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Alloimmunization and Age of Death among Deceased Sickle Cell Disease Patients

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Alloimmunization and Age of Death among Deceased Sickle Cell Disease Patients

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Bachelor of Science

University of Utah

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Abstract

Alloimmunization and Age of Death among Deceased Sickle Cell Disease Patients

By Kyron L Nielsen

Transfusion therapy remains a primary treatment for Sickle Cell Disease (SCD). Alloimmunization frequently occurs as a result of the incompatibility of donor and recipient blood types. The association between alloimmunization and morbidity and mortality is not completely understood. Some studies have suggested an association between alloimmunization and increased morbidities as well as decreased life expectancy, though research in this area is lacking. We investigated the association between alloimmunization and the age at death among a cohort of 136 patients who died from SCD in Georgia from 2004-2008. Patients were identified from a previous study and were linked to existing medical records in several hospital systems. Variables of interest included birth date, death date, alloimmunization status, antibody profile, phenotype, number of transfused units, type of hemoglobinopathy, cause of death, ABO/Rh blood type, and date of last antibody screen. A red blood cell index was calculated to estimate the number of units needed to crossmatch in order to find a compatible unit based on the patient's phenotype, antibody profile, and the average donor pool. The mean age at death of those who were alloimmunized was 38.5 years, compared to 39.5 years for those who were not alloimmunized ($p=0.72$). The mean age at death was 40.3 years for those with a low (<20) number of transfusions, compared to 33.4 years for those with a high (20 or more) number of transfusions ($p=0.05$). Neither the total number of antibodies, nor the presence of specific antibodies affected the age at death among alloimmunized patients. Multivariate analysis showed that decreased age at death was not associated with the red blood cell index, total number of antibodies, or number of transfusions. These findings differ from some earlier studies due to a number of limitations in study design. To further assess the association between alloimmunization and survival, a prospective cohort study is needed.

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Table of Contents

Introduction

Background

Sickle cell disease (SCD) is a genetically acquired defect in the beta-chain of hemoglobin (Hgb). The alteration of the Hgb beta-chain occurs when glutamic acid is substituted with valine at codon 6 (1). The defective hemoglobin protein is known as hemoglobin S (HgbS), as opposed to normal hemoglobin A (HgbA) (2). When HgbS is in a low oxygenation state the red blood cells (RBCs) appear narrow and elongated, classically presenting in the shape of a crescent or "sickle" (3). Unlike normal RBCs, which are elastic and can easily change shape to pass through small blood vessels, sickled RBCs are rigid and may block the vessels and bind to the endothelium, thereby compromising proper blood flow, decreasing pH, activating coagulation, and promoting vasoconstriction (3, 4), which may lead to vascular complications and organ damage (2, 5). Deformed cells are destroyed by hemolysis at an increased rate; this results in chronic anemia (6).

The term SCD refers to several genotypes that are distinguished by their hemoglobin structures and the presence of other blood disorders. When a person inherits both HgbS alleles this leads to a homozygous HgbS/S variant (3), which manifests as classic sickle cell anemia (SCA) (6). It is the most common and most severe form (6, 7). Sickle cell trait (8) is the term given to variants that have one HgbS gene and one non-HgbS gene, such as in HgbS/A or HgbS/C. The most common heterozygous variant is HgbS/C (6), a moderately severe form of SCD (6). Other blood disorders may also combine with SCD to influence disease severity (2, 3, 6). The most common of those is β-thalassemia, a genetic disorder that affects the production of the beta-chain of hemoglobin. Whereas SCD affects the quality of the beta-chain, β-thalassemia affects the quantity of beta-chains produced (9). A defective hemoglobin S gene may be combined with a very low production beta-chain gene, as in HgbS/β^0, or with only moderately reduced beta chain production, as in HgbS/β^+ (6).

