Supporting Statement B for

Characterization of risk of HIV and HIV outcomes in the Brazilian Sickle Cell Disease (SCD) population and comparison of SCD outcomes between HIV sero-positive and negative SCD patients

March 2016

Sponsored by:

The National Heart, Lung, and Blood Institute
Blood Epidemiology and Clinical Therapeutics Branch
National Institutes of Health

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B.1. Respondent Universe and Sampling Methods

This project is part of the Recipient Epidemiology and Donor Evaluation Study (REDS)-III research program. REDS-III is a National Heart, Lung, and Blood Institute funded multicenter research program that is conducted in the United States, Brazil, China, and South Africa. The objectives of REDS-III are to ensure safe and effective blood banking and transfusion medicine practices through a comprehensive strategy involving basic, translational and clinical research. One ongoing research project within the REDS-III Brazil program is a cohort study of Sickle Cell Disease (SCD) patients (Establishing a Brazilian SCD Cohort and Identifying Molecular Determinants of Response to Transfusions, and Genetic Determinants of Alloimmunization, clinical exemption number 2013-03-001). The goals of the cohort study are to characterize SCD outcomes, blood utilization, and genetic determinants of red blood cell alloimmunization in the Brazilian SCD population and perform targeted studies focused on transfusion and HIV outcomes within the cohort. SCD patients receive outpatient care and transfusion therapy at sites called Hemocenters within Brazil. It is estimated there are approximately 30,000 SCD patients treated at the approximately 31 Brazilian Hemocenters. There are 5 Hemocenters participating in the REDS-III research in the cities of Belo Horizonte, Rio de Janeiro, Montes Claros, Juiz de Fora, and Recife that collectively treat approximately 10,000 SCD patients. The REDS-III infrastructure will be utilized to identify eligible patient populations for the proposed research. The objectives of this proposed research are listed below.

Objective 1: case-control study to compare HIV risk behaviors between SCD and non-SCD subjects in Brazil
Objective 2a: case series description of HIV outcomes in Brazilian SCD patients over the past 10 years
Objective 2b: case-control study to compare SCD outcomes between HIV+ and HIV- SCD patients in Brazil

For the primary objective, which is to compare HIV risk behaviors in SCD and non-SCD subjects in Brazil, a case control study of 150 SCD patients and 150 non-SCD controls will be performed. Cases will be selected from the REDS-III Brazilian SCD Cohort. There were 1241 SCD patients $\geq$18 years enrolled in the cohort. SCD cohort patients were randomly selected from the REDS-III Hemocenters' population; we therefore expect the cohort population to be representative of the overall SCD population at each Hemocenter within Brazil. Controls will be recruited from the SCD patient advocacy associations at each Hemocenter. These associations host friends and family of SCD patients during appointments and coordinate outreach efforts in SCD patients' communities. Therefore, we anticipate that the controls recruited though the advocacy associations will be from a culturally and socioeconomically similar population as cases. Controls will be matched by region (Hemocenter) and age (within 5 years of cases). All cases and controls will have only one study visit. At this visit, an assessment will be administered to cases and controls to measure HIV risk behaviors using a self-administered audio computer-assisted self-interview (ACASI). A blood sample will also be collected to confirm HIV status for cases and controls and to test for sickle cell disease/trait in controls.

For the secondary objective (Objective 2a), a case series description of HIV outcomes in Brazilian SCD patients, all SCD patients diagnosed with HIV as part of routine care at participating Brazilian Hemocenters, in the previous 10 years will be identified by reviewing Hemocenter records.

