Original Article

Effectiveness of confidential unit exclusion in screening blood donors of the regional blood bank in Londrina, Paraná State

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The research was carried out in the Regional Blood Bank of Londrina, Londrina, Paraná State Brazil

Background: For transfusion purposes, blood donors must be accepted both in clinical and serological evaluations and must not have excluded their own donation using the confidential unit exclusion.

Aim: The objective of this study was to verify whether blood donors who choose self exclusion are more likely to be positive in serological tests than donors who do not.

Methods: A cross-sectional analysis was carried out of 51,861 consecutive whole blood donations from January 2004 to December 2008 at a public blood bank in Londrina, Southern Brazil.

Results: Self exclusion was chosen in 1672 (3.2%) donations, most frequently by first-time blood donors (p-value < 0.0001), by blood donors from external collections (p-value < 0.0001), by men (p-value < 0.0001) and by under 30-year-old donors (p-value < 0.0001). The frequency of positive serology was 5.3% in the group that chose self exclusion and 3.5% in the group that did not choose self exclusion (p-value < 0.0001).

Conclusions: These results show that confidential unit exclusion used in this blood bank is effective and is inexpensive. However, the diagnostic power to detect blood-borne infections was low and resulted in the discard of a high number of blood bags without any direct or indirect serologic markers of pathogens. The use of confidential unit exclusion could be replaced with molecular tests to screen blood donors.

Keywords: Donor selection; Serologic tests; Diagnosis; Blood transfusion

Introduction

Blood donation is a complex process with screening criteria designed to protect the health of both the donor and transfusion recipient. Three steps should be observed for a safe donation: a pre-donation interview conducted by a healthcare professional; a confidential unit exclusion (CUE) form spontaneously given to the donor; and the laboratorial screening of blood for the main parenterally transmitted diseases using highly sensitive methods.[1-3] In Brazil, the use of CUE is mandatory for all blood donations; however, the design of the CUE form is defined by each blood bank. When donors self exclude themselves, the blood bag must be discarded, that is, it cannot be used for transfusion even if the donor serum is not reactive to the serological tests used in laboratorial screening.[4]

In the international literature, several studies have evaluated the use of CUE; some were favorable to its use and others considered this procedure to have low cost-benefit with loss of many potentially healthy donors.[5-9] Few studies have been conducted in Brazil to evaluate the validity of CUE. Usually these studies were regional that were conducted during a short period and showed contradictory results.[10] The aim of this study was to verify whether the CUE procedure that is currently used in the Regional Blood Bank in Londrina is effective to identify donors at risk of transmitting blood-borne diseases.

Methods

The Regional Blood Bank in Londrina is a public health institution that collects blood within its own facility (internal collection) and in local companies, churches, schools and other places located in the city of Londrina (external collections). It receives an average of 12,000 donors annually; however, approximately 15.0% of these donors are deferred because they do not fill the eligibility criteria. The blood bank provides blood products for outpatients and hospitalized patients in government hospitals of Londrina and region. The blood supply is maintained through contributions from both repeat

donors and constant campaigns to stimulate blood donation. These campaigns are responsible for about 40.0% of the blood collected and are intended to increase repeat donations.

This study has a cross-sectional design with retrospective data collection of serological tests and CUEs from individuals clinically eligible for blood donation. Data from 51,861 consecutive donations during the period of January 2004 to December 2008 were analyzed using the database of the blood bank. The frequencies of the replies to the CUE were calculated in respect to gender, age, category of donation and local of collection. The CUE is one step in the donation process that, in this blood bank, is between the interview to evaluate the donor's clinical and epidemiologic conditions and the donation procedure itself. Donors receive a form of four questions related to same-sex partners, sex as a profession, multiple sex partners and intravenous drug use to which they can answer either yes or no. CUE is a confidential procedure and does not interfere in blood collection; the donation is always discarded when the CUE is positive.

The 51,104 donations successfully completed with the collection of blood bags were analyzed by comparing CUE results with serologic tests carried out in agreement with RDC 153/2004 of the National Agency of Sanitary Surveillance (ANVISA) of the Brazilian government.[4] According to this resolution, highly sensitive serologic tests are performed on all donations using commercial products registered in ANVISA to detect surface antigens of the hepatitis B virus (HBsAg), IgG antibodies against core antigens of the hepatitis B virus (anti-HBc), antibodies against the hepatitis C virus (anti-HCV), antibodies against the human immunodeficiency virus types 1 and 2 (anti-HIV 1/2), antibodies against the human T lymphotropic virus types I and II (anti-HTLV I/II), non-treponemic serologic test for syphilis (VDRL) and IgG antibodies against Trypanosoma cruzi. Reactive results obtained in laboratorial screening tests were confirmed using the same sample, in duplicate, with the same diagnostic reagents.

