

CME Transfusion complications in thalassemia patients: a report from the Centers for Disease Control and Prevention

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BACKGROUND: Transfusions are the primary therapy for thalassemia but have significant cumulative risks. In 2004, the Centers for Disease Control and Prevention (CDC) established a national blood safety monitoring program for thalassemia. This report summarizes the population and their previous nonimmune and immune transfusion complications.

STUDY DESIGN AND METHODS: The CDC Thalassemia Blood Safety Network is a consortium of centers longitudinally following patients. Enrollment occurred from 2004 through 2012. Demographics, transfusion history, infectious exposures, and transfusion and non-transfusion complications were summarized. Logistic regression analyses of factors associated with allo- and autoimmunization were employed.

RESULTS: The race/ethnicity of these 407 thalassemia patients was predominantly Asian or Caucasian. The mean \pm SD age was 22.3 ± 13.2 years and patients had received a mean \pm SD total number of 149 ± 103.4 units of red blood cells (RBCs). Multiorgan dysfunction was common despite chelation. Twenty-four percent of transfused patients had previous exposure to possible transfusion-associated pathogens including one case of babesia. As 27% were immigrants, the infection source cannot be unequivocally linked to transfusion. Transfusion reactions occurred in 48%, including allergic, febrile, and hemolytic; 19% were alloimmunized. Common antigens were E, Kell, and C. Years of transfusion was the strongest predictor of alloimmunization. Autoantibodies occurred in 6.5% and were associated with alloimmunization ($p < 0.0001$). Local institutional policies, not patient characteristics, were major determinants of blood preparation and transfusion practices.

CONCLUSION: Hemosiderosis, transfusion reactions, and infections continue to be major problems in thalassemia. New pathogens were noted. National guidelines for RBC phenotyping and preparation are needed to decrease transfusion-related morbidity.

Worldwide, there are more than 60,000 births annually of serious forms of thalassemia.^{1,2} The World Health Organization considers thalassemia to be a major health burden.^{1,2}

Transfusions are the primary therapy for thalassemia but have significant risks including hemosiderosis, transfusion reactions, alloimmunization, and infections. In 1998, Congress and the Centers for Disease Control and Prevention (CDC) established a blood safety monitoring surveillance system called the Universal Data Collection Project.

ABBREVIATIONS: α -thal = α -thalassemia;
E-thal = E- β -thalassemia; TI = thalassemia intermedia;
TM = thalassemia major.

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In 2004, this program was expanded to include thalassemia. The goals of the program are to monitor blood safety and to develop and test strategies for the prevention and management of complications in patients with thalassemia.

The distributions of the phenotype and genotype of North American thalassemia patients today, as well as their transfusion management, are dramatically different from those in the past decades.³ The majority of patients, previously of Mediterranean descent, are now largely of Asian and Middle Eastern origin.³ The diverse thalassemia phenotypes found in this population result in different transfusion exposure than the Mediterranean population. There are limited data on the transfusion complications in this population including alloimmunization rates. Pilot data suggest that this diverse population in North America may be at greater risk for alloimmunization.³⁻¹³ Knowledge of other transfusion complications such as anaphylaxis and hemolytic reactions is also incomplete because these events are rarely compiled and reported. Importantly, the thalassemia population, which has the highest transfusion exposure of chronic diseases, provides the opportunity to study emerging transfusion-associated infections.

The purpose of this report is to summarize the patient population of the CDC Thalassemia Blood Safety Network as well as previous nonimmune and immune complications at the time of enrollment into the program. A focus on factors associated with allo- and autoimmunization in chronically transfused patients and a description of blood product preparation and transfusion practices at the participating institutions are included.

MATERIALS AND METHODS

The CDC Thalassemia Blood Safety Network (Network) is a consortium of thalassemia centers in the United States that has a longitudinal cohort study of patients to determine risk factors for transfusion-related complications. This report describes the population at the time of their enrollment between 2004 and 2011. Individuals from thalassemia centers located in Boston, Massachusetts; Chicago, Illinois; Philadelphia, Pennsylvania; New York, New York; Oakland, California; Los Angeles, California; and Atlanta, Georgia, participated. Appendix S1 (available as supporting information in the online version of this paper) lists additional information on site and staff participation.

Patient demographics and disease complications

Demographic, physical examination, and laboratory data were collected at enrollment and entered into a central database at the CDC. Demographic data collected included clinical diagnosis, sex, race, ethnicity, genotype, and phenotype. Historical data included information regarding immunizations, infection exposures, surgical

procedures, organ dysfunction, age at transfusion initiation, chelation therapy, type of RBC product, transfusion reactions, and antigen matching. Data were collected from chart review, patient interviews, blood banking records, and laboratory testing. Organ dysfunction was defined as a history of requiring medical treatment for heart disease, diabetes (Type 1 or 2), hypoparathyroidism, hypothyroidism, growth hormone deficiency, or gonadal failure.

Infection

Information on previous exposure to infectious pathogens was collected on enrollment intake forms. In addition, specimens were mailed to the CDC annually for pathogen screening. The protocol and standard assays are provided in Appendix S2 (available as supporting information in the online version of this paper). Institutional review board approval of the protocol and consent forms was obtained at all participating institutions and consent forms were signed by each patient before enrollment.

Transfusion complications

Adverse transfusion reactions were reported according to a Manual of Operations based on National Healthcare Safety Network Hemovigilance Module—published transfusion complication definitions including hemolytic transfusion reactions (acute, delayed), febrile non-hemolytic, allergic, transfusion-associated circulatory overload, transfusion-related acute lung injury (TRALI), posttransfusion purpura, transfusion-associated graft-versus-host disease, transfusion-transmitted infection, hypotensive reactions, and transfusion-associated dyspnea.^{14,15} Alloimmunization was defined as having an antibody to a foreign red blood cell (RBC) antigen while autoimmunization was defined as the presence of an antibody to an antigen on the patients' own RBCs.