Symptoms and Complications

The hallmark symptoms of SCD are vaso-occlusive crises (VOC) or "pain crises" (10-12). These crises are characterized by general pain, especially in the extremities, but they often do not have other physical manifestations (6, 11). One of the most common complications is acute chest syndrome (ACS), a type of acute lung injury characterized by a new pulmonary infiltrate (13). Infection, or sepsis, is another common complication (12) that is often the result of damage to the spleen, reducing its ability to remove bacterial organisms from the body (11). Damage to the spleen may also increase RBC sequestration leading to a dangerous drop in Hgb level (11). Renal complications can arise when cells sickle within the kidney, causing blockage and tissue destruction (6). Other complications include coronary heart disease, pulmonary hypertension, and stroke (6, 12, 14).

Prevalence

In the United States, sickle cell disease (SCD) affects nearly 100,000 individuals (15), of which about 97% are African American (16). The estimated prevalence of SCA in African Americans ranges from 1 in 365 (5, 16) to 1 in 600 (11, 15), but prevalence of sickle cell trait (8) in this population may be as high as 1 in 13 (17). By comparison, the prevalence of SCT among Caucasians is only about 1 in 333 (18). There is considerable variation in SCD occurrence across the U.S., with particularly high prevalence reported in the Southern states (15). Some of this variation can be attributed to the higher proportion of African Americans living in the south; however elevation differences may also contribute to regional variations in prevalence, as high elevation can lead to VOC (19).

Treatment

Treatment of sickle cell disease has improved in recent years, leading to decreased death rates among certain age groups. The rates of pain episodes and hospitalizations have also been decreasing (16). Despite these improvements, some have estimated SCD life expectancy to be about 30 years less than that of the general population (15, 16). It has also been suggested that poor transition from childhood care to adult care may increase risk for young adults (20).

Transfusion therapy remains a primary treatment for SCD (21, 22), but hydroxyurea is also frequently used and has been shown to be highly effective (16, 23). Known complications of transfusion therapy include iron overload (24) and alloimmunization (25). Alloimmunization occurs when the recipient's immune system responds to foreign antigens on the donor RBCs (26). Patients may also respond by creating autoantibodies against their own RBCs, a phenomenon known as autoimmunization. This may occur as a result of alloimmunization because the patient's immune system is essentially hypersensitized (25). Autoantibodies are less common in SCD than alloantibodies, though both can complicate the course of treatment (27, 28). Rates of alloimmunization range from 7% to 47%, depending on recipient's age, blood type, and number of previous transfusions (29, 30). To minimize the risk of alloimmunization, both donor and recipient blood samples are screened and matched for compatibility (31). Rh and Kell antigens are of special concern because they are most often associated with alloimmunization (11, 22, 29). Alloimmunization events are more likely when African American SCD patients receive blood from Caucasian blood donors because of known racial differences in blood antigens (11, 32). However, some studies have suggested that even race "compatible" blood transfusions may induce an immune response due to other differences, especially those involving the Rh group mismatch (29). Each successive unit of blood transfused to a patient increases the chance of having an alloimmunization

event. The totality of those transfusions is sometimes called the "transfusion burden" (22). A high transfusion burden can increase the probability of alloimmunization (33).

Effects of Alloimmunization

The mechanistic link between alloimmunization and health status of SCD patients is not completely understood. While it is believed that alloimmunization can directly affect SCD morbidity and mortality, it is also possible that both alloimmunization and SCD severity are linked via common underlying factors. Some have theorized that alloimmunization among SCD patients results from an overall heightened immune response characterized by inflammation, increased white blood cells, and high levels of C-reactive protein (34). Others have suggested that there is an increased risk of alloimmunization based on the patient's human leukocyte antigen (HLA) alleles (35). It is still unknown exactly how these mechanisms may independently affect morbidity and mortality, though inflammation appears to play an important role (36). Only a few studies have examined the association between alloimmunization and SCD mortality. One recent study found that alloimmunization was associated with organ failure and opioid use, but not with vaso-occlusive episodes and number of previous transfusions (37). The study also reported that life expectancy of alloimmunized SCD patients was ten years lower than that of non-alloimmunized patients (37). Alloimmunization is also likely to be associated with difficulty finding compatible blood units, due to the donor-recipient matching issues and an overall shortage of blood products. The resulting delay in transfusion could also have negative impacts on patient care. Anecdotally, some physicians may even hesitate to order a transfusion, even when medically indicated, in fear that the patient will develop a delayed hemolytic transfusion reaction (DHTR). In this study we will further investigate the association between alloimmunization and mortality using a cohort of deceased sickle cell

patients. Based on the findings of previous studies, we expect that alloimmunized patients will have died younger, on average, than non-alloimmunized patients.