The investigation of anti-HIV 1/2 antibodies was carried out according to Brazilian legislation using two different commercial kits for sample screening.[4] In this study, a sample was defined as anti-HIV 1/2 reactive when it was reactive in both serologic tests and anti-HIV 1/2 inconclusive when the results obtained in the laboratorial screening tests were discordant (a negative result using one method and a reactive or gray zone result using the other).

Positive CUE donors were followed up in their subsequent donations to verify probable seroconversion. With serologically reactive results after the index donation, a new blood collection was requested to confirm the result with supplementary methods.

Parameters of sensitivity, specificity, positive and negative predictive values and accuracy were calculated for CUE using serologic tests as references. Statistical analysis was carried out by using EPI-INFO software and Microsoft Excel. Chi-square (χ^2) or Fisher exact tests were used to compare proportions. Absolute and relative frequencies of the parameters and Odds Ratio (OR) with a 95% confidence interval (95% CI) were also calculated. The study was approved by the Ethics Committee of the Universidade Estadual de Londrina.

Results

Blood donor population

Of the qualified donors, 32,073 (61.8%) donated at the blood bank (internal collections) and 19,788 (38.2%) donated blood at external collections; 18,876 (36.4%) donations were from first-time donors and 32,985 (63.6%) donations were from individuals who had already donated blood two or more times. A total of 29,824 (57.5%) donors were male and 22,037 (42.5%) were female. More than half of the donations (27,999; 54.0%) were from individuals aged from 18 to 29 years old; 23,862 (46.0%) were from over 30-year-old donors.

Self-exclusion and the frequency of reactivity in serologic tests

Of 51,861 donations evaluated, 1672 (3.2%) presented positive CUEs. Table 1 shows the CUE frequency according to gender, age, donation type and place of collection.

In 757 donations, the CUE form was answered but blood was not collected due to difficulty of vein access. Of the 51,104 remaining donations, 1672 (3.3%) presented positive CUEs and 1833 (3.6%) presented reactive results for at least one of the serological screening tests. Only 89 donations (0.2%) presented both parameters positive. The comparison of CUE with serological test results showed sensitivity of 4.9%, specificity of 96.8%, a positive predictive value of 5.3%, a negative predictive value of 96.5% and accuracy of 93.5% (Table 2).

The frequencies of reactivity to serological tests for donors who indicated their blood should be transfused or not were 3.5% and 5.3%, respectively ($\chi^2 = 15.07$; p-value < 0.0001 – Table 3). The highest frequencies were obtained for the anti-HBc serologic marker (2.2%), followed by anti-HCV (0.6%), VDRL (0.3%) and antibodies against *Trypanosoma cruzi* (0.2%). For the anti-HIV 1/2 serological marker, frequencies were 0.2% and 0.1% for inconclusive and reactive results, respectively.

The frequencies of reactive results were higher for all serological markers for donors who indicated their blood should not be transfused except for anti-HCV; they were significantly high for reactive anti-HBc, inconclusive anti-HIV 1/2, reactive anti-HIV 1/2 and reactive anti-*Trypanososma cruzi* results.

Table 1 - Frequency of self-exclusion in blood donors from Regional Blood Bank of Londrina, Paraná State, Southern Brazil, according to the local of collection, age, gender and type of donor

Variable	Total of donations	Self-exclusion n	Self-exclusion %	OR (95% CI)	χ^2	p-value
Local of collection						
Internal collection	32,073	948	3.0%	reference	-	-
External collection	19,788	724	3.7%	1.25 (1.13-1.38)	19.16	<0.0001
Type of donor						
First-time donor	18,876	881	4.7%	1.99 (1.80-2.20)	197.42	<0.0001
Repetition donor	32,985	791	2.4%	reference	-	-
Gender						
Male	29,824	1,228	4.1%	2.09 (1.87-2.33)	178.91	<0.0001
Female	22,037	444	2.0%	reference	-	-
Age						
18 to 29 years old	27,999	1,140	4.1%	1.86 (1.67-2.07)	139.52	<0.0001
30 to 65 years old	23,862	532	2.2%	reference	-	-
Total	51,861	1,672	3.2%	-	-	-

n: number of self-excluded donations; %: frequency of self-exclusion
OR (95% CI): odds ratio and 95% confidence interval

