antigen-matched transfusions to 28.6 days after antigen-matched transfusions.

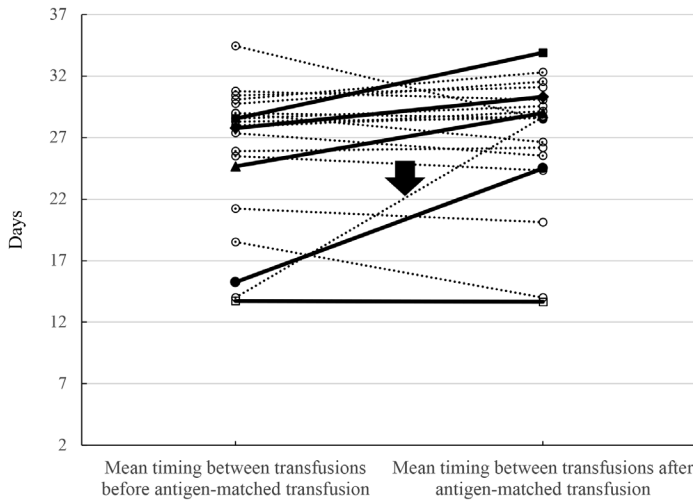


Fig. 2 Mean timing between transfusions before and after antigen-matched transfusions. The timing between transfusions in 22 regularly transfused patients with thalassemia is shown in days. Seventeen patients with no improvement in mean pre-transfusion hemoglobin (Hb) are represented by dashed lines. However, one of these patients showed markedly longer intervals between transfusions after antigen matching (arrow). Five patients who showed significant improvement in the mean pre-transfusion Hb levels (≥ 1.0 g/dL) are represented by solid lines.

Responses to Crossmatch-Positive but Antigen-Matched Transfusions

Although antigen-matched units for Rh (D, C, c, E, e), Mi^a, M, S, Jk^a, Jk^b, Fy^a, Fy^b, and Di^a were issued for all patients, six patients (27.3%, 6/22; patients 2, 4, 8, 9, 14, and 18) showed positive crossmatch reactions with some units. Five of them had autoantibodies, and the other had anti-Chido; all resulted in positive crossmatches. In these six patients, there were 210 transfusion episodes, 74 of which (35.2%) showed positive crossmatch results. The grades of the crossmatch reactivity ($n = 74$) were not more than 2+ (median: 1+).

Before this study was performed, autoantibody or antibody to a high-prevalence antigen often complicated the pre-transfusion process and delayed the transfusion schedule. During this study, antigen-matched units for Rh (D, C, c, E, e), Mi^a, M, S, Jk^a, Jk^b, Fy^a, Fy^b, and Di^a could be prepared in advance; therefore, the patients received transfusions on time.

For the patient with anti-Chido, Chido-RBCs were not provided because it is not a clinically significant antibody. For the patients with autoantibodies, antigen-matched units were found ahead of the transfusion date and the least incompatible antigen-matched unit(s) were selected for transfusion on the day of transfusion.

In the 22 patients with negative versus positive crossmatch results, the responses to antigen-matched transfusions were not statistically different ($p = 1.000$). The average increase of pre-transfusion Hb in individuals with negative versus positive crossmatch was 0.20 g/dL (SD 0.74) versus 0.18 g/dL (SD 1.22), respectively. Moreover, no acute or delayed hemolytic transfusion reactions were detected in those six patients with positive crossmatch transfusions.

Predictors of Responses to Antigen-Matched Transfusions

We hypothesized that patients with three or more antibodies would respond well to antigen-matched transfusions. However, the response to antigen-matched transfusions between those patients with versus those patients without three or more antibodies was not statistically significant among the 22 patients ($p = 0.523$). The average increase of pre-transfusion Hb in these two patient groups was 0.15 g/dL (SD 0.91) versus 0.25 g/dL (SD 0.86), respectively.

This study determined that the age of the patient before this enrollment could not be a predictor for successful antigen-matched transfusions ($p = 0.651$). The mean age of individuals with versus individuals without increases in pre-transfusion Hb after enrollment was 22.3 years (SD 11.7) versus 26.8 years (SD 16.4), respectively.

Discussion

To the best of our knowledge, this is the first report of clinical outcomes of phenotype-matched transfusions to multiply transfused patients with thalassemia guided by patient DNA-based typing. Of the 22 patients without serologic phenotypes, data before the first transfusion were included, and no new alloantibodies were detected. Hb levels of five patients were increased, and one patient required less frequent transfusions after receiving antigen-matched transfusions, suggesting a prolongation of transfused erythrocyte survival (5 of 22 evaluable patients, or 22.7% response rate), although there was no significant change for the entire group. The fifth patient with Hb improvement also underwent a splenectomy and, therefore, the effect of this protocol cannot be determined.

