Update article

**Contribution of the Retrovirus Epidemiology Donor Study (REDS) to research on blood transfusion safety in Brazil**

Paula Loureiro\(^a,b\), Cesar de Almeida-Neto\(^c\,*\), Anna Bárbara Carneiro Proietti\(^d\), Ligia Capuani\(^e\), Thelma Terezinha Gonçalez\(^f\), Claudia Di Lorenzo de Oliveira\(^g\), Silvana Carneiro Leão\(^b\), Maria Inês Lopes\(^b\), Divaldo Sampaio\(^a,b\), Giuseppina Maria Patavino\(^e\), João Eduardo Ferreira\(^h\), Paula Fraiman Blatyta\(^c\), Maria Esther Duarte Lopes\(^1\), Alfredo Mendrone-Junior\(^e\), Nanci Alves Salles\(^c\), Melissa King\(^j\), Edward Murphy\(^l\), Michael Busch\(^l\), Brian Custer\(^f\), Ester Cerdeira Sabino\(^e\)

\(^a\) Faculdade de Ciências Médicas, Universidade de Pernambuco (UPE), Recife, PE, Brazil
\(^b\) Fundação Hemope, Recife, PE, Brazil
\(^c\) Fundação Pró-Sangue – Hemocentro de São Paulo, São Paulo, SP, Brazil
\(^d\) Fundação Hemominas, Belo Horizonte, MG, Brazil
\(^e\) Faculdade de Medicina, Universidade de São Paulo (USP), São Paulo, SP, Brazil
\(^f\) Blood System Research Institute, San Francisco, California, USA
\(^g\) Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil
\(^h\) Instituto de Matemática e Estatística, Universidade de São Paulo (USP), São Paulo, SP, Brazil
\(^i\) Fundação Hemorio, Rio de Janeiro, RJ, Brazil
\(^j\) Westat, Rockville, Maryland, USA
\(^l\) University of California San Francisco, California, USA

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**ABSTRACT**

The Retrovirus Epidemiology Donor Study (REDS) program was established in the United States in 1989 with the purpose of increasing blood transfusion safety in the context of the HIV/AIDS and human T-lymphotropic virus epidemics. REDS and its successor, REDS-II were at first conducted in the US, then expanded in 2006 to include international partnerships with Brazil and China. In 2011, a third wave of REDS renamed the Recipient Epidemiology and Donor Evaluation Study-III (REDS-III) was launched. This seven-year research program focuses on both blood banking and transfusion medicine research in the United States of America, Brazil, China, and South Africa. The main goal of the international programs is to reduce and prevent the transmission of HIV/AIDS and other known and emerging infectious agents through transfusion, and to address research questions aimed at understanding global issues related to the availability of safe blood. This article describes the contribution of REDS-II to transfusion safety in Brazil. Articles published from 2010 to
Introduction

Retrovirus Epidemiology Donor Study (REDS) is the name of a multicenter transfusion safety research program funded by the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health (NIH) in the United States of America. A review was recently published about the accomplishments of the REDS program, and its successor, REDS-II.1

The NHLBI REDS project started in 1989 during the HIV epidemic, and had as principal objectives the improvement of transfusion safety in the light of retroviral infections. Its successor, REDS-II, was launched in 2004 and expanded in 2006 to include two large international research components consisting of partnerships between US Institutions and blood center networks in Brazil and China. In 2011, a new 7-year program was initiated, the Recipient Epidemiology and Donor Evaluation Study-III (REDS-III), which extended the focus of REDS and REDS-II to include not only research in blood banking (focused on blood donors and their donations) but also clinical transfusion research (focused on transfused recipient outcomes). REDS-III includes large research collaborative efforts conducted in the US, Brazil, China, and South Africa. The REDS programs have all been structured to establish donor and donation databases that serve as the basis for conducting detailed analyses of blood safety and availability in each country, and to conduct specific protocols that directly address key research questions in blood safety and transfusion medicine in each country.2

In Brazil, the REDS-II project started in three blood centers: Fundação Pré-Sangue (FPS) in São Paulo, Fundação Hemominas in Belo Horizonte and Fundação Hemope in Recife. Later, Hemorio in Rio de Janeiro was also included. The program collected and analyzed data from the operational systems of the blood centers and from specific research protocols. Table 1 summarizes the overall data sources and the studies performed in Brazil under the REDS-II program.