Table 2 - Distribution of complete donations from Regional Blook Bank of Londrina, State of Paraná, Southern Brazil, according to performance of confidential unit exclusion (CUE) and laboratorial screening tests results

Confidential unit exclusion (CUE)	Screening serological test		
	Reactive n (%)	Non-reactive n (%)	Total
Positive	89 (0.2%)[a]	1,583 (3.1%)[b]	1,672 (3.3%)
Negative	1,744 (3.4%)[c]	47,688 (93.3%)[d]	49,432 (96.7%)
Total	1,833 (3.6%)	49,271 (96.4%)	51,104 (100.0%)

Sensitivity: a/(a+c) = 4.9%; Specificity: d/(b+d) = 96.8%; Positive Predictive Value: a/(a+b) = 5.3%;
Negative Predictive Value: d/(c+d) = 96.5%; Accuracy: (a+d)/(a+b+c+d) = 93.5%

Table 3 - Frequency of serological markers in blood donors from Regional Blood Bank of Londrina, State of Paraná, Southern Brazil, that used self-exclusion and did not use self-exclusion during the period from January 2004 to December 2008

Serological Marker	Self-exclusion (+) n	Self-exclusion (+) %	Self-exclusion (-) n	Self-exclusion (-) %	Total of donations n	Total of donations %	OR (95%CI)	χ	p-value
Anti-HBc reactive	54	3.23	1,091	2.21	1,145	2.24	1.48 (1.11-1.97)*	7.26	0.0070
Anti-*Trypanossoma cruzi* reactive	9	0.54	101	0.20	110	0.22	2.64 (1.25-5.40)*	ND	0.0102 [1]
HBsAg reactive	5	0.30	89	0.18	94	0.18	1.66 (0.60-4.25)	ND	0.2384 [1]
Anti-HCV reactive	7	0.42	283	0.57	290	0.57	0.73 (0.32-1.60)	0.43	0.5104
Anti-HIV 1/2 reactive	5	0.30	52	0.11	57	0.11	2.85 (1.00-7.44)*	ND	0.0384 [1]
Anti-HIV 1/2 inconclusive	7	0.42	78	0.16	85	0.17	2.66 (1.12-5.98)*	ND	0.0214 [1]
Anti-HTLV I/I reactive	1	0.06	18	0.04	19	0.04	1.64 (#)	ND	0.4685 [1]
VDRL reactive	9	0.54	145	0.29	154	0.30	1.84 (0.88-3.72)	2.47	0.1163
Any serological marker reactive	89	5.32	1,744	3.53	1,833	3.60	1.54 (1.23-1.92)*	15.07	<0.0001
Total of samples	1,672	100.00	49,432	100.00	51,104	100.00	-	-	-

(+): positive self-exclusion; (-): negative self-exclusion; n: number of donations with a reactive serological marker; %: frequency of the serological marker in the sample;
OR (95% CI): odds ratio and 95% confidence interval; (*): p < 0.05; (1): Fisher exact test; (#): invalid confidence interval; Anti-HBc: antibodies against hepatitis B
virus core antigen; HBsAg: hepatitis B virus surface antigen; anti-HCV: antibodies against hepatitis C virus; anti-HIV 1/2: antibodies against human immunodeficiency
virus types 1 and 2; anti-HTLV I/II: antibodies against human T lymphotropic virus types I and II; VDRL: Venereal Disease Research Laboratories for antibodies against
cardiolipin; ND: not done.

Self-exclusion donor profile and investigation of probable seroconversion

Between January 2004 and December 2008, a total of 1478 donors indicated that their 1672 donations should not be used in transfusions. Of these, 1059 (71.7%) were men and 419 (28.3%) were women. Most of them were young; 987 (66.8%) were from 18 to 29 years old and 491 (33.2%) were over 30 years old at the time of the donation. According to the donor's history, the number of times these donors answered the CUE in the affirmative varied from one to seven in the period from January 1999 to December 2009. Most of the donors excluded themselves just once (1269 donors; 85.9%), 158 (10.7%) donors excluded themselves twice, 30 (2.0%) of them three times and the other 21 donors (1.4%) four times or more. The number of donations of these donors varied from one to 31. A total of 609 donors (41.2%) donated blood just one time and indicated that their blood should not be transfused. Another 309 donors (20.9%) that marked the CUE in the affirmative had donated blood on two previous occasions. The use of positive CUE in three, four and five or more donations was observed in 178 (12.0%), 92 (6.2%) and 290 donors (19.6%), respectively.

Eight donors were investigated for seroconversion because they had reactive serological screening tests after a positive CUE donation. All of them were male; six donated at internal collections and two at external collections. Just one donor was younger than 30 years old. Three donors were reactive to the serological screening test for anti-*Trypanosoma cruzi*, two for VDRL, two for anti-HCV and one for anti-HIV 1/2. Future collections of samples to confirm these reactive serological results showed that four cases were false-positive and these donors were readmitted for donation. The other donors were permanently deferred, one due to inconclusive diagnosis for Chagas' disease, two because of reactivity to VDRL and one did not return for further anti-HIV 1/2 tests. Of the two donors that were reactive for the non-treponemic serologic tests (VDRL), the result of one was confirmed by a treponemic method (FTA-ABS), while the other presented a false-positive result. The donor with inconclusive result for anti-HIV 1/2 did not provide another blood sample to check the result and so it was not possible to confirm seroconversion. This donor had donated blood 20 times and responded the CUE positively on two different occasions.

Discussion

The objective of this study was to analyze the effectiveness of the self-exclusion form used in the Regional Blood Bank in Londrina. Characteristics of the donor population and the effectiveness and limitations of the CUE instrument as a diagnostic method were assessed.

The results showed that most of the donors at the blood bank are male, in agreement with previous studies.[11-13] The majority of donors are very young which can be explained by the fact that Londrina has many universities and technology colleges where regular campaigns encourage first-year students to donate blood. About 3.6% of collected bags were seropositive with the most frequent marker being anti-HBc, followed by anti-HCV, VDRL and anti-*Trypanosoma cruzi*. These data are in agreement with those reported in 1997 (United States) where approximately 4.0% of blood units collected were discarded due to reactive screening results.[14] In Scotland, 1.0% of all donations are reactive for at least one of the serological markers tested.[15] In a study carried out in Curitiba, Paraná State in Southern Brazil, 7.2% of blood bags were discarded because of seropositivity; the most frequent markers were anti-HBc, followed by anti-*Trypanosoma cruzi,* VDRL and anti-HIV 1/2. Anti-HCV was not included because the test was not mandatory at that time.[11]

The frequency of self-exclusion in the Regional Blood Bank in Londrina was higher than that observed in donors from Curitiba (0.91%) but very similar to donors in Uberaba, Minas Gerais State in Southeast Brazil (2.7%).[11,16] High frequencies are related to low socioeducational conditions of donors, poor explanations from the staff about the correct use of CUE or misunderstanding of the self-exclusion instrument.[6,16] Some authors showed that the correct use of CUE is related to the level of the donor's educational.[17,18] Although this association may be true, the present study did not evaluate the association between the CUE and the donor's education due to the lack of information on the database of the institution. Self-exclusion was more frequent in male donors, those under 30 years old and those who were donating blood for the first time; this is in agreement with previously described results.[7] In the Regional Blood Bank of Uberaba, there was a similar profile, but over 30-year-old donors were more likely to respond affirmatively to the CUE than the younger group.[16]

Among the donors that excluded themselves, the frequencies of reactivity to anti-HBc, anti-HIV 1/2 and anti-*Trypanosoma cruzi* were significantly higher than in the group that did not. In Uberaba, the donors that indicated that their blood should not be transfused also had greater seropositivity for anti-HIV 1/2.[16] In a study conducted in 2001 (United States) that evaluated more than 6.5 million donations, donors that presented positive CUE had a higher frequency of reactivity to serological markers, except for anti-HTLV I/II. These authors reported that donors that excluded themselves had a 13-times greater chance of seropositivity for anti-HIV 1/2 than donors that did not and the chances were seven, five and four times greater for HBsAg, anti-HCV and syphilis markers, respectively.[19]

Although CUE considers direct and indirect markers of risk associated with sexually or intravenous transmissible diseases, this study also included Chagas' disease serology, because this is a mandatory screening test in Brazil and its exclusion from the statistical analysis would result in an incorrect number of discarded bags reactive in serological tests. Unexpectedly, a statistical significant association was

observed between anti-*Trypanosoma cruzi* reactivity and donors who indicated that their blood should not be transfused a fact that is impossible to explain and which needs to be further investigated in other cohorts of blood donors.