Successful maintenance of pre-transfusion Hb in chronically transfused patients with thalassemia is an important goal for better quality of life, lower frequency of transfusion, lower serum ferritin, and fewer transfusion reactions.^{15,17} RBC alloimmunization is a challenge when providing compatible blood units. Although many factors

contribute to this undesirable event, the most important factor is the exposure to foreign RBC antigens.^{18,19} Therefore, antigen-matched transfusion is a goal for success. Additionally, the efficacy of transfusion for chronically transfused patients with thalassemia could be affected by the timing between transfusions and host factors, such as splenectomy and comorbidities, as well as the presence of precipitating factors, such as infections.

Because the baseline serologic phenotype of most patients is unavailable, a report of regularly transfused Thai patients with thalassemia showed that the presence of circulating donor RBCs leads to mixed-field reactivity and inaccurate results.²⁰ Discrepancies between serologic phenotypes performed by different laboratory personnel at different time points of testing were also observed. Therefore, DNA-based typing offers an advantage over traditional serologic typing to accurately predict the identity of the patient's RBC antigens after recent transfusion. Moreover, DNA-based typing may provide benefits to patients with autoantibodies, multiple alloantibodies, antibodies to high-prevalence antigens, or antibodies against antigens with unavailable antisera.⁸

The extended molecular-matched RBC transfusion for chronically transfused patients can improve the effectiveness of transfusion by decreasing the alloimmunization rate.^{17,21} Despite these benefits, this approach is currently not widely available in Thailand because of the high cost for genotyping a large inventory of donor RBCs. Therefore, DNA-based typing is only performed for patients, and phenotype-matched units are provided according to patient genotyping data.

Finding compatible blood units for patients with multiple alloantibodies is laborious and time-consuming. After protocol implementation, antigen-matched units were procured before the planned patient visits. Although some crossmatches revealed serologic incompatibility due to autoantibody or clinically insignificant antibody to a high-prevalence antigen, blood units were issued on time because all clinically significant antigens were matched. The least incompatible antigen-matched units from the donor inventory would be transfused to the patient, and the antibody identification retested on every visit. The transfusion of incompatible RBC units is not a standard practice. However, it is not possible in some patients with autoantibody or clinically insignificant antibody to high-prevalence antigen to provide compatible units. In this case, administration of antigen-negative RBC units is the recommended approach. Interestingly, hemolytic transfusion reactions were not detected in our patients under this protocol.

Prophylactic antigen-matching has been shown to reduce new antibody formation.^{11,22} A recent study in patients with sickle cell disease in the United States showed new antibody formation for the unmatched antigens.²¹ We matched only common clinically significant RBC antigens in Thailand, but no new alloantibodies were detected. One explanation is the ethnic differences between donors and patients in the United States.²³ Sickle cell disease affects mainly African Americans, whose blood donation rates are only 25–50 percent of those of white individuals.²⁴ In Thailand, blood donors share the same ethnicity with the patients. Therefore, there is a better chance to obtain phenotype-matched blood units for Thai patients with thalassemia.

The predictors of good responses to antigen-matched blood could not be identified. The number of patients in our study was probably too small to find weak predictors. Therefore, *p* values need to be interpreted carefully. Future multicenter studies are required. Another limitation of our study was the frequency of blood transfusions. One patient did not receive a blood transfusion as scheduled after the enrollment, which affected the mean pre-transfusion Hb level.

To apply this study into our routine work, DNA-based typing should be performed for chronically transfused patients with thalassemia who have no available phenotyping data. The extended antigen-matched transfusion could apply to patients with multiple antibodies, autoantibody, or a clinically insignificant antibody to a high-prevalence antigen but not for patients without antibody. This practice will help the majority of patients with difficult pre-transfusion testing to receive blood on schedule.

In conclusion, DNA-based typing is helpful in transfusion management of chronically transfused patients with thalassemia, especially when phenotype data are unavailable. The genotype data allow us to provide antigen-matched units despite a positive crossmatch. In this study, no new alloantibodies were detected after the median follow up of 25.5 months and average number of 50 units/patient. A subgroup of patients showed increases in pre-transfusion Hb. A larger prospective clinical trial is needed to evaluate the definitive role of DNA-based typing in transfusion-dependent patients with thalassemia.

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