SCD patient are screened approximately annually for HIV and a register of all HIV+ SCD patients is maintained at each Hemocenter. All HIV+ SCD patients who are alive and treated by the Brazilian Hemocenters will be invited to participate in the study. Eligible subjects will have the option to consent to a medical record review and risk factor interview or only a medical record review. Key data related to HIV and SCD outcomes will be collected from the subject's medical record. Approval will also be sought to perform a medical record review on all HIV+ SCD patients lost to follow up or deceased at time of study start to limit survival bias in the analysis. Finally, to compare SCD outcomes between HIV+ and HIV- SCD patients in Brazil (Objective 2b), we will randomly select 2 HIV negative controls matched by age (± 2 years), gender, Hemocenter and SCD genotype from the REDS-III cohort for each one identified HIV+ SCD patient. Cohort patients have already consented to inclusion of their clinical/transfusion data in the REDS-III database for use in REDS-III studies, therefore the HIV negative controls selected from the database will not require re-contact or new informed consent. Key SCD outcomes will be compared between HIV+ SCD patients enrolled into Objective 2 of this study and the HIV- matched controls selected from the REDS-III cohort.

Table 1: Recruitment Populations

Study Population	Recruitment Population	Estimated Size of Recruitment Population	Number to Enroll
Objective 1: HIV risk compared between SCD and non-SCD			
Case (SCD)	REDS-III Cohort	1500	150
Control (non-SCD)	SCD Advocacy Groups	3000	150
Objective 2. Case series description of HIV+ SCD subjects			
HIV + SCD	HIV+ SCD treated at Hemocenters	25-35	All eligible will be recruited, estimate 25
Objective 3. SCD outcomes compared between HIV+ and HIV- SCD			
Case (HIV+)	Objective 2 Cases	25-35	All eligible will be recruited, estimate 25
Control (HIV-)	REDS-III Cohort	1500	2 controls for each identified case

Inclusion & Exclusion Criteria Objective One (Characterize risk of HIV in SCD):

SCD Cases

- SCD (any genotype) patients enrolled into the REDS-III Brazilian SCD Cohort
- Age ≥18 years

Non-SCD Controls

- Age≥ 18 years with the number of controls matching the number of cases in each 5-year age stratum
- Frequency matched by Hemocenter
- No sickle cell disease by self-report or by confirmatory testing

Inclusion & Exclusion Criteria Objective Two (Characterize HIV Outcomes in SCD and Compare SCD Outcomes Between HIV+ and HIV- SCD):

HIV sero-positive Cases (Included in Objectives 2a and 2b)

- SCD (any genotype) treated by REDS-III Hemocenter for at least 1 year in the past 10 years
- Diagnosed with HIV within 10 years of study start. Diagnosis of HIV must be confirmed by positive results on two separate HIV testing platforms including any two of the below:
  - HIV nucleic acid test (DNA or RNA)
  - HIV1 p24 antigen
  - HIV nucleotide sequence
  - HIV antibody testing
    - Enzyme immunoassay
    - Western Blot
  - Combination HIV antigen/antibody test

HIV sero-negative Controls (Included only in Objective 2b analysis)

- 2:1 matched to HIV+ case on the following criteria
  - Age $\pm$ 2 years
  - Hemocenter
  - Gender
  - SCD genotype
- No history of HIV in REDS-III database
- HIV negative by most recent infectious screening results

Subject Enrollment:

Objective One: Patients with SCD (Cases)

A list of all REDS-III SCD cohort patients $\geq$18 years will be placed in random order and subjects will be called consecutively according to this list to invite subjects to participate in the study. Details of the study will be explained over the phone and interested subjects will be scheduled for a Hemocenter visit to complete informed consent and study procedures. Patients will be enrolled consecutively at all Hemocenters until 150 patients have completed enrollment. No Hemocenter specific enrollment targets will be established.

Objective One: Non-SCD Controls

The enrollment of cases into objective 1 will trigger enrollment of a similar number of controls from the same Hemocenter in the same 5 year age stratum (frequency matched). A list of required controls with eligible ages will be updated daily based on ages of enrolled cases at each Hemocenter. Controls will be matched only on region (Hemocenter) and age strata. Other matching criteria will not be included to avoid overly restrictive/narrow matching criteria that would impede enrollment. We anticipate enrollment of males and females will be approximately equal based on previous REDS

research, however we will monitor gender distribution of cases and controls and add gender matching if necessary. Flyers that describe the study will be distributed in the SCD patient advocacy association rooms and at advocacy events. REDS-III research staff will also attend functions sponsored by SCD patient advocacy associations to give presentations describing the study. Any interested subjects will contact REDS-III staff to determine eligibility. Interested subjects who meet age eligibility will be scheduled for a research visit. Research assistants will maintain a list of names and contact information of all interested potential controls not meeting eligibility criteria at this point of contact and call the subject in the future if s/he becomes eligible.