It is well known that syphilis transmission by blood transfusion is very rare, but the detection of treponemic or non-treponemic antibodies in blood donors is still justified because of the association of high risk sexual behavior and the transmission of HIV infection.[20] In the current study, 154 donations were reactive for VDRL and nine of them responded the CUE positively. One donor presented syphilis/HIV co-infection, and had excluded himself on five occasions. This case shows the importance of CUE in blood banks as a way to detect test-seeking donors.

Inconclusive results were observed in 85 donors for anti-HIV 1/2 and seven donations were accompanied by positive CUEs. In a low prevalence population such as blood donors, discordant results in screening serological tests are rarely associated with true HIV infection, that is, most are false-positive results. The correct conduct, due to possibility of seroconversion for HIV 1/2, is to follow up these donors with additional serological investigations.[21] No supplementary tests for diagnosis are done in the Regional Blood Bank in Londrina. Supplementary tests are performed only when the donor returns for an interview in the outpatient clinic. In this study, the results for anti-HIV 1/2 were separated in reactive and inconclusive in an attempt to differentiate probable true positive cases from false-positive ones because of the lack of confirmatory tests.

Donors that answered affirmatively to the CUE had greater chance of presenting reactive results in some of the laboratorial screening tests indicating the effectiveness of the current instrument to identify individuals with added risk for blood-borne infections. However, the low sensitivity and the low positive predictive value demonstrate that the device has a low power for diagnosis. A similar conclusion was reached in the United States where the use of CUEs showed sensitivity and positive predictive values of 2.3% and 3.5%, respectively.[7] It is difficult to compare CUE effectiveness among different blood banks because studies seldom describe the exact process. Sometimes it is not possible to evaluate the degree of confidentiality of the process, the wording of the instrument, the instructions offered by professional staff and the characteristics of the population studied.[22] Some authors alert about the clarity of the instrument, with the use of letters of an appropriate size and text that is easy to read and comprehend.[6,9] These aspects were well evidenced when the CUE form used in the Regional Blood Bank in Londrina before the period of this study was compared with the current one.[23]

CUE is an inexpensive procedure, but it increases the discard of blood bags without serological evidence of pathogen contamination. In this blood bank, about 3.0% of bags collected are discarded under these conditions. The follow up of donors who had affirmatively responded to the CUE showed poor evidence of seroconversion after self-exclusion. So, the question that should be asked is whether the cost-benefit compensates the discard of bags from potentially healthy individuals. Donor selection should offer a balance between the high safety for the transfusion and the low rejection of healthy donors.[1] Nowadays, the donor selection policies continue to include procedures of low specificity in an effort to increase the transfusional safety. The CUE was very important at the beginning of the HIV epidemic as a tool to identify donors that were potentially in the serologic window period. However, with the increase in the sensitivity of the serological methods currently used in screening evaluations, the access to anti-HIV 1/2 diagnostic tests to the general population, and the possibility of the use of molecular methods to screen blood donors, the use of CUE as a mandatory process in blood banks in Brazil should be reviewed. Some studies have been carried out to quantify the residual risk in blood donations and to review the impact of new selection criteria in terms of donor loss, costs for the blood banking system and consequences on the donor's life.[22] The impact of the CUE on blood stocks was examined in 3.7 million American blood donors where 322 blood donors presented seroconversion. This study showed that the process has a minimum impact in transfusional safety because of the rare number of donations in the serologic window period and the rare frequency of CUE use by donors in seroconversion, although donors that use CUE have 21 times greater probability of anti-HIV positivity.[8] In conclusion, some individuals are more likely to present positive CUE and should be instructed about safe donation. The high frequency of serologic reactivity in positive CUE donors indicates that the instrument is effective to identify individuals with high risk for blood transmissible infections, but it has low power for diagnosis purposes. The use of CUE leads to a high discard of blood bags from healthy individuals indicating that the method rarely contributes to transfusional safety and should be discontinued, mainly after the introduction of nucleic acid tests in the Brazilian blood bank screening routine.

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