Transfusion protocol guidelines and institutional practices

Data were collected regarding institutional practices related to transfusion. A prospective questionnaire was sent to the participating institutions and their principal investigator regarding their institution's preparation methods of RBC products and transfusion practices. Preparation methods of RBCs were classified as leukoreduced (by filtration methods), washed, and/or irradiated. Data regarding screening for alloantibodies and autoantibodies at each thalassemia center were obtained. Antigen matching was determined as one of three categories: ABO/D; ABO/D, C, E, Kell; and "extended."

Statistical analysis

Patients were characterized as "chronically transfused" if they had a history of chronic transfusions and received

TABLE 1. Thalassemia patient characteristics in the CDC hemovigilance study

	Chronic Tx (n = 327)	Intermittent Tx (n = 38)	Never Tx (n = 42)	Total (n = 407)
Mean \pm SD age (years)*	22.8 \pm 13.1	21.9 \pm 16.7	9.8 \pm 10.2	21.3 \pm 13.8
Diagnosis				
β -TM	82 (267/327)	29 (11/38)	14 (6/42)	70 (284/407)
β -TI	6 (20/327)	21 (8/38)	21 (9/42)	9 (37/407)
α -Thal syndromes†	6 (20/327)	39 (15/38)	55 (23/42)	14 (56/407)
E-thal	6 (20/327)	11 (4/38)	10 (4/42)	7 (28/407)
Sex				
Female	53 (174/327)	76 (29/38)	48 (20/42)	55 (223/407)
Male	47 (153/327)	24 (9/38)	52 (22/42)	45 (184/407)
Race				
Asian	48 (157/327)	68 (26/38)	57 (24/42)	51 (207/407)
White	49 (159/327)	24 (9/38)	17 (7/42)	43 (175/407)
Other	3 (11/327)	8 (3/38)	26 (11/42)	6 (25/407)

* Median ages for chronic Tx (transfusion), intermittent Tx, and never Tx, respectively: 31.3 (<1-58), 17.3 (1-61), and 7 (1-53).
† HbH, HbHCS.

eight or more transfusions within a 12-month period, “intermittently transfused” if they had not been chronically transfused but had received one to seven transfusions within a 12-month period, or “never transfused” at the date of study entry. Patients were classified as β -thalassemia major (TM), β -thalassemia intermedia (TI), hemoglobin (Hb) E- β -thalassemia (E-thal), or α -thalassemia (α -thal) syndrome (including HbH disease and HbH/Constant Spring) based on their diagnosis, clinical course, and genotype. Demographic variables such as age, sex, and race were summarized for the total population and for each transfusion group. For chronically transfused patients, the years of transfusion exposure was calculated by subtracting the age at which the patient began transfusion from their age at enrollment. Continuous variables such as current age, age at start of transfusion therapy, and number of transfusions in the year before enrollment were compared between the transfusion groups utilizing ANOVA or t tests. Discrete variables, such as the prevalence of alloimmunization, were compared across groups utilizing chi-square or Fisher’s exact tests. Logistic regression models were used to identify independent predictors of allo- and autoimmunization status in chronically transfused patients. All analyses were performed at the data coordinating center (CDC) with computer software (SAS/STAT Software, Version 9, SAS Institute, Cary, NC). p values less than 0.05 were considered significant.

RESULTS

Patient characteristics

Of the 407 thalassemia patients enrolled in the CDC transfusion surveillance study between 2004 and 2011, a total of 284 (70%) patients were diagnosed as TM, 58 (14%) α -thal syndromes, 37 (9%) TI, and 28 (7%) E-thal. Fifty-five percent of participants were female. There were two major racial groups: Asian (207) and white (175),

which accounted for 94% of the population. An additional 4% were classified as black (see Table 1). Seventy-three percent of the population was born in the United States, and 27% outside the United States (India, Pakistan, Thailand, China, Vietnam, and Iran accounting for 57%). Of the 110 patients born outside of the United States, 104 were transfused and the majority started transfusion therapy before their first visit to a US Thalassemia Treatment Center (TTC), although data about the location or other details of these transfusions are not available.

Eighty percent (327) of the patients had been chronically transfused, 9% (38) had only been intermittently transfused, and 10% (42) were never transfused (Table 1). Diagnostic categories of the patients who had been chronically transfused included TM (82%), TI (6%), E-thal (6%), and α -thal syndromes (6%). Forty-nine percent of the chronically transfused patients were white and 48% were Asian. At study entry, the median age of the chronically transfused patients was 31.3 years (range, <1 to 58 years). The median age at transfusion initiation was 1 year with a mean age of 4.5 ± 8.2 years. The mean estimated years of transfusion exposure was 18.5 ± 12.3 . The mean \pm SD number of transfusions in this group during the year before enrollment was 15.6 ± 5.8 .

Among the 38 intermittently transfused patients, there were 15 α -thal syndromes, 11 TM, 8 TI, and 4 E-thal patients. The median age was 17.3 years with a range of 1 to 61 years. The proportion of Asians in the intermittently transfused group was higher than in the chronically transfused group: 68% vs. 48% ($p = 0.02$). Also, the percentage of females was higher: 76% compared to 53% ($p = 0.007$). Most patients with β^0 -TM were chronically transfused; 4%, however, were included in the intermittently transfused population receiving fewer than eight annual transfusions in some years. As transfusion was intermittent in this group, an estimate of the years of transfusion exposure was not possible.

The diagnostic categories in the patients who had never been transfused were 23 patients with α -thal syndromes, nine patients with TI, six TM, and four E-thal. Untransfused patients were younger with a median age of 7 years (range, 1-53 years). TM patients in the nontransfused category were young infants under the age of 1 year who had not yet begun chronic transfusion at study enrollment.

