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Red Cell Alloimmunization in a Diverse Population of Transfused Patients with Thalassaemia

Alexis A. Thompson¹, Melody J. Cunningham², Sylvia T. Singer³, Ellis J. Neufeld⁴, Elliott Vichinsky³, Robert Yamashita⁵, Patricia Giardina⁶, Hae-Young Kim⁷, Felicia Trachtenberg⁷, and Janet L. Kwiatkowski⁸ for the Thalassemia Clinical Research Network Investigators

¹Children's Memorial Hospital, Chicago Illinois

²University of Tennessee, Memphis, Tennessee

³Children's Hospital and Research Center Oakland, Oakland, California

⁴Children's Hospital Boston, Boston, Massachusetts

⁵California State Univ. San Marcos, San Marcos, California

⁶Weill Cornell Medical College, New York City, New York

⁷New England Research Institutes, Watertown, Massachusetts

⁸Children's Hospital of Philadelphia, Philadelphia, Pennsylvania

SUMMARY

Red blood cell (RBC) transfusion is the primary treatment for severe forms of thalassaemia. Pre-storage screening has resulted in decreased transfusion-transmitted infections, but anti-RBC antibodies remain a major problem. We report on 697 participants who had ever received transfusions. Allo- and autoantibody rates were compared with respect to splenectomy status, ethnicity, diagnosis, duration of transfusions, treatment centre, and age at transfusion initiation, together with rates before and after 1990, when leucoreduction methods were routine at thalassaemia treatment centres. Allo- and autoantibodies were reported in 115 (16.5%) and 34 (4.9%) subjects, respectively. Splenectomized patients were more likely to have alloantibodies (Odds Ratio [OR] = 2.528, $p < 0.0001$), or autoantibodies (OR = 2.590, $p = 0.0133$). Alloantibodies occurred in 19 of 91 (21%) splenectomized subjects who started transfusion after 1990, and only 18 of 233 (7.7%) nonsplenectomized subjects ($p < 0.001$). Data from this study demonstrate that RBC antibodies continue to develop in chronically transfused thalassaemia patients at a high rate. Splenectomy preceded the development of antibodies in most cases. Increased rates of RBC sensitization among splenectomized patients is concerning and deserves further study.

Keywords

thalassaemia; alloimmunization; splenectomy; transfusion; leucocyte depletion

INTRODUCTION

Red cell (RBC) transfusions are the mainstay of treatment for severe forms of thalassaemia. While improved pre-storage screening methods have resulted in a dramatic decrease in the frequency of transfusion-transmitted infections, the development of anti-RBC antibodies (both allo- and autoantibodies) remains a major problem. The development of alloantibodies and/or autoantibodies against RBC antigens complicates RBC crossmatching, shortens *in vivo* survival of transfused cells, delays provision of safe transfusions and may accelerate tissue iron loading (Charache 1990, Higgins and Sloan 2008, Singer, *et al* 2000). The literature reports various frequencies of alloimmunization depending on the homogeneity of the donor-recipient population, RBC phenotype matching policy, and age at transfusion initiation. Reported alloimmunization rates ranged from 4 to 50% in thalassaemia, and were lower in more homogenous populations (Pahuja, *et al* 2010, Singer, *et al* 2000, Sirchia, *et al* 1985, Spanos, *et al* 1990, Wang, *et al* 2006).

Splenectomy has often been employed in the management of patients with thalassaemia intermedia to relieve hypersplenism or to alleviate other manifestations of chronic anaemia. In thalassaemia major, splenectomy can also stabilize annual red cell requirements and iron accumulation (Cohen, *et al* 1989, Graziano, *et al* 1981). The concern of post-splenectomy sepsis more recently has been supplanted by the association of splenectomy with thromboembolic events and pulmonary hypertension in patients with thalassaemia intermedia and to a lesser degree with thalassaemia major (Aessopos, *et al* 2001, Cappellini, *et al* 2000, Tripodi, *et al* 2009).

