

Logistic model for determining factors associated with HIV infection among blood donor candidates at the Fundação HEMOPE

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Conflict-of-interest disclosure:
 The authors declare no competing financial interest

Submitted: 2/1/2012
 Accepted: 2/8/2012

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DOI: 10.5581/1516-8484.20120053

Objective: To determine factors associated with HIV infection in blood donor candidates in Recife, Brazil.

Methods: A transversal study was performed of 106,203 blood donor candidates found eligible by the routine clinical screening process in the blood bank in Recife of the Fundação de Hematologia e Hemoterapia de Pernambuco (HEMOPE) in the period from January 1998 to November 2003. Additional indirect immune and western blot confirmation tests for HIV infection were performed and the candidates were classified as HIV positive or negative. The Chi-squared test and stepwise multiple logistic regression were conducted to examine any association between HIV infection and age, gender, place of residence, schooling, number of donations and serological tests for core hepatitis B antigen virus (anti-HBc), the hepatitis C antiviral (anti-HCV), human T-type antiviral lymph cells (anti-HTLV 1 and 2), serological tests for syphilis (VDRL) and the hepatitis B virus surface antigen (HBsAg).

Results: In the observed sample, 0.204% of blood donor candidates were found to be HIV positive. Among the studied variables, the age, education level, residency, donation type, and serologic status for anti-HBc and VDRL tests were found to be associated with HIV infection.

Conclusion: The younger, spontaneous donor candidates living in the Recife metropolitan area with a low level of education and positive for anti-HBc and VDRL have a higher risk of HIV infection than the other candidates. Data such as these are useful to understand the dynamics of infection and to guide healthcare policies.

Keywords: HIV Infections; Blood donation; Risk factors; Logistic models

Introduction

Despite the advances in world medicine, it has not yet been possible to find a cure for acquired immune deficiency syndrome (AIDS). The etiological agent of the syndrome, human immunodeficiency virus (HIV), was first identified in 1983 by the French scientist Luc Montagnier. It is a retrovirus belonging to the *Retroviridae* family, occurring in two forms HIV-1 and HIV-2. The clinical spectrum of HIV infection ranges from asymptomatic to advanced AIDS. Transmission of the virus may occur through sexual contact, contact with contaminated blood (parenteral) and from mother to child through breastfeeding (perinatal). Although every sexually active individual runs a risk of contamination, such risks are not evenly distributed, but vary according to certain practices constituting risk behavior involving greater contact with etiological agents. Examples of this are homosexual relationships with multiple partners, the use of illicit injectable drugs and prostitution. This paper focuses on parenteral infection, specifically transmission through blood transfusions which require the following precautions: avoiding unnecessary blood transfusions, screening out blood donors belonging to HIV risk group (increasing the number of low-risk donors) and the systematic detection of HIV antibodies in all donated blood⁽¹⁾.

As in most countries, Brazilian blood banks have, over the last few decades, adopted a number of strict practices governing the selection of blood donors, with clinical screening (questionnaires and interviews) being introduced as early as 1983. The aim has been to screen out candidates with the highest risk for infection on the basis of behavioral and serological tests, aiming at lowering the risk of transmission by transfusion. Historically, there has been a low prevalence of HIV-positive tests among blood donors⁽²⁻⁷⁾. However, owing to the immunological window, in which serological tests are unable to detect infection, there remains a residual risk of transmission, for which clinical screening still needs to be improved, as this "window" is related to false negative results in serological screening, implying a possibility of contamination of the recipient^(8,9). Post-transfusion AIDS, though rare, should be a constant concern of all people handling blood and its derivatives.

With the aim of contributing to knowledge on the dynamics of HIV infection among blood donors, this study was conducted using a cross-section sample of 106,203 blood donor candidates from January 1998 to November 2003, taking into account the routine clinical screening in one blood bank. A binary logistical regression model was used to identify variables

associated with HIV infection at the clinical screening stage (gender, age, schooling, marital status, place of residence and donation type) and those at the serological testing stage [tests for core hepatitis B antigen virus (anti-HBc), the hepatitis C antiviral (anti-HCV), human T-type lymphotropic virus antibodies (anti-HTLV 1 and 2), serological tests for syphilis (VDRL) and the hepatitis B virus surface antigen (HBsAg)], thereby quantifying the degree of risk presented by each group.