Database analyses

Most blood centers in Brazil carry out their operations using computerized information systems. However, these systems are heterogeneous, so obtaining nationwide data is not an easy task. In the Brazilian project, the variables from the US

Table 1 - Sources of data and the studies performed in Brazil under the Retrovirus Epidemiology Donor Study II (REDS-II) program

<table>
<thead>
<tr>
<th>Studies</th>
<th>Goals</th>
<th>Type of study</th>
<th>Number of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Establishment of a Comprehensive Donor and Donation Database</td>
<td>Describe donor demographics, prevalence and residual risk of transfusion-transmitted disease</td>
<td>Database analysis</td>
<td>1,900,000</td>
</tr>
<tr>
<td>The HIV Case-Control and Molecular Surveillance Study</td>
<td>Describe risk and motivation among HIV-positive donors and characterize the HIV strains</td>
<td>Case control</td>
<td>1342</td>
</tr>
<tr>
<td>Chagas Natural History among Blood Donors</td>
<td>Describe the natural history of Chagas among positive blood donors</td>
<td>Retrospective 1088 cohort study</td>
<td></td>
</tr>
<tr>
<td>Motivation to Donate</td>
<td>What motivates people to donate blood in Brazil?</td>
<td>Cross sectional</td>
<td>7635</td>
</tr>
<tr>
<td>Deferral study</td>
<td>Understand if the deferral criteria questionnaire improves blood safety</td>
<td>Cross sectional</td>
<td>3933</td>
</tr>
</tbody>
</table>

REDS program were adapted to the Brazilian context and a data warehouse was developed by the Institute of Mathematics and Statistics (USP) to receive and validate the data.2 During REDS-II, data were transferred to a central data-coordinating center. A web application was developed to streamline the process, including a system for data management and quality control checking for consistency. The development of this tool was a major accomplishment as it allows the inclusion of data from other centers in Brazil and allows the Brazilian group to continue this hemovigilance effort on a larger scale and outside of the context of the REDS research programs.

Blood donor characteristics in Brazil

In the initial publication of the Brazilian REDS-II project, Carneiro-Proietti et al. documented that the studied donor population is relatively young with more

2013 are summarized, including database analyses to characterize blood donors, deferral rates, and prevalence, incidence and residual risk of the main blood-borne infections. Specific studies were developed to understand donor motivation, the impact of the deferral questions, risk factors and molecular surveillance among HIV-positive donors, and the natural history of Chagas disease. The purpose of this review is to disseminate the acquired knowledge and briefly summarize the findings of the REDS-III studies conducted in Brazil as well as to introduce the scope of the REDS-III program that is now in progress and will continue through 2018.
than 60% below 35 years of age. Males are predominant, accounting for 66% of the donors; and 39% are replacement donors: donors who give either at the request of or on behalf of a specific recipient to replace units in the overall supply. The average number of donations per donor/year for two years was 1.48 at FPS; 1.58 at Hemope, and 1.42 at Hemominas.

The return rates and inter-donation intervals were analyzed by Almeida-Neto et al. who showed that 28.8% of first-time donors and 56.5% of repeat donors returned to donate within two years of initial donation, rates not particularly different from those observed in the US. Community donors, donors who give to the general blood supply, were more likely to return than replacement donors; Hemope had the highest return rate.

Currently there are no national policies stipulating blood donation based on the number of sexual partners prior to donation. The three primary REDS-II Brazil centers had introduced a question about the number of sexual partners in the past year during the donor screening procedure. The centers adopted different cut-off points: São Paulo – up to six sexual partners; Recife – up to three sexual partners; Belo Horizonte – up to two sexual partners in the previous year. Patavino et al. evaluated the relationship between the number of heterosexual partners and infectious diseases at the three blood centers listed above. A correlation was found between the number of heterosexual partners in the year previous to the donation and the prevalence of HIV and syphilis. These analyses highlight the importance of keeping questions related to heterosexual activities in Brazilian blood centers as well as provided clues about possible cut-off values (or example, FPS has now decreased the deferral rule to a maximum of 3 sexual partners in the past year).