We examined a broadly distributed, ethnically diverse population of patients with transfusion-dependent thalassaemia to identify risk factors for antibody development as well as possible strategies for risk reduction that may impact the clinical care of these patients. The primary aim of the current study was to determine the prevalence of allo- and autoimmunization of subjects in a multi-national clinical research network and to assess potential clinical factors associated with RBC antibody formation. We assessed allo- and autoantibody rates and the effect of splenectomy status, ethnicity, transfusion initiation age, and transfusion duration on RBC antibody rates. We also compared rates before and after 1990, when universal methods for leucocyte reduction were in wide use by blood banks, to determine if changes in practices over time impacted antibody formation.

METHODS

The Thalassemia Clinical Research Network (TCRN) is a National Heart, Lung and Blood Institute (NHLBI)-funded consortium of centres in North America and the United Kingdom that developed a cross-sectional registry and subsequent longitudinal observational cohort study to characterize demographic and clinical features of patients with thalassaemia, to highlight areas requiring clinical research, and to identify candidates eligible for clinical research protocols (see Appendix I for participants). The cross-sectional registry enrolled patients from May 2000 until October 2006. In May 2007, the TCRN launched the Thalassemia Longitudinal Cohort (TLC) study to extend these observations based on annual

assessments of clinical and laboratory information on relatively severe thalassaemia subtypes in North America, and also at thalassaemia treatment centres in London. Criteria for inclusion in the Registry are described elsewhere (Cunningham, *et al* 2004). The inclusion criteria for the TLC were similar, however, conditions with relatively few clinical sequelae, such as HbH with baseline haemoglobin >90 g/l, and heterozygous beta thalassaemia with Hgb C or D, were excluded. Data were assembled from retrospective chart review and by patient self-report using case report forms covering demographic information, family history, transfusions, chelation, surgical procedures and disease- or treatment-related complications. Data were also collected regarding institutional practices related to transfusions. Institutional review boards approved the protocol at each site and each subject or a parent or guardian gave informed written consent. Assents were also obtained based on institutional requirements.

Definitions

For the purposes of this report, beta thalassaemia major was defined as homozygous (or compound heterozygous) beta thalassaemia requiring eight or more transfusions in the 12 months prior to enrollment in the Registry and the TLC. Patients with beta thalassaemia who required fewer than eight transfusions annually were considered to have thalassaemia intermedia. This included several patients who might otherwise have been considered beta thalassaemia major but had suspended regular transfusions prior to enrollment (e.g., due to transfusion reactions or use of alternative therapies). Alpha thalassaemia syndromes (severe Haemoglobin H disease, HbH/Constant Spring, homozygous alpha thalassaemia) as well as Haemoglobin E/beta thalassaemia were also eligible to participate in the both studies. Patients with successful engraftment of transplanted stem cells at the time of Registry or TLC enrollment were excluded from this analysis. Alloantibodies were defined to include all antibodies reactive to specific RBC alloantigens. Direct antiglobulin testing was used to detect autoantibodies. Clinically significant allo-antibodies are those that are known to cause haemolytic transfusion reactions or haemolytic disease of the fetus or newborn (Poole and Daniels 2007).

Statistical Methods

Predictors considered for analysis were age, gender, race, age at start of regular transfusion, year starting regular transfusion, duration of regular transfusion, and, splenectomy. Continuous variables were analysed using Wilcoxon two-sample tests. Categorical variables were analysed by chi-squared test or Fisher's exact test. Logistic regression was used to test for associations between predictors and allo- or auto-immunization status. Multivariate effects of predictors significant in univariate analysis were modelled using logistic regression for the presence of alloantibody. All analyses were performed at the data coordinating centre (New England Research Institutes, Watertown, MA) with SAS statistical software (9.2, SAS Institute, Cary, NC). P-values less than 0.05 were considered statistically significant.