General complications

Hemosiderosis occurred in all groups. As expected, the average measurement of ferritin was significantly higher in the chronically transfused patients (median, 1375.85 $\mu\text{g/L}$) compared to the other two groups (intermittent, 356 $\mu\text{g/L}$; nontransfused, 80.5 $\mu\text{g/L}$; $p < 0.0001$). Seventy-eight percent of all patients had received chelation therapy. In the total population, a history of organ dysfunction requiring medical treatment was common and included cardiac disease (13%), gonadal failure (17%), growth hormone deficiency (8%), hypothyroidism (8%), hypoparathyroidism (1%), diabetes (10%), cirrhosis (2%), and thrombotic events (7%). Sixteen and a half percent had more than one organ system involved. Thrombotic events were often serious and included six cases of pulmonary embolism, five superior vena cava syndromes, four deep vein thromboses, three cardiac thromboses, two portal vein thromboses, and two patients with renal thrombosis. Of the 26 chronic transfusion patients with thrombosis, 25 had a splenectomy and 21 had concomitant central venous access. Overall, 31 patients were on chronic anticoagulant therapy. Most complications occurred in the chronically transfused population; however, cardiac, gonadal, thrombotic, and other complications were noted in 16% of the intermittently transfused.

Surgical procedures were common in this population. Splenectomy was reported in 45% of patients and was more prevalent in the transfused patients: 52% in chronically transfused, 29% in intermittently transfused, and 7% nontransfused ($p < 0.0001$). The mean \pm SD age at the time of splenectomy was 10 ± 6.3 years. The majority of splenectomies occurred before 15 years of age, with 20% occurring before age 5. Asians were less likely than whites to have undergone splenectomy (32% vs. 65%, $p < 0.0001$). However, Asians were younger (17.9 ± 9.6 years) compared to Caucasians (27.5 ± 14.7 years). Other procedures included central venous access devices in 27% and cholecystectomy in 14% of patients.

Infections

At study entry, 86 patients' intake forms revealed previous exposure to possible transfusion-associated infectious diseases, including hepatitis C (61), hepatitis B (20), hepatitis A (3), parvovirus (9), human immunodeficiency virus

(HIV; 4), malaria (1), *Staphylococcus aureus* (1), and babesia (1). In total, 24% of transfused patients had laboratory evidence of previous exposure to one or more infectious diseases.

Transfusion complications

A history of transfusion reactions was reported in 48% of transfused patients. Chronically transfused patients were more likely to have had a reaction than intermittently transfused patients (50% vs. 22%, $p = 0.002$). Transfusion reactions occurred in 55% of males compared to 42% of females ($p = 0.02$) but were not related to race or splenectomy status.

Fifty-two percent of patients with transfusion reactions reported having only an allergic reaction, 16% experienced only febrile reactions, and 27% had multiple transfusion reactions of varied types. Most allergic reactions were mild to moderate in severity; however, there were two anaphylactic reactions, one transfusion-associated hypotensive event, and one episode of transfusion-associated dyspnea. There were 17 reported hemolytic transfusion reactions. Source documents were able to confirm that nine of these were hemolytic immunologic transfusion reactions. The other cases occurred with allergic and febrile reactions but did not provide immunologic testing or laboratory evidence of hemolysis. Other complications included transfusion-associated tachycardia, acute vertigo, and transient vomiting. There were no episodes of TRALI.

Alloantibodies

Overall, 19% (68/365) of all transfused patients had alloantibodies. Twenty-three percent of chronically transfused patients were alloimmunized, compared to 13% of the intermittently transfused ($p = 0.30$). Forty-seven percent of alloimmunized patients had multiple antibodies: anti-E, anti-K, or anti-C were identified in 70% of these patients (Table 2). One TM patient of Chinese ancestry developed anti-Mi^a. The hemolytic transfusion reactions that occurred were caused by anti-E, anti-Jkb, anti-c, anti-Jka, anti-S, anti-Kell, and anti-f and two cases of warm autoantibodies.¹⁵ Current age, race, and splenectomy were also associated with alloimmunization. The mean \pm SD age of patients with alloantibodies was 29.4 ± 13.3 years ($n = 68$), and in those without, 20 ± 12.5 years ($n = 261$). Caucasians were more often alloimmunized than Asians (29% vs. 13%, $p < 0.0001$). Alloimmunization was found in 31% of splenectomized patients vs. 11% of nonsplenectomized patients ($p < 0.0001$).

Predictors of alloimmunization in chronically transfused

In the chronically transfused patients only, the age at which patients began transfusion was significantly

TABLE 2. RBC antigen specificity of alloantibodies detected*

Alloantibody	Frequency
E	29
K	17
C	12
Jka	6
c	5
HLA	5
KP ^a	4
V	4
D	3
I	3
S	3
Bg ^a	2
Cw	2
e	2
Jkb	2
Lea	2
WA1	2
f	1
Fya	1
Fyb	1
G	1
HTLA	1
Js ^a	1
LU ^a	1
M	1
Mi	1
Sd ^a	1
York A	1

* There were 10 antibodies without identified specificities. A total of 47% of patients had multiple antibodies (see text for details).