Gonzalez et al. analyzed the rates and reasons for deferral in each center. The centers use different codes and approaches for donor deferral so the first step was to group them into similar categories. The overall deferral rate was 22.5%; Belo Horizonte had the highest rate (27%), followed by Recife (23%) and São Paulo (19%). Females were more likely to be deferred than males (30% vs. 18%, respectively). The three most common deferral reasons were low hematocrit or hemoglobin, medical diagnosis, and high-risk behavior. Notably, the types and frequencies of deferral varied substantially among the three blood centers. The results indicated that blood donor deferral in Brazil has regional aspects that should be considered when national policies are developed. This study also highlighted the potential value in standardizing codes and procedures among centers.

Gonzalez et al. also reviewed 700,000 whole blood donations to assess adverse reactions among blood donors and observed an overall reaction rate of 2.2%. However, the rates were very different in each center: 53, 290, and 381 per 10,000 donations in Recife, São Paulo, and Belo Horizonte, respectively. Reactions were classified as: mild grade for presyncopal vasovagal adverse reactions (VVRs) such as pallor, sweating and anxiety, in the majority of situations (95%); 4.6% were considered moderate grade for hypotension, vomiting, and transient loss of consciousness; and 0.4% were considered severe reactions when there is loss of consciousness associated to other signs and symptoms such as recurrent vomiting, prolonged pulse and/or blood pressure recovery times, incontinence, and convulsions, among other signs and symptoms. Females were twice as likely to have an adverse reaction as males.

Almeida-Neto et al. conducted an analysis at FPS to assess the demographic profile and infection disease markers in donors who said their blood should not be used when asked at the time of the donation (Confidential Unit Exclusion or CUE) process. At FPS the policy was to discard all subsequent donations from donors who self-excluded their donations using the CUE process. A total of 265,550 donations from 181,418 donors were evaluated and a total of 3.6% of the units were discarded – 1.1% by the current CUE, and 2.5% due to CUES in previous donations. Self-excluded donors had a tendency to be male, with poor schooling (less than eight years), non-Caucasian and first-time donors. Donors who selected CUE on the current donation were more likely to have positive infectious disease marker than donors who had never selected CUE [Odds ratio (OR) = 1.41; 95% confidence interval (CI) 1.13-1.77], whereas donors who had previously used CUE (but not for the current donation) were not more likely to have a positive marker than donors who had never selected CUE [OR = 1.04; 95% CI: 0.75-1.45]. Thus the results suggest that discontinuing the ongoing discard of the unit of donors who self-excluded in the past but not for the current donation should be considered.

Transfusion related acute lung injury (TRALI) is one of the main causes of severe adverse reactions and post-transfusion death in recipients. One of the options for Brazilian blood centers to mitigate this problem is to use male plasma only and/or to defer multiparous female donors from apheresis platelet donation. Blaytta et al. analyzed the impact of only deferring multiparous females from donating apheresis platelets and found that there would be a tolerable 5% decrease of all apheresis platelet collections. However, 30.8% of whole blood plasma and 24.1% of apheresis platelet donations would not be available if only male donor plasma was issued for transfusion and all female donors were deferred from apheresis donation, respectively, suggesting TRALI mitigation by selecting only male donor plasma products would have an unacceptable impact on plasma and platelet availability.