Methods

Study design and population

This observational study focuses on people who died from SCD in the state of Georgia between January 1, 2004 and December 31, 2008. IRB approval was waived by Emory University, Children's Healthcare of Atlanta (CHOA), and Grady Memorial Hospital (GMH), as the study was determined to be a public health surveillance effort. This study used data from a previous study by Paulukonis and colleagues (38). In that earlier study, participants were selected using a process that combines state birth and death information with insurance claims, hospital inpatient and emergency department data, and data from treatment centers that participate in the Registry and Surveillance System for Hemoglobinopathies (RuSH) (39). To be eligible, participants had to have a diagnosis of SCD confirmed by laboratory testing or have SCD listed as a cause of death on their death certificate. There were 288 SCD deaths recorded in Georgia during the 2004-2008 study period. For the purpose of the current analyses we included only those subjects who received blood products from the American Red Cross in Georgia or were treated at Augusta University Medical Center (AUMC), CHOA, or GMH at any point, and whose records still remained in the electronic database or as a paper file (n=155). Of those, 19(12%) subjects had causes of death that were determined to be unrelated to SCD, such as Motor Vehicle Accident, Gun Shot Wound, HIV/AIDS, Hepatitis, etc., and were removed from the study. The remaining 136(88%) subjects with SCD-related causes of death were included in the sample.

Data collection

Previously identified patient information included date of birth, date of death, type of hemoglobinopathy (HgbS/S or HgbS/β^-thalassemia, HgbS/C, HgbS/β^+thalassemia, or genotype unknown), county of death, causes of death (primary, secondary, underlying, and other), and

healthcare system(s) the subject visited (GHS, CHOA, or AUMC) (38). Age at death was calculated by subtracting date of death from date of birth. We linked subjects to their medical, transfusion, and laboratory records at each site using name and date of birth as unique identifiers. From those records we retrieved relevant study information, including alloimmunization status, antibody profile, phenotype, number of transfused units, ABO/Rh type, and date of last antibody screen. Number of antibodies was calculated as the sum of all antibodies identified for each subject. Type of hemoglobinopathy was collapsed into three categories: HgbS/S or HgbS/β^{-}thalassemia, other genotype, or genotype unknown. Healthcare system was expanded to include an "other" category for subjects that had received care at other hospitals and who had blood work done at the American Red Cross (ARC), from whom we retrieved this information. Subjects were considered to be adults if they were at least 21 years old at death. We were able to determine ABO/Rh for 86(63%) subjects, but the phenotype was only available for about 50% of all subjects. Alloimmunization profiles were found for all but 32(21%) subjects. For those who had no alloimmunization profile, they were assumed to be negative for alloantibodies. An RBC compatibility index was calculated to estimate the number of units needed to crossmatch in order to find a compatible unit based on the patient's phenotype, antibody profile, and the average donor pool. The RBC index was dichotomized at the median (high 7+/low <7) for analysis due to extreme skewness. Transfusion burden was calculated by dichotomizing the number of transfused units as high or low using at least 20 units as the cutoff. If subjects had no history of transfusion then their transfusion burden was estimated to be zero. Death records were reviewed to determine if sepsis was a contributing cause of death, and were recorded as sepsis-related death (yes/no). Study data were collected and managed using REDCap electronic data capture tools hosted at Emory University (40).

Analysis

Bivariate analyses compared patients with and without evidence of alloimmunization using two-sample *t*-tests for continuous variables and chi-square tests for categorical variables. Fisher's exact test was used for categorical variables with group counts <5.

The distributions of age at death were examined in relation to sex, number of transfusions (low vs. high), sepsis related death, total number of antibodies (low vs. high), RBC index (low vs. high), and presence of selected antibodies (E, C, Kell, S, Fy(a), and warm autoantibody). Multivariate logistic regression model was used to further examine the association between age at death (dichotomized at median age of 40.6 years) as the outcome and total number of antibodies, number of transfusions, and RBC index (low vs. high) as predictors. The results of logistic regression analyses were expressed as adjusted odds ratios (OR) and the corresponding 95% confidence intervals (CIs). Statistical significance was set at two-sided α-error level of 0.05. All analyses were performed using SAS 9.2 (SAS Institute, Inc., Cary, NC).

Results

We were able to obtain information for 136 out of 288 potentially eligible subjects (47%) from the Georgia study site (Figure 1). Death from causes not related to SCD accounted for 19 ineligible participants. All participants whose records were stored by the American Red Cross were eligible.

Table 1 presents results of the analyses comparing different patient characteristics by their alloimmunization status. The mean age at death of those who were alloimmunized was 38.5 years, compared to 39.5 years for those who were not alloimmunized (p=0.72). The mean numbers of RBC units received were also similar in the two groups (20.8 units vs. 22.1 units; p=0.91). The proportion of alloimmunized patients was lower among those with HgbS/S or HgbS/β^-thalassemia than in patients with other types of hemoglobinopathy (25% vs. 40%), although the difference was not statistically significant (p=0.08). The proportion of alloimmunized subjects did not differ by sex, sepsis-related death, or healthcare system.

When the study group was stratified by adult/pediatric status (using 21 years as the cutoff), the difference in mean age at death between alloimmunized and non-alloimmunized patients was more pronounced in the adult age group (40.3 vs. 43.9 years), but not statistically significant (p=0.13). The corresponding difference was absent in the pediatric age group.

As shown in Table 2, the mean age at death was 40.3 years for those with a low (<20) number of transfusions, compared to 33.4 years for those with a high (20 or more) number of transfusions (p=0.05). Mean age at death for those with and without an Anti-E antibody was 38.1 and 44.2 years, respectively (p=0.08). Age at death distributions did not differ by sex, sepsis related death, total number of antibodies (low vs. high), RBC index (low vs. high), or presence of select antibodies.

Multivariate logistic regression analyses are presented in Table 3. Decreased (below median) age of death was not associated with the total number of antibodies, number of transfusions, or RBC index (dichotomized as high/low). The previously noted association between age of death and the number of transfusions (dichotomized as <20 vs. 20+) was not evident when the number of transfusions was used as a continuous variable (OR=1.01; 95% CI: 0.99-1.03)

Discussion

One previous study has suggested an association between alloimmunization and decreased life expectancy (37). In that study, 319 adults (18 years or older) from two health centers in North Carolina were selected for chart review and their data was compiled with a larger study that calculated survival curves over a 9.3 year average follow-up period (41). While this study only included adults, our study included all ages, which could help explain why our results differed. One such explanation is that the likelihood of alloimmunization increases with increasing number of transfusions, suggesting that younger people might have lower rates of alloimmunization, which would drive down the mean age at death in the non-alloimmunized group. This is further complicated when considering those who died from non-SCD causes, which was the case with 19 subjects initially identified for this study. Those 19 subjects died an average of 9 years younger than those with SCD-related causes of death ($p=0.01$), but were not included in our final sample.

One major limitation of this study is misclassification bias. Most notably, among 53 subjects for whom data were available, there was a median of 300 days between the most recent antibody screen and death. It is possible that some subjects developed an alloantibody since the last recorded antibody screen (at another healthcare facility, for instance), and were misclassified as non-alloimmunized. On the other hand, many SCD patients produce alloantibodies earlier in life (26), and for this reason it is unlikely that a large proportion of our study subjects became alloimmunized in their late 20s or later. Further, there were 32 subjects who had no antibody profile available, and thus their alloimmunization status is unknown. We categorized these individuals as non-alloimmunized under the assumption that the records for alloimmunized individuals would have been preserved and available based on historical practices of transferring only alloantibody positive blood bank records following the implementation of new software systems at several of the participating hospitals. Another significant issue is that the number of transfusions was an under-

representation of the true number of transfused units, which was almost certainly much higher (10), because those records were rarely preserved. Consequently, we are unable to accurately determine a high or low transfusion burden. Further misclassification may have occurred when assigning the type of hemoglobinopathy. Due to limited sample size, those without documented HgbS/S or HgbS/β^-thalassemia were included in the "other and unknown" category. It is likely that most of the "unknowns" actually had the HgbS/S or HgbS/β^-thalassemia genotype.

The above limitations are the result of sparse data and limited resources in the various hospital systems. This is not unexpected given that many of the records were more than ten years old. During the transition to electronic records, many paper charts were destroyed and were not transferred to the electronic system. This made it very difficult to gather historical data relating to blood banking or transfusion medicine.

Further, the proportion of alloimmunized subjects in our study was 32%, which is higher than many estimates of alloimmunization prevalence (26, 42), but not as high as some (30). This is because our study hospitals retained the records of unusual cases, such as alloimmunized patients, but typically disposed of other records.

In summary, the limitations of the present analysis preclude us from drawing conclusions about the effect of alloimmunization on survival of SCD patients. To properly assess this association, a prospective cohort study is needed. Such a study would include participants who are screened for SCD at birth and followed through successive transfusions, alloimmunization events, and complications to eventual death. Time delays in receiving blood units as a result of previous alloimmunization could be measured along with any associated complications. This study design would allow for the calculation of survival curves, and allow for consideration of possible confounders. Further, it would help identify factors that contribute to alloimmunization and common morbidities, and the subsequent effects on quality of care. It could also be used to further

investigate underlying genetic abnormalities, the contributions of HLA alleles, and the role of inflammation in SCD. Further research is also needed to determine the role of delayed blood transfusions, especially due to difficulties in finding compatible blood units, the role of immune system factors, such as C-reactive protein and inflammation, and the impact of genetic factors and antigenic differences.

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Tables and Figures

Table 1. Descriptive characteristics of deceased SCD patients, Georgia 2004-2008

Characteristics[a]	Alloimmunized (n=43)	non-Alloimmunized (n=93)	p-value[b]
Age at Death, years	38.5 (13.5)	39.5 (16.1)	0.72
Adults (>=21)[c]	40.3 (12.1)	43.9 (12.3)	0.13
Children (<21)[d]	14.4 (3.9)	12.5 (7.7)	0.68
Number of Transfusions	20.8 (66.7)	22.1 (61.6)	0.91
Sex			0.58
Male	20 (29)	48 (71)	
Female	23 (34)	45 (66)	
Type of Hemoglobinopathy			0.06
Hgb S/S or Hgb S/β- thalassemia	19 (25)	57 (75)	
Other or Unknown Genotype	24 (40)	36 (60)	
Sepsis Related Death			0.33
Yes	11 (39)	17 (61)	
No	32 (30)	76 (70)	
Healthcare System			0.47
Grady Healthcare System	19 (28)	50 (72)	
Children's Healthcare of Atlanta	4 (29)	10 (71)	
AUMC or Other	20 (38)	33 (62)	

a. Descriptive characteristics are shown as mean(sd) for continuous variables and N(%) for categorical variables

b. P-values were obtained from chi-square tests for categorical variables and t-tests for continuous variables

c. Alloimmunized n=40, non-alloimmunized n=80

d. Alloimmunized n=3, non-alloimmunized n=13

Table 2. T-tests for the Mean Age at Death across bivariate demographic variables in 136 deceased SCD patients.