TABLE 3. Age at initiation of transfusion therapy and alloimmunization (n = 297)*

Age (years) at transfusion start	Rate of alloimmunization (p = 0.0021)
<1	11% (10/91)
1-10	28% (47/169)
11-20	31% (4/13)
21+	18% (3/17)

* On univariate analysis, infants initiating chronic transfusion therapy before 1 year of age had a lower rate of alloimmunization suggesting antigen tolerance as a mechanism.

associated with the alloimmunization and reflected transfusion exposure. However, in those who began transfusion before 1 year of age, the proportion of alloimmunized patients was lower despite greater transfusion burden: 11% vs. 18% to 31% in the older age groups (Table 3). The mean ± SD years of transfusion exposure in alloimmunized patients was higher (25.9 ± 13.1 years, n = 64) versus the nonalloimmunized (16.4 ± 11.1 years, n = 226; p < 0.0001). The mean ± SD number of transfusions received the year before enrollment in alloimmunized patients was 18 ± 6.6 (n = 64) versus 15.2 ± 5.2 (n = 235) in patients without alloantibodies (p = 0.003). In a stepwise multivariate logistic regression of alloantibody

TABLE 4. Univariate predictors of alloimmunization in chronically transfused patients (n = 299)

Current age		p < 0.0001
With alloantibody	29.5 ± 13.1 (64)	
Without alloantibody	20.5 ± 12.3 (235)	
Years of transfusion		p < 0.0001
With alloantibody	25.9 ± 13.0 (64)	
Without alloantibody	16.4 ± 11.1 (226)	
Sex		NS
Male	23% (32/137)	
Female	20% (32/162)	
Race		p = 0.001
White	30% (43/145)	
Asian	13% (19/144)	
Splenectomy		p < 0.0001
Yes	30% (48/158)	
No	11% (16/141)	

* Data are reported as mean ± SD (number) or alloimmunization percent (number/number). In a stepwise multivariate logistic regression of alloantibody formation in chronically transfused patients—which included age at study enrollment (left as a continuous variable), years of transfusion, race (Asian vs. white), and splenectomy status—only years of transfusion remained a significant independent predictor of alloimmunization. NS = not significant.

formation in chronically transfused patients—which included age at study enrollment (left as a continuous variable), years of transfusion, race (Asian vs. white), and splenectomy status—only years of transfusion remained a significant independent predictor of alloimmunization (Table 4).

Predictors of autoimmunization

Autoantibodies occurred in 6.5% of patients; chronically transfused and intermittently transfused patients had a similar risk (6.4% vs. 6.9%). Patients with autoantibodies were significantly older than those without (27 ± 13 years vs. 21 ± 13 years, p = 0.0395). In chronically transfused patients, the risk of autoantibody formation was 10% in splenectomized patients compared to 3% in nonsplenectomized patients (p = 0.02). Years of transfusion exposure, race, and sex were not associated with the rate of autoimmunization. Eighty-four percent of patients with autoantibodies were alloimmunized, in contrast to only 17% of those without autoantibodies (p < 0.0001). A stepwise multivariate logistic regression analysis of autoantibody formation in chronically transfused patients included age at study enrollment, splenectomy status, presence of alloantibodies, and years of transfusion exposure. Years of transfusion was included in the model because previous literature has shown an association between transfusion burden and formation of autoantibodies. In our model, only the presence of an alloantibody remained a significant independent predictor of autoimmunization.

Current blood processing and transfusion practices

For patients transfused in the year before study entry ($n = 330$), 31% received blood matched for ABO/D only; 38% were also matched for C, E, and Kell; and 10% received extended phenotypically matched RBCs. The extent of matching for 21% was unknown or variable. Additional processing included leukoreduction in 94%, washed RBCs in 35%, and irradiated cells in 33%.

Local blood banking practices varied. Two sites utilized standard ABO-Rh typing as their transfusion policy, with extended matching only once an antibody occurred. One site matched preventatively for C and E antigens, and three sites routinely matched for C, E, and Kell antigens. Extended RBC phenotypic matching, including Jkb antigen, was utilized at one site. All blood products were irradiated at four sites regardless of individual patient risk. Three sites restricted radiation therapy to only thalassemia patients undergoing transplantation. One site utilized RBC washing as a standard technique of leukoreduction while others restricted washing to patients with a history of allergic reactions.

DISCUSSION

As the life span of thalassemia patients has dramatically increased, their cumulative exposure to RBC transfusions has resulted in this disease being the most heavily RBC-transfused syndrome worldwide. This report is an assessment of the morbidity of transfusion therapy in 407 thalassemia patients monitored by the CDC. It is the first report focusing only on the US thalassemia population. In this report, half the study population is of Asian ethnicity and 27% immigrated to the United States. It includes information concerning transfusion complications not routinely studied in thalassemia such as serious allergic and hemolytic reactions. These complications and the variability in institutional approaches to blood typing and processing may provide an impetus for developing national transfusion guidelines for thalassemia.

Hemosiderosis was the most common complication in this population.¹⁶ This cross-sectional report of patients at the time of enrollment at thalassemia treatment centers across the United States was not designed to collect annual detailed information about iron intake, chelation, and iron loading to sensitive organs. However, despite new chelation therapies in this young population, hemosiderosis-induced organ dysfunction remains a serious problem.^{12,15,17-19} The prevalence of cardiac disease and endocrine dysfunction is lower than in cross-sectional data from previous decades, but its interpretation is limited by lack of laboratory confirmation, age at diagnosis, and detailed information regarding iron exposure, chelation compliance, treatment, and screening practices.¹⁷ Serious thrombotic complications occurred

particularly in patients who had undergone splenectomy and central line placement. Cappellini and others²⁰ have recommended that these high-risk patients should receive preventative anticoagulation therapy.

At enrollment, 24% of the transfused patients reported previous exposure to serious pathogens. While the actual source of infections cannot be unequivocally attributed to transfusion exposure given that some of the pathogens are endemic in areas outside of the United States, this rate of exposure is likely an underestimate as exposure data were unknown in some cases and enrollment forms did not query about all possible pathogens. High rates of hepatitis and HIV are expected in this population, which includes adults transfused before adequate blood bank testing was in place.^{14,16,21-24} However, since 27% of the thalassemia patients have immigrated from areas that are still not providing uniform infectious screening of RBC transfusions, active infections are likely to occur in immigrant patients of all ages.^{25,26} The observations in our study of malaria and babesia are concerning. These organisms are among several that threaten the transfusion blood supply and blood banks do not routinely test for them.²⁷⁻³³ Recently, the CDC reported a marked increase in the number of imported malaria cases.³² Babesia is a growing transfusion risk in the United States and has been implicated in 10 of 28 deaths from blood products between 2005 and 2008.^{27-29,34,35} Hemoglobinopathy patients are at particular risk for severe hemolysis that has been associated with babesia.^{15,36} The development of sensitive, specific assays for mass screening and a clear understanding of the risks of these infections is essential.

Almost half the transfused patients had experienced a transfusion reaction. Allergic reactions occurred in one-third of patients, including at least two episodes of anaphylaxis. The frequency and severity of allergic reactions in chronically transfused thalassemia have not been well studied. It appears much higher than reported risk in the general population of approximately 1% for allergic reactions and 1:25,000 to 1:150,000 for anaphylaxis.^{23,37-41} However, longitudinal studies are needed to confirm this. Although immunoglobulin A deficiency is thought to be the most common cause of anaphylaxis, recently haptoglobin antibody has been identified as a more common cause, particularly in people of Asian descent.⁴² These deficiencies have not been studied in the thalassemia population.

Alloimmunization is a serious adverse consequence of transfusion therapy. The overall proportion of alloimmunized patients in our study was 19%, with almost half the patients having multiple antibodies and several hemolytic transfusion reactions. Understanding the predictors of alloimmunization would enable the implementation of a cost-effective extended matching program for at-risk patients. In our study, logistic regression analysis

found that transfusion exposure was the strongest predictor of alloantibody formation.⁴³ While there are genetic and immunologic differences between patients that affect alloimmunization rate, it is likely that alloimmunization will continue to increase with age and transfusion burden in this thalassemia cohort.⁴⁴⁻⁵² Transfusion exposure is the major factor in cumulative antibody risk.^{9,12} E, Kell, and C antigens accounted for the majority of hemolytic antibodies identified in this US cohort of patients. Matching for these antigens has reduced alloimmunization and complications in other studies.^{6,9-12,53} Newer technology is making such preventative alloimmunization policies more cost-effective and supports their widespread implementation.^{51,53,54}

Certain antibodies are unique to the Asian population and are likely to become an emerging transfusion-related complication in the United States.^{4,5,7} Our Chinese patient with an anti-Mi^a is one of the first North American thalassemia patients reported to have this antibody. In contrast, it accounts for more than 30% of antibodies in thalassemia patients in China. The Mi^{a+} antigen is almost uniquely found in Asian donors.^{4,5,7} In China, blood banks routinely screen donors for Mi^{a+} RBCs. As the Asian donor population increases in the United States, anti-Mi^a are likely to increase and donor screening may become necessary.

Our study supported the concept of immune tolerance developing in very young children under 1 year of age. The alloimmunization rate in children who began transfusion before 1 year of age was only 11%, in contrast to 27% who began transfusion after 1 year of age. Immune tolerance as a factor in the rate of RBC alloimmunization has previously been suggested.^{11,43,50} Recently, genetic factors influencing immune tolerance and alloimmunization have been identified.⁵⁰

The effects of splenectomy on alloimmunization are unclear.^{9,12} Splenectomy markedly increases antigen and microparticle exposure in hemolytic anemias.^{55,56} This splenectomy-induced antigen overload likely increases alloimmunization rate. Thompson and coworkers¹² found on multivariate analysis that duration of transfusion and splenectomy were risk factors for alloimmunization; however, they found that splenectomy was not a significant risk factor in the older, chronically transfused patients. In our study, we observed splenectomy to be a risk factor on univariate analysis, but on multivariate analysis, only transfusion exposure was an independent predictor of alloimmunization. Differences between the two study populations and statistical modeling may account for the divergent findings. A large percentage of the Thalassemia Clinical Research Network included patients from international sites with differences in the percentage of thalassemia diagnostic categories as well as age at onset and duration of transfusions.^{3,12} Clearly, both studies underscore transfusion exposure as a key predictor of alloimmunization.⁴³

Autoantibodies are a serious problem, occurring in 6.5% of this population. This is much higher than in the general population.^{9,38,41,57} Their presence makes serologic cross-matching difficult and often masks an undetected alloantibody, which if not recognized, can result in a hemolytic transfusion reaction. Eighty-one percent of patients with an autoantibody had been alloimmunized. Multivariate analysis confirmed alloimmunization as the only significant independent predictor of autoimmunization. These data suggest that the control of alloimmunization may help to minimize autoantibody formation.

There is no national standardized procedure for preparation of RBCs and no consensus among participating thalassemia centers. Transfusion policy for thalassemia is determined by generalized blood banking policies within each institution.⁵⁸ The risk/benefit of extended RBC antigen matching for transfusions in hemoglobinopathies is debated.^{51,53,59,60} Prospective studies indicate that antigen matching for Cc, Ee, D, and K reduces alloimmunization.^{6,51,53,61} Molecular RBC phenotyping of donors and recipients may increase the efficacy and efficiency of RBC matching and decrease blood inventory requirements.^{37,51,62} Prospective outcome studies, including cost analysis, are needed.

The routine policy to irradiate all blood products received by thalassemia patients at four of the participating centers is controversial.^{37,63} Radiation adds monetary cost and alters RBC metabolism, causing accelerated cellular potassium loss, elevated free plasma Hb levels, and shortened RBC survival.^{37,64,65} The British Blood Transfusion Task Force and the Serious Hazards of Transfusion Program (SHOT), a UK hemovigilance effort, do not recommend irradiated RBC units for hemoglobinopathies.⁶⁴ However, some centers with large populations of immunocompromised patients, cancer patients, and premature infants use universal radiation as a local policy. The overall best policy for use of irradiated RBCs in hemoglobinopathy patients needs to be addressed.