Objective 2: HIV+ SCD Cases

SCD patients followed at REDS-III Hemocenters are screened annually for HIV. Hemocenter records will be reviewed to identify all SCD patients with confirmed HIV in the 10 years prior to study start. As HIV is relatively rare in the SCD population, HIV seropositive cases will be recruited from the entire Hemocenter SCD population (not limited to patients enrolled in the REDS-III cohort) in order to capture the majority of HIV positive patients. All living HIV seropositive SCD patients receiving care at the Hemocenters will be recruited via phone or at routine Hemocenter visits. Details of study will be explained and interested subjects will be scheduled for visits to become informed about the study, complete the informed consent and the HIV risk behavior assessment (only for HIV+SCD patients $\geq$ 18 years). A review of patients' medical record will be performed to capture HIV outcomes. Eligible patients will be given the option to consent solely to the medical record review or to the medical record and the completion of the HIV risk behavior assessment if $\geq$ 18 year old. IRB/ethical committee approval will be requested to perform a medical record review of all HIV seropositive patients no longer living or otherwise lost to follow up at the Hemocenters.

Two of the participating REDS-III Hemocenters (Hemominas Belo Horizonte and Hemorio) have estimated that between 0.16-0.45% of their current SCD population are HIV seropositive (Table 1). Therefore, we anticipate that approximately 25-35 HIV positive SCD cases from the past 10 years at the five participating Hemocenters will be eligible for objective 2 and will attempt to enroll 25 HIV positive cases.

Table 1: Current estimates of HIV at Two REDS-III Hemocenters

	Number HIV+	Total SCD Population	Percent SCD Population HIV positive
Hemominas	5	3211	0.16%
Hemorio	17	3791	0.45%

Subjects will be enrolled until target enrollment of 150 cases and 150 controls for objective 1 is achieved and all identified HIV+ patients for objective 2 are recruited. We anticipate approximately one year will be required to achieve target enrollment, from March (or when OMB approval is received) 2016 –March 2017.

Potential Nonresponse Bias

During data collection, we will monitor participation and response rates to identify any potential problems that are indicated by differential response rates across sites. The demographics and SCD severity of the population from which the Objective 1 SCD cases will be recruited are known,

therefore we will be able to compare the age, gender, SCD type and key indicators of disease severity between responders and non – responders. If significant differences between the populations are detected, specific effort to recruit under-represented sub-populations will occur.

We expect low levels of item nonresponse within the ACASI for this study because the ACASI allows the subjects to answer questions confidentially on a touch-screen computer. Our use of ACASI was very successful in previous REDS-II studies with little evidence of important levels of nonresponse to any of the questions asked during the interview. For example, when subjects were asked if they had ever been tested for HIV outside of blood donation only 1 person out of 1244 respondents (<0.1%) refused to answer this question. Similarly, for a clearly social sensitive question in which we asked respondents to classify their sexual orientation, only 18 out 1244 respondents (1.4%) refused to answer. The rate of unanswered questions in the study ranged from <0.1% - 5% across all questions. These data suggest that the use of ACASI was successful in eliciting responses to stigmatizing or socially sensitive questions, and we expect the same to be true for this very similar interview in the REDS-III HIV SCD study.

Sample Size Calculations:

The number of sexual partners in the previous year was chosen as the primary outcome (HIV risk factor) on which to power study. A simulation study was conducted to explore statistical power for a comparison of rates of sexual activity among patients with sickle cell disease (cases) and age matched persons without sickle cell disease (controls). It was assumed that subjects would be classified into three levels of sexual activity: 0, 1-2 or 3+ partners in the preceding year to produce a 3-level multinomial distribution. The distribution in the controls was based on the distribution observed in an HIV negative control population in a previous REDS-II study in Brazil: 6.3% in the zero activity group, 86.6% in the low activity group and 7.1% in the high activity group. The simulations proceeded as follows:

1) Specify a distribution of activity among sickle cell patients.
2) Take random samples of 500 simulated subjects from the multinomial distributions for the cases and controls.
3) Perform a chi square test to compare the two distributions and note whether the resulting p value was <0.05. Repeat using the first 250 subjects per group, the first 200 subjects per group, the first 150 and the first 100 subjects per group.
4) Repeat this 2,000 time; the proportion of the replicates with p<0.05 is an estimate of statistical power for the comparison in question at samples sizes of 500, 250, 200, 150 and 100 per group.