Prevalence, incidence and residual risk of transfusion transmitted disease

The REDS-II dataset enabled us not only to define the prevalence, incidence and residual risk of infectious disease, but to evaluate the associations between infections and donor demographic characteristics. Table 2 summarizes the main findings. HBsAg was the marker with the highest prevalence among first time donors. HIV had the lowest prevalence among first time donors but in comparison to the other markers, the incidence was the highest among repeat donors (although it was not possible to define the incidence for HBV). São Paulo had the highest prevalence among first time donors for Hepatitis C virus (HCV) and Chagas, while HIV and human T-lymphotropic virus (HTLV) was higher in Recife. The use of parallel blood donor screening for HIV with two enzyme immunoassays (EIAs), as is mandatory in Brazil.
was also reviewed and concluded that parallel testing did not appear to improve the ability to capture and interdict HIV-positive blood donations.14

Inconclusive serological test results for Chagas Disease are a major challenge for blood donor counseling in Brazil. By using a second recombinant EIA (EIAr) assay, Sabino et al. classified the inconclusive samples into two groups: low reactive Chagas (EIAr positive) and probably other parasite infections (EIAr negative).13 The data suggest that a second recombinant EIA may be used as a confirmatory assay.

In collaboration with Honduras and the US, T. cruzi EIA signal to cut-off values (S/CO) of the Ortho EIA was compared to polymerase chain reaction (PCR) results. PCR was not positive among individuals with S/CO below 2, suggesting that low-level T. cruzi antibody reactive samples may represent seroreversion after parasite clearance.15

### Table 2 - Prevalence and incidence of blood-borne diseases in blood donors at FPS in São Paulo, Hemominas in Belo Horizonte and Hemo in Recife from 2007 to 2009.

<table>
<thead>
<tr>
<th></th>
<th>Prevalence per 100,000 1st time donations</th>
<th>Incidence per 100,000 repeat donations</th>
<th>Residual risk per 1,000,000 persons-year</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>92.2</td>
<td>22.55</td>
<td>11.3*</td>
</tr>
<tr>
<td>HBsAg+Anti-HBC</td>
<td>289</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HCV</td>
<td>191</td>
<td>3.11</td>
<td>5.0</td>
</tr>
<tr>
<td>HTLV</td>
<td>135.2</td>
<td>3.59</td>
<td>5.0</td>
</tr>
<tr>
<td>Chagas</td>
<td>140</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>São Paulo</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>84.91</td>
<td>37.49</td>
<td>6.4</td>
</tr>
<tr>
<td>HBsAg+Anti-HBC</td>
<td>213</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HCV</td>
<td>287</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HTLV</td>
<td>100.7</td>
<td>1.73</td>
<td>-</td>
</tr>
<tr>
<td>Chagas</td>
<td>206</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Belo Horizonte</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>70.98</td>
<td>33.36</td>
<td>6.0</td>
</tr>
<tr>
<td>HBsAg+Anti-HBC</td>
<td>270</td>
<td>ND</td>
<td>-</td>
</tr>
<tr>
<td>HCV</td>
<td>78</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HTLV</td>
<td>82.7</td>
<td>2.82</td>
<td>-</td>
</tr>
<tr>
<td>Chagas</td>
<td>119</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Recife</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>119.15</td>
<td>43.88</td>
<td>7.8</td>
</tr>
<tr>
<td>HBsAg+Anti-HBC</td>
<td>419</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HCV</td>
<td>131</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HTLV</td>
<td>222.4</td>
<td>7.16</td>
<td>-</td>
</tr>
<tr>
<td>Chagas</td>
<td>60</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

HCV: hepatitis C virus; HTLV: human T-lymphotropic virus; ND: no data.

* Ag + Ab IA = immunoassay that detect antigen p24 and anti-HIV. Ag: antigen; Ab IA: antibody immunoassay.

### Use of the database to assess the effectiveness of interventions

The ability to collect systematic blood donor data allowed us to conduct a trial to evaluate the effectiveness of an educational intervention among blood donors. González et al. assessed the impact of an educational brochure in enhancing blood donors’ knowledge about the screening test window phase, which consists of the time frame after infection when a person can transmit HIV through blood donation but still has not produced antibodies detectable by serology, reducing at-risk individuals from donating.16 This trial compared an educational intervention as a blood center’s usual practice. A brochure explaining the HIV window, the importance of the deferral questions and where the donor could get free testing at other sites was distributed in alternating months to all donors. After donating, sampled participants completed questions about their HIV window period knowledge. While the educational pamphlet increased window period knowledge, contrary to expectations this information alone was not enough to make donors self-defer or acknowledge potential behavioral risk. This study highlights the difficulties in increasing donor adherence to the screening questionnaire.