This study has several limitations. Data collected at enrollment were retrospective and not all of the information could be verified. While not the focus of this report, analysis of iron-induced tissue injury is limited by a lack of actual tissue iron concentrations and specific vital organ functions as well as incomplete information regarding initiation and maintenance of chelation regimens. The intake forms did not quantitate the number of transfusion reactions per patient unit of exposure, which limits the determination of actual transfusion complication rates. Also, the laboratory testing for immune reactions was not centralized.

In summary, the thalassemia population in the United States is a multiethnic community including many recent immigrants who have a high rate of transfusion-related complications that exceeds that of other transfused populations.^{14,23,39,40,43} Transfusion

reactions (including allo- and autoimmunization, hemolytic, and anaphylactic) are serious problems. Despite advances in iron chelation and blood safety, major improvements in hemosiderosis and transfusion-acquired infections are still needed. There is no standard practice among thalassemia centers on blood product preparation designed to limit transfusion reactions. This longitudinal surveillance project will enable the development of recommendations for interventions to prevent transfusion morbidity and evaluate long-term effectiveness. Ultimately, this will improve the safety of the national blood supply and its delivery.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to **TRANSFUSION**.

REFERENCES

- Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. *Bull World Health Organ* 2008;86:480-7.
- Weatherall DJ. The inherited diseases of hemoglobin are an emerging global health burden. *Blood* 2010;115:4331-6.
- Vichinsky EP, MacKlin EA, Wayne JS, Lorey F, Olivieri NF. Changes in the epidemiology of thalassemia in North America: a new minority disease. *Pediatrics* 2005;116:e818-25.
- Cheng CK, Lee CK, Lin CK. Clinically significant red blood cell antibodies in chronically transfused patients: a survey of Chinese thalassemia major patients and literature review. *Transfusion* 2012;52:2220-4.
- Chu C, Ho H, Lee H, Chan YS, Chang FJ, Wang CL, Lin M. Anti-“Mi(a)” immunization is associated with HLA-DRB1*0901. *Transfusion* 2009;49:472-8.
- El Danasoury AS, Eissa DG, Abdo RM, Elalfy MS. Red blood cell alloimmunization in transfusion-dependent Egyptian patients with thalassemia in a limited donor exposure program. *Transfusion* 2012;52:43-7.
- Lee CK, Ma ES, Tang M, Lam CC, Lin CK, Chan LC. Prevalence and specificity of clinically significant red cell alloantibodies in Chinese women during pregnancy—a review of cases from 1997 to 2001. *Transfus Med* 2003;13:227-31.
- Pahuja S, Pujani M, Gupta SK, Chandra J, Jain M. Alloimmunization and red cell autoimmunization in multitransfused thalasseemics of Indian origin. *Hematology* 2010;15:174-7.
- Singer ST, Wu V, Mignacca R, Kuypers FA, Morel P, Vichinsky EP. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly Asian descent. *Blood* 2000;96:3369-73.
- Sirchia G, Zanella A, Parravicini A, Morelatti F, Rebulla P, Masera G. Red cell alloantibodies in thalassemia major. Results of an Italian cooperative study. *Transfusion* 1985;25:110-2.
- Spanos T, Karageorga M, Ladis V, Peristeri J, Hatziliami A, Kattamis C. Red cell alloantibodies in patients with thalassemia. *Vox Sang* 1990;58:50-5.
- Thompson AA, Cunningham MJ, Singer ST, Neufeld EJ, Vichinsky E, Yamashita R, Giardina P, Kim HY, Trachtenberg F, Kwiatkowski JL; Thalassemia Clinical Research Network Investigators. Red cell alloimmunization in a diverse population of transfused patients with thalassaemia. *Br J Haematol* 2011;153:121-8.
- Wang L, Liang D, Liu H, Chang FC, Wang CL, Chan YS, Lin M. Alloimmunization among patients with transfusion-dependent thalassemia in Taiwan. *Transfus Med* 2006;16:200-3.
- American Association of Blood Banks. Infectious complications of blood transfusions. In: Vengelen-Tyler V, editor. AABB technical manual. Bethesda (MD): American Association of Blood Banks; 2008. p. 601-30.
- Centers for Disease Control and Prevention (CDC). The National Healthcare Safety Network (NHSN) manual. In: CDC, editor. NHSN biovigilance component protocol. Atlanta (GA): Division of Healthcare Quality Promotion. National Center for Emerging and Zoonotic Infectious Diseases (proposed). Centers for Disease Control and Prevention; 2010. p. 30.
- Cunningham MJ, Macklin EA, Neufeld EJ, Cohen AR; Thalassemia Clinical Research Network. Complications of beta-thalassemia major in North America. *Blood* 2004;104:34-9.
- Fung EB, Harmatz P, Milet M, Ballas SK, De Castro L, Hagar W, Owen W, Olivieri N, Smith-Whitley K, Darbari D, Wang W, Vichinsky E; Multi-Center Study of Iron Overload Research Group. Morbidity and mortality in chronically transfused subjects with thalassemia and sickle cell disease: a report from the multi-center study of iron overload. *Am J Hematol* 2007;82:255-65.
- Lal A, Goldrich ML, Haines DA, Azimi M, Singer ST, Vichinsky EP. Heterogeneity of hemoglobin H disease in childhood. *N Engl J Med* 2011;364:710-8.
- Pakbaz Z, Fischer R, Fung E, Nielsen P, Harmatz P, Vichinsky E. Serum ferritin underestimates liver iron concentration in transfusion independent thalassemia patients as compared to regularly transfused thalassemia and sickle cell patients. *Pediatr Blood Cancer* 2007;49:329-32.
- Cappellini MD, Motta I, Musallam KM, Taher AT. Redefining thalassemia as a hypercoagulable state. *Ann NY Acad Sci* 2010;1202:231-6.
- Ansar MM, Kooloobandi A. Prevalence of hepatitis C virus infection in thalassemia and haemodialysis