Results are summarized in table 2 below. The estimates of statistical power are grouped according to the assumed proportion with zero activity in the cases. Within each assumed proportion with zero activity, estimates of power are listed in order of descending proportions in the low activity group. Estimates of power that are ≥0.80 are shown in bold. Power calculations were restricted to situations in which the cases (SCD patients) have lower sexual activity than the controls meaning that (1) the sum of the percentages in the low and high activity groups was lower in the cases than the controls and (2) the percentage in the high activity group was lower in the cases than in the controls.

Table 2 Power Simulations

Distribution in the cases	Power			
	100 per	150 per	200 per	250 per

	group	group	group	group
15/85/0	**0.92**	**0.99**	**0.995**	
15/84/1	0.76	**0.92**	**0.98**	
15/83/2	0.63	**0.825**	**0.92**	
15/82/3	0.53	0.72	**0.86**	
15/81/4	0.475		**0.80**	**0.885**
15/80/5	0.415		0.75	**0.835**
15/79/6	0.42		0.715	**0.818**
15/78/7	0.41		0.725	**0.83**
14/86/0	**0.90**	**0.99**	**0.995**	
14/85/1	0.705	**0.89**	**0.965**	
14/84/2	0.58	0.775	**0.89**	**0.95**
14/83/3	0.47		0.79	**0.885**
14/82/4	0.405		0.71	**0.81**
14/81/5	0.37		0.66	0.765
14/80/6	0.355		0.65	0.75
14/79/7	0.355		0.645	0.74
13/87/0	**0.89**	**0.99**	**>0.995**	
13/86/1	0.665	**0.87**	**0.95**	
13/85/2	0.53	0.725	**0.85**	**0.925**
13/84/3	0.395		0.73	**0.830**
13/83/4				0.745
13/82/5				0.66
13/81/6				0.610
13/80/7				0.625
12/88/0	**0.86**	**0.985**	**>0.995**	
12/87/1	0.635	**0.83**	**0.93**	
12/86/2	0.465	0.655	**0.80**	**0.89**
12/85/3	0.35		0.65	0.755
12/84/4				0.625
12/83/5				0.55
12/82/6				0.505
12/81/7				0.505
11/89/0	**0.84**	**0.98**	**>0.995**	
11/88/1	0.585	**0.80**	**0.91**	
11/87/2	0.425	0.595	0.76	**0.855**
11/86/3				0.69
11/85/4				0.54
11/84/5				0.425

A 1984 survey administered to 52 females with SCD and 80 controls demonstrated 39% of SCD subjects were sexually active compared to 81% of controls, and the mean age at first sexual encounter was 17.7 years in the SCD group vs. 17.0 years in the control population. More updated preliminary data is not available for utilization in power calculations. Therefore an assumption of

differences on the order of magnitude of 11, 88, 1% (0, 1-2, 3+ partners, respectively) in the cases compared to the presumed distribution of 6.3%, 86.6%, 7.1% in controls is reasonable. As 80% power is achieved with 150 subjects per group in this distribution, this was chosen as the sample size to achieve study objectives and maintain feasibility within resources.