Methods

This study involved 106,203 prospective first-time blood donors in the period from January 1998 to November 2003 who had been approved in the clinical screening stage and proceeded to the serological testing stage. The criteria governing both clinical screening and serological testing were not devised specifically for this survey, but were the criteria routinely used in selecting blood donors in accordance with Brazilian legislation in force during the stated period⁽¹⁰⁻¹²⁾.

The clinical screening procedure consisted of questions to check factors to ensure the safety of donor and recipient by classifying the donor as suitable or unsuitable. The most important aspect of the questionnaire was that it showed whether the candidate engaged in behavior that is associated with a high risk of blood-transmitted diseases. All the blood donor candidates had already been approved at the clinical screening stage and were therefore considered fit donors; they had not reported exposure to HIV/AIDS risk situations. The aim, therefore, was to seek variables that might be indirectly related to behavior conducive to an enhanced risk of HIV infection including gender, age group, schooling, marital status, place of residence and donation type (autologous, specific, spontaneous or replacement). An autologous donation is made by the patient for their own use, a specific donation is for the use of a particular patient, spontaneous donations are those made as an act of altruism for general use and a replacement donation is that made by a donor who has a connection with an in-patient. The Recife metropolitan region comprises fourteen municipalities, Abreu e Lima, Araçoiaba, Cabo de Santo Agostinho, Camaragibe, Igarassu, Ilha de Itamaracá, Ipojuca, Itapissuma, Jaboatão dos Guararapes, Moreno, Olinda, Paulista, Recife and São Lourenço da Mata.

The serological tests for sexually transmitted diseases involves testing for the presence of anti-HBc, anti-HCV, anti-HTLV 1 and 2, VDRL and HBsAg with candidates being classified as positive, inconclusive and negative. During the study period, the "ELISA" method was employed in blood analysis; this is an immunoenzymatic test to detect HIV antibodies (Murex HIV Ag/Ab combination-Abbott, Murex HIV.1.2.0, Abbott), HB_c (Ortho-Clinical Diagnostics, Johnson-Johnson, New Jersey), HCV (Murex anti-HCV 4.0, Abbott), HBsAg (Murex HBs-Ag-Version 3, Abbott) and HTLV (Murex HTLV I+II, Abbott), while the VDRL flocculation test (Laboclin-VDRL, Brás) was used for the detection of syphilis. Further confirmatory tests following the screening tests were conducted only in the case of HIV-positive results. These included Indirect Immuno-fluorescence (Biomanguinhos, Oswaldo Cruz Foundation, Brazil) and Western Blot (Genelabs Diagnostics, Singapore) according to the protocol

for the confirmation of HIV at blood banks⁽¹¹⁾. Following the screening, each donor was diagnosed as HIV-positive or HIV-negative. All donors classified as inconclusive were excluded from this study. These criteria were adopted because in other studies they were generally accepted as associated with HIV infection and also with other sexually transmitted diseases.

A specific program was developed for this survey, enabling an ASCII text file to be retrieved containing all relevant data from the database of the blood bank of the Fundação de Hematologia e Hemoterapia de Pernambuco (HEMOPE) in Recife. The database holds data on all routine procedures in the blood donation process. This project was submitted to the Research Ethics Committee of HEMOPE and was approved on December 3, 2003.

Statistical analysis was conducted using the SPSS 10 statistical package. Backward stepwise logistic regression analysis was conducted to identify any association between the independent variables (gender, age, schooling, marital status, place of residence, donation type, serological tests for Anti-HBc, HBsAg, Anti-HCV, Anti-HTLV and VDRL) and the dependent variable "HIV infection" (Y = 1 for HIV-positive donors and Y = 0 for HIV-negative donors) The verisimilitude ratio test and Wald test were used with the significance level being set for a p-value < 0.05. The variables to be included in the logistic model were chosen on the basis of the biological and epidemiological interpretation and on a significance level for p-value < 0.20 using the Pearson's Chi-squared test. Following selection of "significant" co-variables, the suitability of the logistic model was checked using the Hosmer-Lemeshow test with a significance level of 10%. Finally, the strength of association between each co-variable and HIV infection was calculated and expressed as an estimated value by the adjusted Odds Ratio (OR) with a 95% confidence interval (95% CI).