- patients in north Iran-Rasht. *J Viral Hepat* 2002;9:390-2.
22. Sievert W, Altraif I, Razavi HA, Abdo A, Ahmed EA, Alomair A, Amarapurkar D, Chen CH, Dou X, El Khayat H, Elshazly M, Esmat G, Guan R, Han KH, Koike K, Largen A, McCaughan G, Mogawer S, Monis A, Nawaz A, Piratvisuth T, Sanai FM, Sharara AI, Sibbel S, Negro F et al. A systematic review of hepatitis C virus epidemiology in Asia, Australia and Egypt. *Liver Int* 2011;31 Suppl 2:61-80.
 23. Stainsby D, Jones H, Asher D, Atterbury C, Boncinelli A, Brant L, Chapman CE, Davison K, Gerrard R, Gray A, Knowles S, Love EM, Milkins C, McClelland DB, Norfolk DR, Soldan K, Taylor C, Revill J, Williamson LM, Cohen H; SHOT Steering Group. Serious hazards of transfusion: a decade of hemovigilance in the UK. *Transfus Med Rev* 2006;20:273-82.
 24. Vamvakas EC, Blajchman MA. Transfusion-related mortality: the ongoing risks of allogeneic blood transfusion and the available strategies for their prevention. *Blood* 2009;113:3406-17.
 25. Mansour AK, Aly RM, Abdelrazek SY, Elghannam DM, Abdelaziz SM, Shahine DA, Elmenshawy NM, Darwish AM. Prevalence of HBV and HCV infection among multi-transfused Egyptian thalassemic patients. *Hematol Oncol Stem Cell Ther* 2012;5:54-9.
 26. Omar N, Salama K, Adolf S, El-Saeed GS, Abdel Ghaffar N, Ezzat N. Major risk of blood transfusion in hemolytic anemia patients. *Blood Coagul Fibrinolysis* 2011;22:280-4.
 27. Gubernot DM, Nakhasi HL, Mied PA, Asher DM, Epstein JS, Kumar S. Transfusion-transmitted babesiosis in the United States: summary of a workshop. *Transfusion* 2009;49:2759-71.
 28. Gubernot DM, Lucey CT, Lee KC, Conley GB, Holness LG, Wise RP. Babesia infection through blood transfusions: reports received by the US Food and Drug Administration, 1997-2007. *Clin Infect Dis* 2009;48:25-30.
 29. Herwaldt BL, Linden JV, Bosserman E, Young C, Olkowska D, Wilson M. Transfusion-associated babesiosis in the United States: a description of cases. *Ann Intern Med* 2011;155:509-19.
 30. Leiby DA. Transfusion-transmitted Babesia spp.: bull's-eye on Babesia microti. *Clin Microbiol Rev* 2011;24:14-28.
 31. Leiby DA. Babesiosis and blood transfusion: flying under the radar. *Vox Sang* 2006;90:157-65.
 32. Mali S, Kachur SP, Arguin PM; Division of Parasitic Diseases and Malaria, Center for Global Health; Centers for Disease Control and Prevention (CDC). Malaria surveillance—United States, 2010. *MMWR Surveill Summ* 2012;61:1-17.
 33. Tonnetti L, Eder AF, Dy B, Kennedy J, Pisciotto P, Benjamin RJ, Leiby DA. Transfusion-transmitted Babesia microti identified through hemovigilance. *Transfusion* 2009;49:2557-63.
 34. FDA. Fatalities reported to FDA following blood collection and transfusion. Annual summary for fiscal year 2007. Washington, DC: US Food and Drug Administration (FDA), 2008.
 35. Herman JH, Ayache S, Olkowska D. Autoimmunity in transfusion babesiosis: a spectrum of clinical presentations. *J Clin Apher* 2010;25:358-61.
 36. Bloch EM, Herwaldt BL, Leiby DA, Shaieb A, Herron RM, Chervenak M, Reed W, Hunter R, Ryals R, Hagar W, Xayavong MV, Slemenda SB, Pieniazek NJ, Wilkins PP, Kjemtrup AM. The third described case of transfusion-transmitted Babesia duncani. *Transfusion* 2012;52:1517-22.
 37. Luban NL, McBride E, Ford JC, Gupta S. Transfusion medicine problems and solutions for the pediatric hematologist/oncologist. *Pediatr Blood Cancer* 2012;58:1106-11.
 38. Klein HG, Spahn DR, Carson JL. Red blood cell transfusion in clinical practice. *Lancet* 2007;370:415-26.
 39. Stainsby D, Jones H, Wells AW, Gibson B, Cohen H; SHOT Steering Group. Adverse outcomes of blood transfusion in children: analysis of UK reports to the serious hazards of transfusion scheme 1996-2005. *Br J Haematol* 2008;141:73-9.
 40. Domen RE, Hoeltge GA. Allergic transfusion reactions: an evaluation of 273 consecutive reactions. *Arch Pathol Lab Med* 2003;127:316-20.
 41. Hendrickson JE, Hillyer CD. Noninfectious serious hazards of transfusion. *Anesth Analg* 2009;108:759-69.
 42. Shimada E, Tadokoro K, Watanabe Y, Ikeda K, Niihara H, Maeda I, Isa K, Moriya S, Ashida T, Mitsunaga S, Nakajima K, Juji T. Anaphylactic transfusion reactions in haptoglobin-deficient patients with IgE and IgG haptoglobin antibodies. *Transfusion* 2002;42:766-73.
 43. Rosse WF, Gallagher D, Kinney TR, Castro O, Dosik H, Moohr J, Wang W, Levy PS. Transfusion and alloimmunization in sickle cell disease. The Cooperative Study of Sickle Cell Disease. *Blood* 1990;76:1431-7.
 44. Bao W, Yu J, Heck S, Yazdanbakhsh K. Regulatory T-cell status in red cell alloimmunized responder and nonresponder mice. *Blood* 2009;113:5624-7.
 45. Bao W, Zhong H, Li X, Lee MT, Schwartz J, Sheth S, Yazdanbakhsh K. Immune regulation in chronically transfused allo-antibody responder and nonresponder patients with sickle cell disease and beta-thalassemia major. *Am J Hematol* 2011;86:1001-6.
 46. Hall AM, Cairns LS, Altmann DM, Barker RN, Urbaniak SJ. Immune responses and tolerance to the RhD blood group protein in HLA-transgenic mice. *Blood* 2005;105:2175-9.
 47. Hendrickson JE, Chadwick TE, Roback JD, Hillyer CD, Zimring JC. Inflammation enhances consumption and presentation of transfused RBC antigens by dendritic cells. *Blood* 2007;110:2736-43.
 48. Hoppe C, Klitz W, Vichinsky E, Styles L. HLA type and risk of alloimmunization in sickle cell disease. *Am J Hematol* 2009;84:462-4.

49. McPherson ME, Anderson AR, Castillejo MI, Hillyer CD, Bray RA, Gebel HM, Josephson CD. HLA alloimmunization is associated with RBC antibodies in multiply transfused patients with sickle cell disease. *Pediatr Blood Cancer* 2010;54:552-8.
50. Tatari-Calderone Z, Minniti CP, Kratovil T, Stojakovic M, Vollmer A, Barjaktarevic I, Zhang E, Hoang A, Luban NL, Vukmanovic S. rs660 polymorphism in Ro52 (SSA1; TRIM21) is a marker for age-dependent tolerance induction and efficiency of alloimmunization in sickle cell disease. *Mol Immunol* 2009;47:64-70.
51. Yazdanbakhsh K, Ware RE, Noizat-Pirenne F. Red blood cell alloimmunization in sickle cell disease: pathophysiology, risk factors, and transfusion management. *Blood* 2012;120:528-37.
52. Yu J, Heck S, Yazdanbakhsh K. Prevention of red cell alloimmunization by CD25 regulatory T cells in mouse models. *Am J Hematol* 2007;82:691-6.
53. Lasalle-Williams M, Nuss R, Le T, Cole L, Hassell K, Murphy JR, Ambruso DR. Extended red blood cell antigen matching for transfusions in sickle cell disease: a review of a 14-year experience from a single center (CME). *Transfusion* 2011;51:1732-9.
54. Wilkinson K, Harris S, Gaur P, Haile A, Armour R, Teramura G, Delaney M. Molecular blood typing augments serologic testing and allows for enhanced matching of red blood cells for transfusion in patients with sickle cell disease. *Transfusion* 2011;52:381-8.
55. Pattanapanyasat K, Gonwong S, Chaichompoo P, Nulsri E, Lerdwana S, Sukapirom K, Siritanaratkul N, Fucharoen S. Activated platelet-derived microparticles in thalassaemia. *Br J Haematol* 2007;136:462-71.
56. Westerman M, Pizzey A, Hirschman J, Cerino M, Weil-Weiner Y, Ramotar P, Eze A, Lawrie A, Purdy G, Mackie I, Porter J. Microvesicles in haemoglobinopathies offer insights into mechanisms of hypercoagulability, haemolysis and the effects of therapy. *Br J Haematol* 2008;142:126-35.
57. Young PP, Uzieblo A, Trulock E, Lublin DM, Goodnough LT. Autoantibody formation after alloimmunization: are blood transfusions a risk factor for autoimmune hemolytic anemia? *Transfusion* 2004;44:67-72.
58. Spinella PC, Dressler A, Tucci M, Carroll CL, Rosen RS, Hume H, Sloan SR, Lacroix J; Pediatric Acute Lung Injury and Sepsis Investigators Network. Survey of transfusion policies at US and Canadian children's hospitals in 2008 and 2009. *Transfusion* 2010;50:2328-35.
59. King KE, Shirey RS. Transfusion management of patients with sickle cell disease: the continuing dilemma. *Transfusion* 2010;50:2-4.
60. Osby M, Shulman IA. Phenotype matching of donor red blood cell units for nonalloimmunized sickle cell disease patients: a survey of 1182 North American laboratories. *Arch Pathol Lab Med* 2005;129:190-3.
61. Vichinsky EP, Luban NL, Wright E, Olivieri N, Driscoll C, Pegelow CH, Adams RJ; Stroke Prevention Trial in Sickle Cell Anemia. Prospective RBC phenotype matching in a stroke-prevention trial in sickle cell anemia: a multicenter transfusion trial. *Transfusion* 2001;41:1086-92.
62. Anstee DJ. Red cell genotyping and the future of pretransfusion testing. *Blood* 2009;114:248-56.
63. King KE, Ness PM. How do we prevent transfusion-associated graft-versus-host disease in children? *Transfusion* 2011;51:916-20.
64. Serious Hazards of Transfusion—United Kingdom (SHOT-UK). Serious hazards of transfusion for children. 2011 [cited 2012 Jul 31]. Available from: URL: <http://www.shotuk.org/wp-content/uploads/2010/03/SHOT-for-Children.pdf>
65. Treleaven J, Gennery A, Marsh J, Norfolk D, Page L, Parker A, Saran F, Thurston J, Webb D. Guidelines on the use of irradiated blood components prepared by the British Committee for Standards in Haematology blood transfusion task force. *Br J Haematol* 2011;152:35-51.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Site and staff information.

Appendix S2. Procedures for laboratory testing of infections by the CDC.