B.2. Procedure for the Collection of Information

B.2.1. Questionnaire

A detailed HIV risk factor assessment will be administered to all subjects. This assessment will be based upon an instrument previously utilized and validated by the CDC in its HIV surveillance at U.S. blood banks. This tool has been modified for use in other HIV related REDS-II studies in Brazil, and has been further tailored to the SCD population with input from Brazilian SCD physicians. A self-administered audio computer-assisted self-interview (ACASI) on a laptop computer or netbook will be used in order to maximize reporting of stigmatized behaviors. A research assistant or nurse will provide the ACASI (including earphones to be able to listen to the questions confidentially) to each subject at the Hemocenter. The study subject will be shown how to use the computer to complete the interview by entering basic demographic data with the help of the nurse, but will be given privacy to complete the rest of the assessment. The research assistant or nurse will remain available to answer questions and provide help as necessary. We chose the ACASI to maximize reporting of stigmatized risk behaviors and to streamline the interview as the ACASI allows built in skip patterns depending on initial responses so that subjects are only prompted to answer questions about the details of a specific risk factor if they report having the risk. The ACASI format also utilizes electronic data capture which will reduce data entry errors

B.2.2 Phlebotomy for Clinical Testing

A total of 12 mL of blood will be drawn from consented cases at the time of the enrollment and interview. Specimens will be subjected to testing as summarized below. The remaining specimens will be processed into aliquots and saved in the study repository in Sao Paulo for future testing if the subject consent to have their left over blood specimens saved for future research (separate consent).

Objective 1 SCD Cases: HIV testing
Objective 1 Non-SCD Controls: HIV and SCD/sickle cell trait testing
Objective 2 HIV+ SCD Cases: HIV viral load, HIV genotyping, drug resistance and complete blood count. A CD4 count will be requested if we are able to coordinate this testing with an appropriate local clinical laboratory

B.2.3 HIV Testing

HIV testing will follow the Brazilian algorithm for HIV testing of blood donors. Samples will be screened with a 4th generation EIA (enzyme immunoassay). If repeat reactive, the sample will be submitted to NAT (nucleic acid testing). If the NAT is negative the sample will be submitted to Western Blot. If Western blot is also negative the individual is considered non-infected by HIV.

B.2.4 Sickle cell disease/trait testing in non-SCD controls

Each Hemocenter has established protocols for sickle cell testing of family members of SCD patients. This infrastructure will be utilized to confirm there is no diagnosis of sickle cell disease in the non-SCD controls. One cellular aliquot will be transferred to the local Hemocenter lab for hemoglobin S testing by HPLC. If SCD trait is detected, the subject will be eligible to serve as a control.

B.2.5 Counseling and Medical Referrals

Participating Hemocenters routinely test blood donations for HIV and an infrastructure for confirmatory testing, counseling and medical referral exists at each Hemocenter. Any objective 1 subjects found to be HIV infected will be called back to the Hemocenter for the same counseling, repeat testing and referral that blood donors undergo upon confirmation of HIV positive blood donation.

Objective 1 non-SCD controls with sickle trait (hemoglobin AS pattern on HPLC) will be counseled about the presence and implications of sickle trait by hematologists at the Hemocenter who typically perform this counseling for family members of SCD patients.

B.2.6 Data Management and Analysis

B.2.6 Data Management

Data collected from the ACASI interview is stored on the local netbook or laptop computer used to conduct the ACASI. . The RTI study manager and data analyst will work directly with the staff developing the computer assisted questionnaire program and will test the system to ensure that appropriate skip patterns are followed and that the standard response categories (e.g., don't know, refused, not applicable) are consistent coded throughout the survey. **The ACASI data files will be sent weekly by research assistants in Hemope, Hemorio, Hemominas Juiz de Fora and Hemominas Montes Claros to the lead study coordinator in Hemominas Belo Horizonte (HBH). This designated study coordinator in HBH is responsible for review and basic cleaning of data to identify missing or irregular data. This coordinator will then up load the files to the data coordinating center, RTI, through a secure, encrypted FTP site on the private REDS-III website using the same procedures defined for other REDS-III studies.**

The HIV case report form and SCD case report form (medical record questionnaire) will be programmed into the data entry and storage system that has been developed for the REDS-III Brazil SCD Cohort study. This system utilizes a web-based interface for data entry. Data is then directly transferred to databases within the University of Sao Paulo for storage. Once per month data will be extracted from the USP database and securely uploaded to RTI for quality control checks. RTI will follow data management best practice guidelines means developing and implementing quality control processes from the survey develop stage through analysis. This will minimize data manipulation and allow for quicker evaluation of the data, identification of any data and data retrieval/remediation.