Results

Characteristics of the sample of 106,203 first-time blood donors are shown in Table 1. Two hundred and seventeen (0.204%) of the blood donor candidates, who passed the clinical screening and formed the basis of this study, were diagnosed as HIV-positive. The results obtained from Pearson's Chi-squared independence test indicated that gender (p-value = 0.261) and the following serological markers anti-HCV (p-value = 0.608) and anti-HTLV (p-value = 1.0) had no statistically significant correlation (p-value < 0.20) with HIV infection. For this reason, these three variables were not included in the logistic model.

Of the remaining independent variables, HBsAg (p-value = 0.103) was eliminated in accordance with the verisimilitude ratio test at a significance level of 5%. Also, marital status showed no significance in the Wald test (W = 2.752; p-value = 0.097).

At a significance level of 5%, there is sufficient evidence to reject the statement that none of the factors in the final logistic model was correlated to HIV infection, under the verisimilitude ratio test ($\chi_{11}^2 = 126.579$, p-value = 0.000). Furthermore, the statistical value of the Hosmer-Lemeshow test was found to be C = 5.733 and p-value = 0.571, indicating that the multiple logistic model provides a satisfactory fit for the data in question.

Table 1 - Statistical analysis of HIV infection according to age, schooling, marital status, place of residence, type of donation, results of serological testing for: Anti-HBc, HbsAg and VDRL

	HIV infection					Pearson Chi-squared test p-value	Multiple logistic regression			
	Positive		Negative		Total		Wald test p-value	OR	95% CI	
	n (%)		n (%)						Lower	Upper
Age						0.059				
18 to 28	89	(0.2)	44,720	(99.8)	44,809		0.028	2.392	1.098	5.209
29 to 39	90	(0.25)	36,469	(99.75)	36,559		0.011	2.742	1.266	5.94
40 to 49	31	(0.17)	18,141	(99.83)	18,172		0.181	1.753	0.771	3.988
Over 50	7	(0.11)	6656	(99.89)	6663			1		
Schooling						0.024				
Illiterate	5	(0.41)	1222	(99.59)	1227		0.002	7.935	2.1	29.987
Elementary school	170	(0.21)	80,068	(99.79)	80,238		0.033	2.955	1.092	7.996
High school	38	(0.21)	18,083	(99.79)	18,121		0.039	2.971	1.059	8.335
Higher education	4	(0.06)	6613	(99.94)	6617			1		
Marital status						0.029				
Married/ widowed	87	(0.17)	50,608	(99.83)	50,695					
Single/ divorced/other	130	(0.23)	55,378	(99.77)	55,508					
Place of residence						0.003				
Recife metropolitan area	204	(0.22)	92,221	(99.78)	92,425		0.005	2.234	1.268	3.937
Smaller cities / other states	13	(0.09)	13,765	(99.91)	13,778			1		
Type of donation						0.000				
Autologous/ specific	1	(0.03)	3314	(99.97)	3315			1		
Spontaneous	81	(0.46)	17,661	(99.54)	17,742		0.014	11.799	1.646	84.568
Replacement	135	(0.16)	85,011	(99.84)	85,146		0.147	4.283	0.601	30.54
Anti - HBc						0.000				
Positive	53	(0.37)	14,354	(99.63)	14,407		0.000	1.968	1.43	2.706
Negative	164	(0.18)	91,632	(99.82)	91,796			1		
HbsAg						0.010				
Positive	7	(0.58)	1207	(99.42)	1214					
Negative	210	(0.2)	104,779	(99.8)	104,989					
VDRL						0.000				
Positive	24	(0.69)	3446	(99.31)	3470		0.000	3.232	2.101	4.972
Negative	193	(0.19)	102,540	(99.81)	102,733			1		
Total	217	(0.2)	105,986	(99.8)	106,203					