Variable		N	Mean Age at Death	Standard Deviation	p-value
Sex	Male	68	40.7	14.6	0.26
	Female	68	37.7	15.9	
Number of Transfusions	Low (<20)	114	40.3	15.2	0.05
	High (>=20)	22	33.4	14.2	
Sepsis Related Death	No	108	39.3	15.0	0.91
	Yes	28	38.9	16.6	
Total Number of Antibodies	Low (<3)	21	36.0	11.4	0.23
	High (>=3)	22	41.0	15.1	
RBC Index	Low (<7)	21	36.6	13.4	0.55
	High (>=7)	22	39.1	13.1	
Anti-E	No	112	38.1	15.7	0.08
	Yes	24	44.2	12.2	
Anti-C	No	122	39.1	15.3	0.75
	Yes	14	40.4	15.2	
Anti-Kell	No	122	39.3	15.5	0.80
	Yes	14	38.3	13.3	
Anti-S	No	126	39.4	15.4	0.70
	Yes	10	37.4	14.0	
Anti-Fy(a)	No	126	38.8	15.6	0.27
	Yes	10	44.3	8.6	
Warm Autoantibody	No	125	39.3	15.3	0.76
	Yes	11	37.8	15.2	

Table 3. Logistic Regression comparing above to below median age at death by relevant indicators.

Variable	Beta	OR	95% C.I.	p-value
Total Number of Antibodies	-0.03	0.97	(0.66, 1.43)	0.88
Number of Transfusions	0.01	1.01	(0.99, 1.03)	0.42
RBC Index (high/low)	0.00	1.00	(0.21, 4.70)	1.00

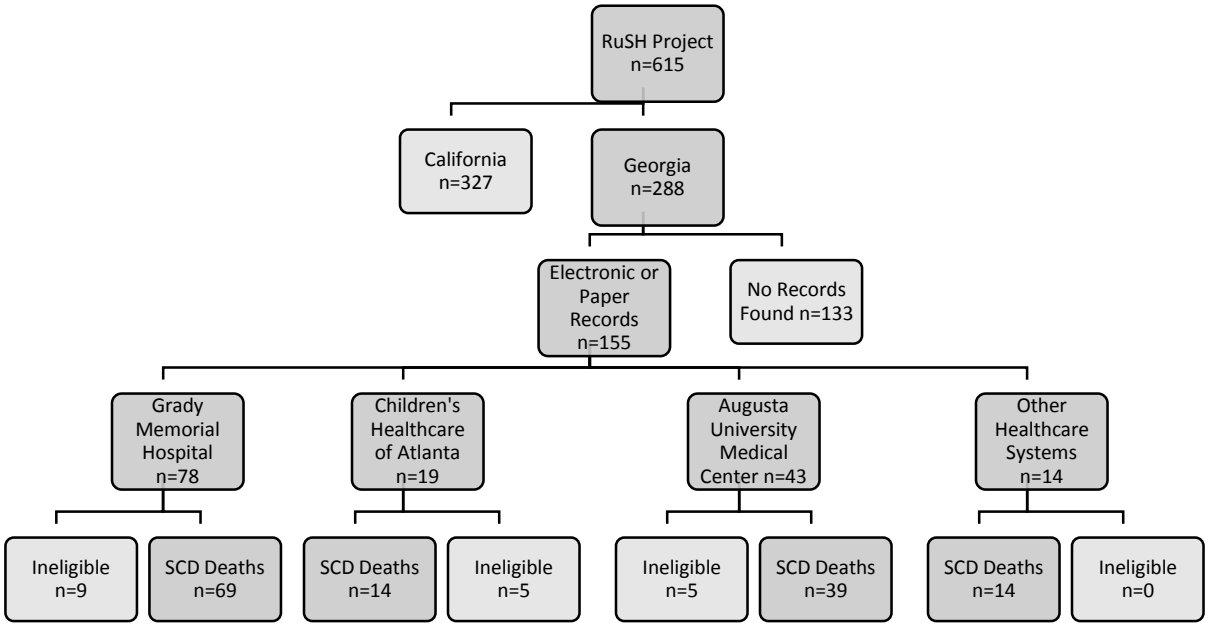

Figure 1. Participants were selected from the RuSH project based on the availability of their records and the healthcare system where they received care. The final sample included 136 subjects from three healthcare systems or who received blood units from the American Red Cross.