B.2.6.1 Analysis of risk behaviors

For analysis of HIV risk behaviors in objective 1, the primary outcome to be compared between cases (SCD) and controls (non-SCD) will be number of sexual partners in the previous year. Other HIV risk factors ascertained by the ACASI will be compared between cases and controls including male to male sex, number of lifetime male and female sexual partners, use of condoms, age of sexual debut, intravenous drug use (IDU), sex with an IDU, and sex with an individual known to be HIV-positive.

For continuous variables, two-sample comparisons of the means such as the t-test or an appropriate non-parametric test for non-normally distributed data will be used. Chi-square will be used for comparison of categorical variables between cases and controls. Fisher's exact test will replace the chi square test if the data do not meet the assumptions of the latter (e.g. the table has too many cells with very low expected frequencies). The matching for objective 1 is intended to be frequency or strata matching, therefore matched-paired statistics will not be used. For multi variable modeling, logistic regression will be utilized by dichotomizing number of sexual partners into high and low groups using appropriate thresholds based on the data.

B.2.6.2 Case-series description of HIV outcomes in SCD patients

HIV outcomes will be reported with descriptive statistics (prevalence with 95% confidence limits) for HIV positive patients included in objective 2a. Specific outcomes to be summarized will include prevalence of long term non-progressors, prevalence of elite controllers (EC) if sufficient viral load and treatment data is available (EC=no evidence of viremia as measured by standard assays, <50 or <75 copies/mL with CD4 count >500 for at least a year), time to onset of AIDS, prevalence of AIDS defining illnesses and CDC classification of disease status at diagnosis and most recent point of contact (see table 3 for CDC classification and table 4 for AIDS defining illnesses).

Table 3: CDC Classification of Disease Stage

Stage	Age at Time of CD4+ Lymphocyte Test*					
	< 1 year		1-5 years		≥ 6 years	
	Cells/microL	Percent	Cells/ microL	Percent	Cells/microL	Percent
1	≥1500	≥34	≥1000	≥30	≥500	≥26
2	750 – 1499	23-33	500-999	22-29	200-499	14-25
3 (AIDS)**	<750	<26	<500	<22	<200	<14

*CD4 lymphocyte count takes precedence over the CD4 percentage. The percentage is considered only if the count is missing.
**If AIDS defining illness (table 4) is diagnosed, the stage is 3 regardless of CD4+ count.

Table 4: AIDS Defining Illnesses

Bacterial infections, multiple or recurrent*
Candidiasis of bronchi, trachea or lungs
Candidiasis of esophagus
Cervical cancer, invasive**
Coccidiomycosis, disseminated or extrapulmonary
Cryptosporidiosis, chronic intestinal (>1 month's duration)
Cytomegalovirus disease (other than liver, spleen or nodes), onset age>1 month
Cytomegalovirus retinitis (with loss of vision)
Encephalopathy, HIV related
Herpes simplex: chronic ulcers (>1 month) or bronchitis, pneumonitis or esophagitis onset age>1 month
Histoplasmosis, disseminated or extrapulmonary
Isosporiasis, chronic intestinal (>1 month)
Kaposi sarcoma

Lymphoma, Burkitt
Lymphoma, immunoblastic
Lymphoma, primary brain
Mycobacterium avium complex or *mycobacterium kansaii*, disseminated or extrapulmonary
Mycobacterium tuberculosis any site
Mycobacterium, other species or unidentified species, disseminated or extrapulmonary
Pneumocystis jirovecii (previously Pneumocystis carinii) pneumonia
Pneumonia, recurrent**
Progressive multifocal leukoencephalopathy
Salmonella septicemia, recurrent
Toxoplasmosis of brain, onset age>1 month
Wasting syndrome attributed to HIV