OR = Odds ratio; 95% CI = 95% confidence interval

In respect to age (Table 1), after adjusting for all other independent variables, the probability that donors in the 18-28 age bracket were HIV-positive was 2.392 times higher than those of the over 50-year-old age group (95% CI: 1.098-5.209). For donors in the 29-39 age bracket, the probability was 2.742 times higher (95% CI: 1.266-5.940) and for the 40-49 age bracket, the probability was 1.753 times higher (95% CI: 0.771-3.988). Furthermore, by the Wald test, it was found that the association with HIV infection was statistically significant only in the two lower age groups (p-value = 0.028 and p-value = 0.011).

It was found that the chances of being an HIV-positive prospective donor were inversely proportional to the level of schooling (Table 1) when all other variables in the model remained constant. The chances of being HIV-positive were 7.935 times higher (95% CI: 2.100-29.987) for individuals classified as illiterate compared to donor candidates with higher education levels. For those with only elementary schooling, the chances were 2.955 times higher (95% CI: 1.092-7.996) than the reference group. And finally, for donors with secondary schooling, the odds were 2.971 higher (95% CI: 1.059-8.335). All three groups had significant associations with HIV infection according to the Wald test.

Donor candidates living in the Recife metropolitan area were 2.234 times more likely to be HIV-positive than those living elsewhere (p-value = 0.005).

No correlation was observed (Table 1) between replacement type donations and HIV infection (Wald test p-value = 0.147), while the chances are 4.283 times in autologous/specific donations (95% CI: 0.601-30.540). For spontaneous donations, the chances of being HIV-positive were 11.799 times those of autologous/specific donors (95% CI: 1.646-84.568), while the correlation with HIV infection was statistically significant (Wald = 6.032; p-value = 0.014).

Anti-HBc-positive candidates had a 1.968 times higher chance of being HIV-positive than anti-HBc-negative candidates (Table 1). VDRL-positive candidates had a 3.232 times higher chance of being HIV-positive than VDRL-negative candidates.

Discussion

Out of the total sample, 217 donors (0.204%) were diagnosed as HIV positive. This is similar to rates reported for Brazilian populations, such as 0.9 to 1.3% for 1993 to 1999⁽²⁾, 1.25% for 2002⁽³⁾, 0.33% for 2003⁽⁴⁾ and an average of 0.28% for 2002 to 2004⁽⁵⁾. In a survey by Andrade Neto et al.⁽⁶⁾ for the 1992-1999 period, a much lower prevalence was found (0.149%). Similarly, Barreto et al.⁽⁷⁾ reported a rate falling from 0.204% in 1995 to 0.131% in 2001.

On the other hand, while it is not possible to state that different rates of prevalence of HIV-positive donors might potentially be associated with different geographical locations, it is known that the spread of the AIDS epidemic in Brazil did not follow the main axes of migration, but rather the communication networks linking places with vulnerable population groups. Geographically, the spread initially occurred in the main metropolitan areas, moving northward in a second stage, and finally southward in the late 1990s. An interesting aspect to be considered has to do with a movement of the AIDS epidemic away from state capitals into smaller towns and cities, though not exactly into rural areas. The fact that most Brazilians now live in urban areas, and bearing in mind that the dynamics of the AIDS epidemic is dependent upon multi-personal relationships, it is highly relevant that locations displaying a greater population density may favor its spread⁽¹³⁾.

While the infection rate among blood donors is relatively low in absolute terms, it may nevertheless be deemed a cause for concern, given that blood donation is a voluntary altruistic act, subject to a strict selection procedure. On the other hand, a higher number of infected donor candidates may be ascribed to the fact that it is not uncommon for people to use blood donations as a means of having their blood tested for purposes of diagnosis⁽¹⁴⁻¹⁶⁾, thus endangering the overall blood donation system, with HIV-positive donations possibly occurring in the “immunological window” phase; serological analysis is incapable of detecting the HIV antibody for up to thirty days following infection^(8,9).

Studies on transfusion safety have pointed to the need of adopting new technologies, with the use of molecular biology for detecting nucleic acids in pathogenic agents as a means of narrowing the “window” thus reducing the residual risk of HIV transmission. This test is capable of detecting the genome of the virus after ten to fifteen days of infection^(16,17). Two years have now passed since the Health Ministry issued its ruling setting up HIV and HCV nucleic acid tests (NAT) for blood samples taken from donors in the national blood bank network⁽¹⁸⁾ but the mechanism has not yet been implemented systematically throughout the whole country. This highlights the importance of clinical screening in the search for risk factors concerning pathogenic agents that can be transmitted by blood transfusions, as in the case of HIV.

This study showed a significant association between age and HIV infection in only two age brackets, namely 18-28 and 29-39, with the highest risk (OR = 2.742) among the latter group. This is in agreement with the findings of other studies⁽¹⁹⁾.

In the present study, no significant difference in infection was observed between men and women confirming the findings of Andrade Neto et al.⁽⁶⁾, Lima⁽²⁰⁾ and Eutáquio⁽²¹⁾.

When the education was analyzed, illiterate individuals were found to present the highest risk of being HIV-positive donor candidates. This confirms the findings of Souza⁽²²⁾ for the Testing and Counseling Centers (CTA), as well as those of Rodrigues Jr. et al.⁽²³⁾ for Pernambuco and other major urban centers in Brazil, pointing towards a tendency in this period for the “pauperization” of the AIDS epidemic. The data collected in different periods point to a trend in the contamination of individuals with lower levels of schooling (an indicator correlated to the socioeconomic level), suggesting the spread of the epidemic toward the less favored sections of society⁽²³⁾.

While spontaneous donors are regarded as ideal, because in theory they are practicing an act of altruism which would therefore suggest a lower incidence of HIV positive individuals, this is not what we found in the present survey. This finding may be ascribed to a misapprehension that is commonly found among high-risk individuals, that the results obtained at blood donor centers are more reliable than those obtained from specialized test laboratories⁽¹⁴⁻¹⁶⁾.

The anti-HBc reaction, when detected in isolation from other markers of the hepatitis B virus, may indicate a recent infection or an HBV infection in the past or a case of variant hepatitis when HBsAg is not detected. The importance of this marker is thus confirmed; it serves as a marker for the risk of other viral infections. Its association with HIV infection in this study is in agreement with the findings of Reiche et al.⁽²⁴⁾; this possibly occurs owing to the fact that these viral infections share a common means of transmission.

In this study, a positive VDRL result was found to be a factor associated with HIV infection. This is in disagreement with the findings of Reiche et al.⁽²⁴⁾ and Herrera et al.⁽²⁵⁾, whose conclusions were that the syphilis test is of little importance as an indirect marker for identifying and excluding donations by individuals presenting a high risk of HIV infection. There seems, however, to be some controversy on this subject in the literature: in Ethiopia, Rahlenbeck et al.⁽²⁶⁾ found that HIV positive donors presented a higher risk of being syphilis positive. In Brazil, Barcellos et al.⁽²⁷⁾ demonstrated a correlation between syphilis and HIV-positive individuals at an unnamed testing center. Almeida Neto et al.⁽¹⁴⁾ observed a correlation between syphilis and infection by the AIDS virus among blood donors, reporting that donors recently infected by syphilis have a forty times higher probability of also being HIV-positive compared to individuals with a longer history of syphilis, and almost fifty times higher probability compared to the overall population of blood donors.

In conclusion, an analysis of first-time blood donors at the Recife Blood Bank shows a high risk factor of HIV infection in younger donors, in donors with lower levels of schooling, for those testing positive for anti-HBc and VDRL, for those living in the metropolitan area and for those making spontaneous donations. These statistics are necessary for a better knowledge of the dynamics of infection and to provide guidelines for public health policies aimed at controlling the spread of the virus. This conclusion also reinforces the need for awareness campaigns to encourage informed blood donations as a responsible voluntary, altruistic gesture, principally among healthy candidates who do not present the risk profile for HIV infection.

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