### Blood recipient outcomes

By linking three databases (blood center donation data, the hospital patient records and the National Death Index), González et al. were able to describe recipient survival and blood utilization according to patients’ diagnoses at Hospital das Clínicas of the Faculdade de Medicina of the Universidade de São Paulo.17 Of the 30,799 hospitalized patients in 2004, 12.4% received blood transfusions. These patients had 10,479 transfusion episodes, consisting of 39,561 transfused components: 42% red blood cells, 40% platelets and 16% plasma.

The median number of components transfused was three per patient admission. Mortality during hospitalization was different for patients whose admissions included transfusion than those that did not (24% vs. 4%). After one year, 56% of transfusion recipients were alive. The most frequent diagnoses in patients who received transfusions were malignant neoplasms, diseases of the gastrointestinal (GI) tract, diseases of the circulatory system, emergencies, poisoning and other external traumatic causes.17

### REDS-II Brazil studies

#### Donor motivation study

During the REDS-II program, a cross sectional study was performed in three centers (FPS, Hemope and Hemominas) to understand the motivation to donate and social capital among blood donors in Brazil. Approximately 7000 participants completed self-administered questionnaires on demographics, social capital, donor motivation, test seeking behavior and knowledge about HIV infection. Donors were enrolled and completed the survey before the screening process. Results showed that the main motivational factors identified were altruism (43.5%), direct appeal to donate...
(41.7%) and personal interest (29.6%). Oliveira et al. analyzed the relationship between test seeking behavior and social capital. Approximately 8.1% of the donors were classified as self-disclosed test seekers. Male gender, less schooling and lower income was associated significantly with donating to obtain test results related to infectious diseases (test seeking). Test seekers had more cognitive social capital, which means that they had more reciprocity, co-operation, sense of belonging and social support. Blood centers in Brazil could leverage this finding to better communicate with prospective donors in ways that convince them to seek testing at locations other than blood banks.

**HIV case control study**

A case-control study was conducted to understand risks and motivations to donate among HIV positive donors. In addition to FPS, Hemominas and Hemope, Hemorio participated in this study. HIV positive cases were invited to participate after being counseled, and a comparison group of infection negative controls was invited to participate after donation. All responded to a questionnaire in an audio computer-assisted structured interview (ACASI) an innovative technology that allows more privacy and interview standardization, eliminating the potential interviewer bias. All respondents can both read text onscreen and listen to pre-recorded questions and answer options and then record his/her responses using a touch screen and/or keyboard. A total of 341 cases (107 had recently acquired HIV infection) and 791 controls completed all study procedures. The most common HIV risk factors in males were sex with HIV positive partners, being an intravenous drug user (IVDU) or being a partner of an IVDU. Among females, predominant risk factors included sex with HIV-positive male partners and unprotected sex with multiple partners. Remarkably, 13% of controls declared a risk factor that would have resulted in deferral had it been disclosed during the face-to-face donor eligibility assessment at the blood center. Thus, ACASI elicited increased disclosure of HIV risk factors among blood donors and may be a valuable modality of interview to be introduced in Brazilian blood banks. The REDS-II study also allowed us to characterize the HIV subtypes and the primary resistance of HIV-positive donors from the three REDS-II Brazilian blood centers and the blood center of Rio de Janeiro, in accordance with their demographic and exposure risk. HIV Subtype B was the most prevalent in these centers, however an increased proportion of non-B subtypes was observed in later years of the study. Resistance mutations were detected in 11.8% of the donors of non-B subtypes was observed in later years of the study. Prevalent in these centers, however an increased proportion of non-B subtypes was observed in later years of the study.