*Only among children aged < 6 years

** Only among children aged ≥ 6 years, adolescents and adults

Table 3 and 4 adapted from *Revised Surveillance Case Definition for HIV Infection — United States, 2014 (*www.cdc.gov/mmwr/preview/mmwrhtml/rr6303a1.htm?s_cid=rr6303a1_e),

B.2.6.3 Objective 2b Comparison of SCD Outcomes between HIV+ and HIV-

Many of the SCD events of interest in objective 2b will be modeled as binary outcomes, such as the presence/absence of a history of stroke, avascular necrosis, kidney failure, pulmonary hypertension, etc. For binary outcomes, conditional logistic regression will be employed for data analysis since a 2:1 matched design is being used. With this approach, subjects are grouped into strata that are defined by the matching variables used to select controls for the study; for objective 2b, the strata are defined by age ± 2 years, hemocenter, sickle cell type and gender.

It is possible, however, that changes in event rates with age will lead to age-related variation in the odds ratio. We will explore this by examining trends with age in the odds ratio. If trends are evident, then a model that takes account of age will be developed in place of the model under which the odds ratio is assumed to be constant allowing the analysis to report age-varying odds of these adverse events.

HIV acquisition and clinical progression status also require consideration. Among the HIV-positive subset, subjects with a history of a given SCD outcome may include a mix of those with the SCD event prior to HIV infection and those with the event after infection. Because the SCD population is annually tested for HIV as well as other transfusion-transmissible infections we will have date of HIV diagnosis to estimate age of HIV infection, and the age at which the SCD event first occurred. We will be able to include HIV status as a time-varying covariate in a survival analysis of time to the SCD event. Age will be treated as the measure of time in this analysis.

Because event times may not be accurately captured in medical records, we recognize that there may be events for which a meaningful survival analysis is not possible. For example, pulmonary hypertension is diagnosed through examinations that may be performed sporadically on an irregular schedule in many patients, which may mean that we cannot perform survival analysis for this outcome. Before beginning any time to event analysis, we will evaluate the completeness of data for each endpoint of interest.

Some SCD events, such as vaso-occlusive pain crisis (VOC) and acute chest syndrome, can occur repeatedly in the same subjects. Poisson regression will be employed to evaluate risk factors for both of these outcomes. The outcome variable will be the count of events in a defined period.

Regression analysis will be conducted using SAS. Conditional logistic regression will be employed for binary outcomes and generalized linear modeling will be used for Poisson regression.

B.3. Methods to Maximize Response Rates and Deal with Non-response

All adult REDS-III subjects will be recruited into this study. Because these subjects have previously agreed to participate in research and must return to the Hemocenter for scheduled visit, we anticipate the required 150 SCD cases can be recruited from the REDS-III cohort. Because the ACASI allows subjects to confidentially answer all questions, we anticipate a high response rate to all items contained within the interview.

B.4. Test of Procedures

The ACASI was pretested prior to use in the REDS-II study and has now been utilized in REDS-II and REDS-III studies and therefore has previously been validated in multiple projects and populations. Because the ACASI utilizes verbal, visual and audio cues and allows subjects to answer using a touch screen, the tool has demonstrated ease of use, even in subjects with minimal education. The average time spent to complete the assessment in REDS-II was 24 minutes, although donors with minimal education took approximately 45 minutes to complete the assessment. SCD patients and controls recruited from a similar population are estimated to be more similar to blood donors with minimal education by physicians and nurses familiar with both populations; therefore, we have used 45 minutes for the calculation of burden hour.

B.5. Individuals Consulted on Statistical Aspects and Individuals Collecting and/or Analyzing Data

The protocol and assessment to be administered by ACASI were developed in consultation with Brazilian SCD physicians familiar with the study population. All study tools and procedures were also modified after input from research nurses who administered assessments to this population for previous REDS-III research. We have consulted biostatisticians from the REDS-III Data Coordinating Center (Research Triangle Institute) on statistical aspects of the study design. Data analysis will be performed by the analytic staff at the Data Coordinating Center that includes epidemiologists and biostatisticians, with assistance and oversight provided by the REDS-III International Advisory Committee (IAC) (Attachment 3.3). In addition, the REDS-III OSMB (Attachment 3.1) will monitor this study.