RESULTS

A total of 697 unique thalassaemia patients who had ever been transfused at 28 institutions were enrolled in the TCRN Registry and TLC observational studies. Two hundred and ninety-nine subjects participated in the Registry and an additional 398 had data collected through the TLC. Information on age, ethnicity, splenectomy and transfusion history, including allo- and autoimmunization status, was collected as part of both studies (see Appendix II for sample questions from the Registry and TLC). The results of univariate data analysis are summarized in Table I. Allo- and autoantibodies were reported in 115 (16.5%) and 34 (4.9%) subjects, respectively. Twenty-two (3.2%) individuals had both allo- and autoantibodies. Most subjects (74.9%) had beta thalassaemia major (n=528). Other thalassaemia phenotypes were beta thalassaemia intermedia (n=54), HbE/beta thalassaemia (n=84), alpha thalassaemia (n=10), HbH or HbH/Constant Spring (n=17) and 3 subjects with other variants. Thirty-two percent began transfusions before the age of 1 year and over 80% had initiated transfusions by 5 years of age. The date or age for transfusion initiation was not available for 26 patients, most of whom (20/26) were older patients with beta thalassaemia major. Participants with alloimmunization who had ever been transfused were significantly older (median age 26.5 versus 17.9 years, $p<0.0001$) and had longer transfusion duration (median 23.7 versus 14.4 years, $p<0.0001$) compared to transfused subjects without antibodies. Alloantibodies were more likely to occur in patients who began transfusions before 1990 (Odds Ratio [OR]=2.08, $p=0.0009$). In examining birth cohorts, those subjects born prior to 1990 were more likely to be alloimmunized compared to those born after 1990 (OR=3.48, $p<0.0001$). Mean ages at time of registry or TLC entry in the pre- and post-1990 birth cohorts were 28.5 ± 9.5 years and 9.5 ± 4.5 years, respectively. Rates of allo- and autoantibody formation were not significantly greater in patients with thalassaemia major, who were transfused regularly, than for thalassaemia intermedia patients who were intermittently transfused. Rates of alloimmunization did not differ significantly by diagnosis, gender, race or Hepatitis C status after controlling for age.

Nearly half (49.4%) of the thalassaemia patients in this analysis had been splenectomized. The average age at which splenectomy occurred was 9.8 years. The frequency with which splenectomy was performed changed over time such that 68.1% of patients who began transfusion before 1990 were splenectomized, but only 28.0% of those who started after 1990 have had their spleens removed. Splenectomy was associated with significantly higher rates of both allo- and autoantibodies. Patients who had no spleen were more likely to have alloantibodies (OR = 2.528, $p = <0.0001$), or autoantibodies (OR = 2.590, $p = 0.0133$) compared to nonsplenectomized subjects. Additional analysis was performed to examine the relationship between antibody development, splenectomy and era of transfusion initiation (Figure 1). Splenectomy was not associated with a statistically significant increased risk of alloantibodies among splenectomized subjects who initiated transfusions prior to 1990 (OR = 1.691, $p = 0.086$). Patients who had no spleen and began regular transfusions after 1990, however, were more likely to have alloantibodies compared to subjects with intact spleens who began transfusions in the same period (OR = 3.152, $p = 0.0013$). The temporal relationship of splenectomy to the first report of alloantibody formation could be ascertained only in a subset of subjects enrolled in the TLC. The majority (20 of 24) participants for

whom complete data was available had undergone splenectomy prior to the detection of alloantibodies or autoantibodies. Alloantibodies were reported in 19 of 91 (21%) splenectomized subjects who started transfusion after 1990, and only 18 of 233 (7.7%) nonsplenectomized subjects in the same cohort ($p < 0.001$). Using variables that were significant on univariate analyses, a multivariate model of allosensitization identified splenectomy and duration of transfusion as significant risk factors (Table II). When compared to patients who had been transfused regularly for 10 years or less, patients who had been transfused 20 or more years were more likely to have alloimmunization. The differences between the cohorts with greater than 20 years transfusion duration (20, 30 or 40 years) were not significant.

Additional centre-specific information was obtained from TCRN sites in the TLC. Rates of alloimmunization and autoantibody production were collected from all participating TCRN centres. Alloimmunization ranged widely among the centres, from 0 to 37.7%. Further analysis to identify risk factors associated with RBC immunization rates were compared among treatment centres with at least 10 TLC enrollees ($N=14$) and are listed in Table III. There were significant differences among sites for alloimmunization ($p=0.001$), autoimmunization ($p=0.0002$) or both ($p < 0.001$), using Chi-square testing. Leucoreduction and phenotypic matching protocols at the centres were examined as well as the proportion of RBC sensitized patients that were splenectomized. All TCRN sites reported using exclusively leucocyte depleted RBCs for transfusions in thalassaemia patients by 1990. The method for leucoreduction (bedside or prestorage filtration) was not specified. RBCs were routinely irradiated at three sites, but this had no significant effect on site differences in alloimmunization ($OR=0.934$, $p=0.765$). Red cells were washed at one site. Extended antigen matching to include C, E and Kell was performed at five sites. There was no statistical difference in alloimmunization rates among sites based on degree of RBC antigen matching [$OR=1.426$ (95% confidence interval: 0.917-2.217, P -value=0.1148)]. Splenectomy rates also varied widely among centres with at least 10 TLC enrollees, from 14.8 to 80%. There was a modest correlation between alloimmunization and splenectomy rates among centres ($r = 0.51$). Sites with splenectomy rates $>50\%$ had higher average rates of alloimmunization (19.26%) compared to sites with less than $<50\%$ splenectomy rates (9.52%), however this difference was not statistically significant. After controlling for splenectomy rates, however the site effect on alloimmunization persisted ($p=0.0043$).

Data regarding antibody persistence and specificity were collected in the TLC. A total of 116 alloantibodies were detected in 60 participants in the TLC. Of the 60 participants who reported ever having alloantibodies, 47 (78.3%) had current antibodies at the time of TLC enrollment. Thirty (50%) of allosensitized participants had more than one antibody detected. The specificity of the antibodies reported included many red cell antigens that are associated with clinically relevant haemolysis (Table IV)(Poole and Daniels 2007). For autoantibodies, 10/20 (50%) of participants from the TLC dataset that had ever had an antibody still reported autoantibodies currently at the time of TLC baseline data collection. Individuals who had both alloantibodies and autoantibodies ever reported in TLC, had persistence of both in 9/18 (50.0%) of cases. Only two subjects reported discontinuation of chronic transfusions because of allosensitization (p =not significant).

DISCUSSION

Patients with thalassaemia major are among the highest individual consumers of red cells on a recurrent basis at many institutions. Their cumulative red blood cell exposure puts this population at increased risk of transfusion-related complications, including blood borne infections, iron overload and sensitization to RBC antigens. The overall prevalence of RBC alloimmunization among enrollees in the TCRN registry and TLC was 16.6%, two-fold higher than rates in patients with haematological malignancies (Blumberg, *et al* 2003, Schonewille, *et al* 2006), and much greater than the prevalence of clinically significant antibodies reported for the general population (Heddle, *et al* 1995, Higgins and Sloan 2008, Hoeltge, *et al* 1995, Schonewille, *et al* 2006). When alloantibodies occurred in our population, they were generally not transient. The impact of antibody detection on transfusions, leading to either transfusion discontinuation or increased transfusion requirement, while concerning, was manageable with current blood banking practices in most cases.

The thalassaemia population in North America and the United Kingdom is quite diverse, with greater numbers of younger thalassaemia patients now of Asian ancestry. Singer et al (2000) reported significant differences in the alloimmunization rate in thalassaemia patients of Asian descent who were exclusively transfused with blood that was phenotypically matched for Rh and Kell RBC antigens at a single large U.S. thalassaemia treatment centre. The current study did not detect significant differences in the rates of RBC sensitization among thalassaemia patients of different races or ethnic groups. The higher alloimmunization rate among Caucasians with thalassaemia in our study population probably reflected greater transfusion exposure, as they tended to be older. The RBC antibodies most commonly associated with alloimmunization in our population were directed against C, E and Kell antigens. This predominance is not unexpected given the strong immunogenicity of these particular antigens. Alloimmunization can result from both donor and recipient factors, or the interaction of the two. Genetic factors (Human Leucocyte Antigen type, expression of RBC antigen variants) as well as acquired epigenetic factors in the host, such as inflammation and splenectomy, impact RBC immunization. Some factors, such as red cell phenotype matching, cytokine release from donor leucocytes can be addressed in the context of current transfusion practices (Blumberg, *et al* 2003). A recent retrospective analysis of large blood bank databases suggested that a subgroup of transfusion recipients are predisposed to develop alloantibodies independent of disease state (Higgins and Sloan 2008). This analysis included patients with thalassaemia but did not assess them as a separate disease state.

The association of leucoreduction and RBC alloimmunization is not well established. A recent survey of transfusion policies suggests that RBC filtration has become a standard practice in many institutions that are likely to care for thalassaemia patients in the U.S. and Canada (Spinella, *et al* 2010). Findings in a retrospective regional study from the Netherlands did not find a link between universal leucodepletion and alloimmunization (Schonewille and Brand 2005), though this procedure has been among the risk factors examined in other reports on transfusions in thalassaemia patients (Ameen, *et al* 2003, Pahuja, *et al* 2010, Singer, *et al* 2000, Wang, *et al* 2006) with discordant results. To the

extent that the contribution by donor leucocytes to RBC sensitization is reduced with filtration, reduction in alloimmunization might have been anticipated, but this was not the case in our thalassaemia population. We predicted that the thalassaemia patients who were exclusively transfused in the era of routine filtration would be less likely to become alloimmunized as reported by Singer *et al*. RBC allo- and autoimmunization continue to develop in chronically transfused thalassaemia patients and it is not clear that the availability of leucoreduced RBC resulted in the lower immunization rate observed in younger patients. Studies of alloimmunization in a murine model suggest that viral-like inflammatory mediators enhance alloimmunization. Interestingly, the effects of the viral-like inflammation on alloimmunization were only seen in nonsplenectomized animals (Hendrickson, *et al* 2007). Splenectomy abrogated the enhanced alloimmunization, suggesting that cellular components of the immune response that reside in the spleen are required for this effect.

The role of the spleen in RBC alloimmunization in mouse models is seemingly contradictory to the pattern observed in our patients. Uptake of transfused RBCs by splenic macrophages and dendritic cells, and subsequent presentation of RBC as immunogens under certain conditions, such as inflammation, results in allosensitization. Surgical splenectomy has been associated with differential alterations in immune cell function in other organs, with increased interleukin (IL)-6 production in macrophages in the liver, peritoneal and alveolar macrophages in humans and mice models (Shih-Ching, *et al* 2004). Elevated IL-6 has also been seen in post-splenectomized patients with thalassaemia (Archararit, *et al* 2000), however, it is not clear if IL-6 levels are different in thalassaemia patients who develop RBC sensitization. When splenectomized mice were exposed to a model RBC antigen, their antibody response was decreased by seven-fold compared to intact (nonsplenectomized) animals, yet we observed in our study that thalassaemia patients who had undergone splenectomy had significantly higher rates of alloimmunization. While alloantibodies were more prevalent in older patients who started transfusions prior to 1990, and therefore probably were exposed to donor leucocytes, splenectomy in the younger birth cohort is also associated with a significantly higher rate of alloimmunization. This suggests that if there are any benefits of leucoreduction, they may be offset by the changes in immunomodulatory capacity following splenectomy. The risk for RBC immunization that splenectomy imparts is concerning and deserves closer analysis.

There have been concerted efforts to standardize care guidelines for patients with thalassaemia, yet variations in centre practices persist and may contribute to RBC antibody formation. We collected data on the initiation of leucoreduction and policies regarding antigen matching in order to understand the differences. We did not include centres enrolling fewer than 10 patients in the Registry or TLC because limited participant numbers might skew the data. There are significant disparities in the rates of alloimmunization at the different centres as well as rates of splenectomy. These differences were not attributable to practices such as leucofiltration, red cell washing, irradiation, or antigen matching. We presume that other factors that were not included in our data collection contribute to this variation because, even after controlling for splenectomy rates, the site effect on allosensitization remained strong. Factors that might contribute to site variation not addressed in this study include other changes of practice over time, changes in quality/ storage conditions of blood products or human immunodeficiency virus/Hepatitis C status of

older patients. Transfusion duration greater than 10 years was a significant risk factor for alloimmunization.

Interestingly, nearly two thirds (65%) of the RBC-specific alloantibodies identified in our chronically transfused thalassaemia cohort were the same spectrum described in patients with sickle cell disease (Vichinsky, *et al* 1990, Vichinsky, *et al* 2001). This raises the possibility that applying a similar strategy of extended red cell antigen typing which has been widely adopted as clinical standard of care in sickle cell disease might also reduce the frequency of alloimmunization in thalassaemia even in the absence of an apparent ethnic disparity. Currently, some but not all of the thalassaemia treatment centres participating in the TCRN report that they routinely performed extended phenotypic antigen matching of RBCs. Racial or ethnic identification of blood donors was not readily available, thus the contribution of donor/recipient ethnic disparity as a risk factor for alloimmunization could not be assessed. While efforts to support transfused sickle cell patients with RBCs exclusively from well-matched African American donors may have merit (Price, *et al* 2009), it is not clear that this strategy is feasible or necessary in thalassaemia.

Over two thirds (79/116) of alloimmunized participants had had their spleens removed. The apparent timing of antibody detection following splenectomy raises the possibility that removal of the spleen may predispose chronically transfused individuals to RBC sensitization. The recent trend toward reduced utility of splenectomy in thalassaemia may mitigate the alloimmunization rate as well as other complications in the future. In general, the total blood exposure for intermittently transfused intermedia would be expected to be lower than in chronically transfused beta thalassaemia major, however cumulative transfusion volume was not collected as part of the TLC or Registry. The analysis of our data did not detect a difference in alloimmunization rates between thalassaemia major and any of the other thalassaemia conditions including thalassaemia intermedia. This may be due to the relatively small numbers of patients with these other diagnoses enrolled.

In summary, we report on the largest cohort to date of transfused thalassaemia patients from diverse ethnic and racial backgrounds across two continents and find that alloimmunization in this population is a persistent problem that exceeds rates demonstrated in other groups. Splenectomy and duration of transfusion were significant risk factors for the development of alloantibodies. While the practice of splenectomy is less common at most sites over time, the alloimmunization rate, if anything, is actually higher in younger splenectomized patients. This observation cannot be adequately explained by other data collected as part of the Thalassaemia Longitudinal Cohort study but deserves further investigation.

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Appendix I

The following institutions and researchers contributed to the Thalassemia Clinical Research Network Registry and Thalassemia Longitudinal Cohort data reported in this paper.

Children's Hospital, Boston: Ellis Neufeld, MD, PhD, Principal Investigator, Jennifer Braunstein, NP, Research Nurse, Amber Smith, Study Coordinator, Latoya Lashley, Study Coordinator; Satellite: University of Texas Southwestern Medical Center at Dallas: Charles Quinn, MD, MS, Principal Investigator, Deborah Boger, RN, MSN, PNP, Study Coordinator, Leah Adix, Study Coordinator, Sandra Richardson, Study Coordinator; Children's Healthcare of Atlanta: Jeanne Boudreaux, MD, Principal Investigator, Leann Hassen, Study Coordinator; Baylor College of Medicine: Brigitta Mueller, MD, Principal Investigator, Bogden Dino, Study Coordinator; Weill Medical College of Cornell University: Patricia Giardina, MD, Principal Investigator, Elizabeth Evans, Study Coordinator; Satellite: Columbia University; Nassau; Schneider; New York Methodist, Long Island; Hackensack; Winthrop University Hospital: Mark Weinblatt, MD, Principal Investigator, Linda Skelly, Study Coordinator. The Children's Hospital of Philadelphia: Janet Kwiatkowski, MD, Principal Investigator, Marie Martin, RN, Research Nurse, Owen Beams and Sage Green, Study Coordinators; Satellite: St. Christopher; Children's Memorial Hospital, Chicago, IL: Alexis Thompson, MD, Principal Investigator, Janice Beatty, RN, Research Nurse, Tiffany Drinkwater, Study Coordinator; Children's National Medical Center; Children's Hospital at Oakland: Elliott Vichinsky, MD, Principal Investigator, Dru Foote, NP, Research Nurse, Nancy Sweeters, Study Coordinator, Olivia Vega, Study Coordinator; Satellites: Central Valley Children's Hospital; Children's Hospital of Los Angeles, Thomas Coates, MD, Principal Investigator, Susan Carson, RN, Research Nurse, Eun Ha Pang, Study Coordinator, Rachna Khanna, Study Coordinator; UC San Francisco; Stanford Hospital, Michael Jeng, MD, Principal Investigator, Kokil Bakshi, Clinical Research Associate; Children's Hospital Orange County; Children's and Women's Health Center of British Columbia, John Wu, Principal Investigator, Heather McCartney, RN, Research Nurse, Colleen Fitzgerald, Study Coordinator, Stephanie Badour, Study Coordinator. Toronto General Hospital, Toronto, Ontario, Canada: Nancy F. Olivieri, MD, Principal Investigator, Vivek Thayalasuthan, Study Coordinator; Satellite: Hospital for Sick Children, Isaac Odame, MD, Principal Investigator, Manuela Merelles-Pulcini, RN, Study Coordinator. University College London, John Porter, MD, Principal Investigator, Cindy Bhagwandin, Study Coordinator; Satellite: Whittington Hospital, Farrukh Shah, MD, Principal Investigator. NHLBI oversight, Kathryn Hassell, MD. Data Coordinating Center: New England Research Institutes, Sonja McKinlay, PhD, Principal Investigator, Lisa Virzi, RN, MS, MBA, Project Director, Felicia Trachtenberg, PhD, Senior Statistician

Appendix II

Sample questions from the Registry and Thalassaemia Longitudinal Cohort

Complication	Registry Questions/specifics
<i>Transfusion</i>	<p>Has the patient ever received regular transfusions (at least 8 times per year)?</p> <p>Age started regular transfusions: (years) ?</p> <p>Is the patient currently receiving regular transfusions?</p> <p>Age stopped regular transfusions: (years)</p> <p>Date stopped regular transfusions: (MM/YYYY)</p>

Complication	Registry Questions/specifics
	Does the patient have autoantibodies? Does the patient have alloantibodies? (Yes, No, Cannot be determined) Has the patient been splenectomized? Age at splenectomy: (years) or Date of splenectomy: (MM/DD/YYYY)?
Thalassemia Longitudinal Cohort	Questions
<i>T 202 Transfusion Form</i>	Has the participant ever received 8 or more transfusions per year as part of a regular transfusions regimen? Age started 8+ regular transfusions per year: (years) Is the participant currently receiving 8 or more transfusions per year as part of a regular transfusion regimen?
<i>T201 Medical History Form</i>	Has the participant had a splenectomy? At what age was the splenectomy performed: (years)
<i>T225 Antibody Form</i>	Has the participant ever had alloantibodies? Does the participant currently have alloantibodies? List the antibody and the date that it developed Did the participant ever have autoantibodies? Does the participant have autoantibodies now? If the antibody resolved, for how long were the antibodies present?

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Figure 1.

Relationship of Transfusion Initiation Era with Splenectomy and Alloimmunization

Alloimmunization rates of subjects who initiated transfusions prior to or after 1990 with spleen removed (grey) or spleen intact (white). The numbers in each bar correspond to total number of transfused subjects in each category. The p-values are derived from comparison of alloimmunization rates for splenectomized and nonsplenectomized patients within each era.

Clinical and Laboratory Characteristics of Transfused Thalassemia Patients in the Thalassemia Clinical Research Network

Table 1

	N	P-value		
		Allo	Both	Auto
Overall	697	16.5%	4.9%	3.2%
Thalassemia Diagnosis				
Beta	582	16.5%	5.0%	3.1%
Alpha	27	14.8%	3.7%	3.7%
E-beta	84	16.7%	4.8%	3.6%
Race				
Asian	335	13.3%	4.2%	3.0%
Caucasian	328	21.2%	5.8%	3.4%
Other race	28	7.1%	3.6%	3.6%
Gender				
Female	364	17.5%	5.3%	3.1%
Male	333	15.8%	4.6%	3.3%
Age at start of regular transfusions				
< 1 year	224	13.0%	5.8%	3.6%
1 to 3 years	245	18.9%	4.9%	3.7%
3 to 5 years	73	19.2%	5.5%	1.4%
5 years	129	15.9%	2.4%	2.4%
Year starting regular transfusions				
pre-1990	346	21.1%	5.6%	3.5%
post-1990	325	11.4%	4.0%	2.8%
Duration of regular transfusions				
0-10 years	208	9.7%	4.4%	2.9%
10-20 years	198	12.1%	4.6%	2.5%
20-30 years	145	24.7%	6.3%	4.2%
30-40 years	89	25.3%	4.6%	3.5%
40-50 years	20	30.0%	5.0%	5.0%
Hepatitis C antibody				
positive	169	25.8%	8.4%	5.4%
Splenectomized				
Yes	343	23.0%	7.1%	4.7%
No	352	10.6%	2.9%	1.7%

Table II

Multivariate Analysis of Risk Factors associated with Alloimmunization

		Adjusted Odds Ratio	Lower 95% CI	Upper 95% CI
Race	Other vs Asian	0.478	0.107	2.135
	Caucasian vs Asian	1.259	0.788	2.013
Splenectomy	Yes vs No	1.851	1.120	3.059
Year starting regular transfusions	pre-1990 vs post-1990	0.559	0.230	1.355
Duration of regular transfusions	10 years vs 0 years	1.437	0.721	2.863
	20 years vs 0 years	3.897	1.348	11.266
	30 years vs 0 years	3.426	1.117	10.507
	40 years vs 0 years	4.071	1.008	16.444

The numbers in boldtype refers to statistically significant risk factors. CI, confidence interval

Table III

Relationship of splenectomy rates to allo- and auto-immunization among thalassaemia treatment centres with >10 enrollees

Site	N	Allo (%) ^o	Auto (%) ^o	Splenectomy (%)
A	46	13.0	0	54.4 [*]
B	12	16.7	16.7	25
C	16	12.5	0	25
D	87	10.3	1.2	77.0 [*]
E	17	5.9	0	52.9 [*]
F	61	37.7	18	65.6 [*]
G	51	19.6	3.9	63.5 [*]
H	82	18.3	9.8	53.7 [*]
I	20	0	10	25
J	27	3.7	3.7	14.8
K	11	0	0	45.5
L	159	19.5	4.4	28.9
M	14	14.3	0	46.7
N	20	30	0	80 [*]

^o Significant differences among sites using chi-square analysis for alloantibodies (p=0.001), autoantibodies (p=0.0002) or both (p<0.001).

^{*} Correlation of alloimmunization rates among sites with splenectomy rates >50%, r= 0.51

Table IV**RBC Antigen Specificity of Alloantibodies Detected**

RBC Alloantibodies	Frequency	%
Anti-E	22	19.0
Anti-Kell	21	18.1
Anti-C	11	9.5
Anti-Kidd (Jka, Jkb)	9	7.8
Anti-HLA	8	6.9
Anti-c	7	6.0
Anti-e	6	5.2
Anti-Kpa	6	5.2
Anti-Lewis	4	3.4
Anti-D	4	3.4
Anti-S	3	2.6
Anti-V	2	1.7
Anti-Duffy (Fya, Fyb)	2	1.7
Anti-M	2	1.7
Other *	9	

* Other includes 1 of each of the following: IgG unspecified, HTLA, F, G, LuA, Bga, Bgb, Sda, Cw.