**Chagas disease retrospective cohort study**

The purpose of this project was to characterize the natural history of Chagas disease among seropositive blood donors. Approximately 500 T. cruzi seropositive donors from the São Paulo (FPS) and Montes Claros (Hemominas) blood centers and seronegative controls, who donated between 1996 and 2002, were enrolled. This allowed an investigation of rates of Chagas cardiac disease. A detailed health history and questionnaire was administered to all seropositive and seronegative donors and clinical patients and electrocardiograms (ECG) and echocardiograms (Echo) were performed to all participants. The project used a refined definition of Chagas cardiomyopathy: All cases and controls with any abnormality in the ECG and Echo found during clinical evaluation were blind reviewed by three cardiologists. The rate of false positive attribution of Chagas among seronegative controls was 5%. Excluding this error, research revealed a moderate annual incidence (1.85%) of Chagas cardiomyopathy in seropositive, asymptomatic donors. Another important finding was that the Echo could be performed only on individuals with particular ECG abnormalities without losing sensitivity to detect the cardiomyopathy cases.

Ribeiro et al. reviewed the ECG results in detail, described the most important abnormalities associated with these cases and tried to find which ones were most associated with decreases in ejection fraction which is today the most important parameter to define prognosis of heart disease.

**Donor deferral study**

The four REDS-III centers also conducted a study of deferred blood donors to assess the effectiveness of donor deferral. During the routine eligibility assessment, if a donor was deferred for reasons related to higher risk behaviors, the donor was invited to participate in a study to assess disease marker rates. Samples were collected and screened for all infections using the routine procedures of the blood centers. In addition, the donors completed an ACASI to further assess both disclosed and undisclosed risks. The study successfully enrolled 3933 donors who completed all study procedures. Preliminary results indicate that donor deferral based on questioning donors about higher risk behaviors does prevent the acceptance of donors with elevated rates of infectious disease markers in Brazil, clearly reducing the risk of transfusion-transmitted infection.

**REDS-III studies – new contributions to blood safety and transfusion outcomes in Brazil**

REDS-III was established in 2011. The structure of REDS-III is similar to REDS-II with the same three former blood centers, and a fourth additional collaborating center, Hemorio, the Blood Center of Rio de Janeiro, contributing donor and donation data to a centralized database that matches the previous REDS-II database. This will allow for a longer term trend analyses of donors and infections over a period of more than ten years when completed. In addition specific protocols have already been completed or are planned. The following studies are currently underway:

1. A study on dengue with the objective of defining the rate of transmission and penetration of the disease in both donors and recipients. This study enrolled more than 40,000 donors and 1000 recipients during the 2012 epidemic in Rio de Janeiro and Recife. Analyses of Dengue RNA status and immune responses are ongoing as well as an assessment of the symptoms of Dengue infection in blood recipients.
2. A longitudinal multicenter study of sickle cell disease (SCD) in Brazil with the objective of understanding the consequence of transfusion among these patients and establishing a large cohort in Brazil. The study plans to enroll a large cohort of SCD patients to understand the epidemiology of the disease in Brazil and also to assess specific questions such as outcomes of transfusion in this population, including factors associated with alloimmunization of SCD patients and the relationship between SCD and HIV infection.

3. An HIV risk factor and molecular surveillance study with the objective to monitor the rate of infection and epidemiological variants among blood donors in Brazil. Using the tools developed for the REDS-II HIV case control study, ongoing monitoring of risk behaviors and an evaluation of the HIV subtypes and primary drug resistance patterns will be conducted.

4. A donor notification study to assess success rates in blood donor notification following donation test results is currently being planned. This study will also seek to follow-up donors who have previously tested HIV positive to determine the rates of and barriers of access to medical care services following a positive HIV test as a result of blood bank screening.

Conclusion

The REDS-II program in Brazil was able to establish a collaborative research network between four large blood banks and created the conditions for the systematic analysis of the data generated by these centers. Additionally, Brazilian participating centers have further developed strong partnerships with researchers from the US and other countries, and are developing the expertise in Brazil necessary to the establishment and continuation of a research network that can serve as an example to other countries. It also established the conditions for the development and realization of multi-center research protocols that address key research priorities in blood banking in Brazil. This program has been remarkably successful in conducting important research to advance blood safety and availability in Brazil. REDS-III will further contribute to this body of knowledge with additional studies of blood recipients in Brazil.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES


