High rate of seromarkers for HIV, HBV and syphilis among blood donors using confidential unit exclusion, before and after HIV-NAT implementation at a major public blood bank in the Brazilian Amazon

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BACKGROUND: Confidential unit exclusion (CUE) was introduced in the 1980's as an additional layer to blood safety, before highly specific and sensitive nucleic acid tests (NAT) for HIV were implemented. The utility of CUE-use in settings that have implemented NAT should be evaluated over time.

STUDY DESIGN, METHODS: Cross-sectional retrospective study carried out from June 2010–November 2015, at Manaus Hemocenter (HEMOAM), Amazonas, Brazil that implemented HIV-NAT in 2012. The HIV, HCV, HBV, HTLV, Chagas disease, and syphilis rates were compared among CUE and non-CUE blood donors, before and after HIV-NAT implementation.

RESULTS: Among 287,588 donations, 2,154 (0.75%) were associated with CUE, mainly voluntary donations (64.2%), by repeat donors (58.4%) from young (median age = 31 years), males (84.4%), unmarried (63.1%). CUE-users compared to non-CUE donors (n = 285,434) had higher seropositivity rates to HIV (OR = 6.09, 95% CI: 3.68–10.07, p < 0.001), HBV (anti-HBc OR = 1.81 95% CI: 1.24–2.64, p = 0.004; HBsAg OR = 5.68, 95% CI: 1.78–18.07, p = 0.017), and syphilis (OR = 1.78, 95% CI: 1.05–3.04, p = 0.030). Most (97.2%) discarded blood units associated to CUE was seronegative for all pathogens. Most donations (73.4%) were tested by HIV-NAT and showed four window period donations, positive by HIV-NAT only among non-CUE donors.

CONCLUSION: A high rate of transfusion transmissible infections/TTIs was observed at HEMOAM especially in CUE-users. CUE-use offered an additional layer of blood safety by its association with anti-HBc/HBsAg and syphilis that are not covered by NAT. For blood banks in highly endemic areas for HIV and TTI, as HEMOAM, the identification of at risk donors, and the orientation to be tested at proper sites remain a great challenge.

Worldwide safe blood transfusions have been continuously challenged by both emerging and known transfusion transmissible infections (TTIs). Brazil, the largest and most populous country in Latin America is also the most affected by HIV/AIDS. From 1980–2017, 882,810 AIDS cases were reported in the country.[1] In the recent decade, the Brazilian Amazon in the North region has shown an uncontrolled AIDS epidemic with growing prevalence and mortality rates.[1] This expansion sharply contrasts with a stable epidemic with decreased prevalence and mortality in its epicenter, located in Southeast Brazil.[1] A recent 21-year historical series from a major public blood bank from Amazonas State, the Hematology and Hemotherapy Foundation of Amazonas State (HEMOAM), described a significant rate of HIV infection in young male donors, suggesting high-risk, test-seeker behavior and potential risk of HIV transmission by blood transfusion.[2] In the isolated geographic context of Amazonas state where there is a high demand for blood transfusions, the control of an expanding AIDS epidemic and the implementation of safe transfusion procedures are both challenging and represent a critical component for the public health network.

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The confidential unit exclusion (CUE) was introduced in the US in the 1980's as an additional layer for blood safety, especially regarding HIV-1 infection, by giving individuals the chance to confidentially exclude their donations, however maintaining serological testing.[3] Studies have shown association of CUE and higher prevalence of serologic markers, however with low sensitivity and positive predictive value to indicate window period of TTI.[4–6] CUE was discontinued in most US blood centers, while in the recent decade it has been reported in Brazil, Belgium and Canada.[7–9] The controversy about the utility of CUE usage has increased especially after the implementation of highly sensitive and specific nucleic acid tests (NAT), which reduce, the risk of transmission during the window period.[6,10] The Brazilian blood bank regulation establishes that blood bank service, at its discretion, may offer the donor the opportunity to self-exclude on the basis of increased risk not informed or deliberately omitted during screening, on a confidential basis. At HEMOAM, CUE was introduced in June 2010 and HIV-NAT was implemented in July 2012. This study describes demographic characteristics and rates of TTI in blood donors (CUE-users, non-CUE users) before and after the HIV-NAT implementation at HEMOAM, located in a highly endemic region for HIV/AIDS in the Brazilian Amazon.

MATERIAL AND METHODS

Study subjects

This is a cross-sectional retrospective study among consecutive blood donations from HEMOAM from June 2010–November 2015, including donations with the CUE-option (CUE-users) and donations not associated with CUE (non-CUE). The HEMOAM is the only public blood bank institution and reference hemocenter linked to the Secretary of Health of the State of Amazonas and it is part of the Brazilian national blood bank network. This hemocenter follows the Ministry of Health guidelines for the National Blood Program[11] and is responsible for the donor recruitment, blood collection, processing, and distribution of blood and components to 24 public and 12 private hospitals in the Amazonas State (personal communication). The HEMOAM collects approximately 45,000–65,000 blood units annually.[12]

The HEMOAM is located in Manaus, the capital of Amazonas State which is a modern city of 2.5 million inhabitants[13] in the middle of the jungle on the Amazon rainforest, 900 mi inland from the Atlantic Ocean, with access primarily by boat or airplane. The Amazonas State located in the North Region of Brazil, with nearly 4 million inhabitants[13] is the largest Brazilian state by area, being greater than the areas of Uruguay, Paraguay, and Chile combined. The Amazonas State is roughly 90% the size of the US state of Alaska and is equivalent to 2.25 times the area of Texas.[13]

An electronic database program (Donor Management System) was implemented at HEMOAM in March 1993; in 20015 it was replaced by the HEMOSys tool (Hemocenter Management System) to supervise and assure safety in the entire blood cycle process. Since May 2017, the HEMOSys started implementing recipient's data. This electronic database aims to capture and track down the donor/donation information including the blood unit labeling/code, type of donation (voluntary/replacement), type of donor (first time/repeat), donor ID, gender, age, address, educational level, civil status, profession, and serological data. Demographic data, coded donor ID, CUE-option, age, gender, type of donor/donation, educational level, profession, address, civil status, and serological data from all donations from June 1, 2010 to November 30, 2015 were extracted from the computer systems of the Manaus hemocenter. From August 2012 on, the data bank included HIV/HCV- NAT results on all donations, which were also collected, prepared, and electronically sent to the authors for compilation and analysis. The HEMOSys tool is available to the institutional staff, according to the area of activity, with restricted access by area to assure data confidentiality.

Measures

After the donor health screening interview, and before the blood collection, as part of the routine CUE process and standard procedures employed at HEMOAM, a trained staff is responsible for providing an explanation about the CUE usage to the blood donor: "You have been approved for blood donation. However, if you have omitted any important information on risky behavior or if you are unsure about the safety of your blood, we are now offering you the opportunity of using the CUE-option. It means that if your answer is: "My blood cannot be used (transfused) to a patient," the blood bank will follow the standard routine laboratory testing and you will have free access to all the results of laboratory tests performed on your blood; however, your blood won't be transfused to a patient, your blood will be discarded for safety reasons. At HEMOAM the CUE-option uses the following grammatical format: "If you are not sure about the information given during the clinical screening interview, use this form to protect the patient who needs blood. This information is confidential and has no personal implication. Answer honestly by marking with (x) the chosen alternative: () My blood can be used (transfused) by the patient; () My blood cannot be used (transfused) by a patient."

At HEMOAM, the CUE form provided to each donor has the donor's adhesive barcode label. The CUE forms are completed privately, placed anonymously in a poll box, and the votes are checked twice a day by the staff in charge of reception/enrollment. The staff identifies and scans with a barcode reader the forms with the CUE-option, which automatically enters the information into the HEMOSys. If the donor does not answer any of the two CUE questions, this is considered a CUE-positive response for using the blood

for transfusion. Only donations associated with (x) My blood cannot be used (transfused) by a patient are discarded. The HEMOAM has no specific policy for repeat donors who have given different CUE-options on previous donations; therefore, repeat blood donors who have once used CUE have no interdiction in further donations.

Serological test procedures

At HEMOAM all blood donations are tested for HIV-1/2, HTLV-I/II, HBV, HCV, syphilis, and Chagas' disease according to the Brazilian Ministry of Health recommendations.[14] At the time of the study, the Brazilian blood bank HIV testing algorithm consisted of two different enzyme immune assays and if either one of the screening tests had a reactive result, the second blood sample required was tested with the same test assays. If a repeat-positive result was obtained, a confirmatory test was performed. The following kits were used for the screening:

- HIV-1: HIV Ag/Ab Combo Reagents Architect System (Abbott, Germany), Murex HIV Ag/Ab combination, (DiaSorin, Italy);
- HBV**:** Anti-HBc II Architect System, (Abbott, Germany), Anti-HBs Reagents Architect System (Abbott, Germany), Murex anti-HBs (Abbott, Germany);
- HBsAg**:** HBsAg Qualitative ll Architect System (Abbott, Germany);
- HCV**:** Anti-HCV Architect System (Abbott, Germany), Murex anti-HCV 4.0 (Abbott, Germany);
- HTLV-HTLV-I/II: Architect System, (Abbott, Germany);
- Chagas' disease: Chagas Architect System (Abbott, Germany), Chagas Test ELISA III (Abbott, Germany);
- Syphilis**:** IMMUTREP (Omega Diagnostics, Germany), Immune-rapid (WAMA Diagnostics, Belgium), VDRL (WAMA Diagnostics, Belgium), Syphilis TP Architect System (Abbott, Germany).

Confirmatory tests included HIV-1 BLOT 2.2 (MP Biomedicals SAS, France), CHIRON RIBA HCV 3.0 SIA (CHIRON, US) and HCV BLOT 3.0 (MP Diagnostics, Switzerland). All commercial kits used were approved by the Ministry of Health and the brands changed over time according to technical and operational availability.

From August 2012 on, a multiplex real time duplex HIV/HCV PCR nucleic acid test/NAT was used to detect HIV/HCV (Kit NAT HIV/HCV, Bio Manguinhos, Rio de Janeiro, Brazil) employing six sample mini-pools (Janus platform, Perkin Elmer). Whenever a positive pool was detected, all samples contained in that pool were re-tested individually to identify the positive one(s).[11]

Statistics and ethical aspects

The database containing demographic characteristics, serologic markers, HIV-NAT, and CUE-option was analyzed using EPINFO (version 7.0). The demographic characteristics and results of serological screening tests refer to blood donations; results from confirmatory tests and HIV-NAT refer to individual blood donors. Chi-square tests were used in univariate analyses to compare proportions in demographic data and of results from the serological screening (HIV-1/2, HTLV-I/II, HBV, HCV, syphilis, Chagas') and data from confirmatory tests (HIV-1/2, HBV, HCV, syphilis) in CUE-users and non-CUE users. Absolute and relative frequencies of the parameters and odds ratio (OR) with 95% confidence interval (95% CI) were also calculated. A probability lower than 0.05 ($p < 0.05$) and a 95% CI of OR that does not contain 1 were considered significant. This study was approved by the institutional review board ("Fundação de Hematologia e Hemoterapia do Estado do Amazonas, Plataforma Brasil" protocol # 1.129.896).

RESULTS

Main socio-demographic characteristics of the CUE and non-CUE associated donations

From June 2010 to November 2015 a total of 287,588 blood donations were given at HEMOAM and the CUE-option was used in 2,154 donations (0.75%) (Table 1). The main features of CUE-associated donations at HEMOAM included: males, young unmarried donors (median age = 31 years), mostly repeat donors, coming for voluntary donations. Regardless of CUE-option, most blood donations came from individuals with at least 9 years of formal education (96.5% CUE-donations, 92% non-CUE, $p < 0.05$). Compared to non-CUE, CUE associated donations had higher rates of males (OR = 1.67, 95% CI 1.49–1.88, $p < 0.001$), singles (OR = 1.46, 95% CI 1.34–1.60, $p < 0.001$), repeat donors (OR = 1.63, 95% CI 1.50–1.78, $p < 0.001$), individuals with at least 9 schooling years (9–11 years OR = 1.59, 95% CI 1.26–2.01, $p < 0.0001$; university degree OR = 4.47, 95% CI 3.54–5.64, $p < 0.0001$). CUE-associated donations had lower chance of including individuals aged 35–54 years (OR = 0.87, 95% CI 0.79–0.95, $p < 0.002$) and coming for voluntary donations (OR = 0.76, 95% CI 0.70–0.83, $p < 0.0001$) (Table 1).

Seroprevalence of TTIs in CUE-donations and non-CUE donations

Among 287,588 blood donations, 11,768 (4.1%) were positive for at least one serologic marker. TTI's seroprevalence has varied among CUE-associated and non-CUE donations (5.9% versus 4.1% respectively) (data not shown). In screening tests, CUE-associated donations were more likely to have positive results for HIV (OR = 4.06, 95% CI 2.62–6.08, $p < 0.0001$), anti-HBc (OR = 1.28, 95% CI 0.99–1.64, $p = 0.033$) and syphilis (OR = 1.59, 95% CI 1.08–2.35, $p = 0.016$) (data not shown). A total of 264 (0.09%) donations were seropositive for Chagas' disease, 3 (1.1%) of them associated with CUE-option.

The odds of having an HIV-positive confirmatory test was 6 times higher in CUE-donors compared to non-CUE

TABLE 1. Main demographic characteristics of CUE-positive and CUE-negative donations (n = 287,588) at HEMOAM, Manaus – AM (2010–2015)

	Total *(n = 287,588)*	CUE-donations (n = 2,154)	Non-CUE (n = 285,434)		
	n (%)	n (%)	n (%)	OR (95% CI)	*p-value*
Gender					
Female	67,887 (23.6)	337 (15.6)	67,550 (23.7)	1	
Male	219,701 (76.4)	1,817 (84.4)	217,884 (76.3)	1.67 (1.49—1.88)	<0.001
Age (years)					
16–34	174,601 (60.7)	1,383 (64.2)	173,218 (60.7)	1	
35–54	104,363 (36.3)	718 (33.3)	103,645 (36.3)	0.87 (0.79–0.95)	0.002
≥ 55	8,624 (3.0)	53 (2.5)	8,571(3.0)	1.29 (0.98–1.70)	0.068
Marital status *					
Married*	132,841 (46.2)	796 (36.9)	132,045 (46.3)	1	
Unmarried	154,747 (53.8)	1,358 (63.1)	153,389 (53.7)	1.46 (1.34–1.60)	<0.001
Donor status					
Repeat	133,188 (46.3)	1,257 (58.4)	131,931(46.2)	1	
First time	154,400 (53.7)	897 (41.6)	153,503 (53.8)	1.63 (1.50—1.78)	<0.001
Donation status					
Replacement	155,295 (54.0)	1,021 (47.4)	154,274 (46.0)	1	
Voluntary	132,293 (46.0)	1,133 (52.6)	131,160 (54.8)	0.76 (0.70–0.83)	<0.0001
Education (years)					
≤ 8 years	22,929 (8.0)	76 (3.5)	22,853 (8.0)	1	
9 ≤ 11 years	191,630 (66.6)	1,008 (46.8)	190,622 (66.8)	1.59 (1.26–2.01)	<0.0001
university	73,029 (25.4)	1,070 (49.7)	71,959 (25.2)	4.47 (3.54–5.64)	<0.0001

n = absolute frequency; p-value < 0.05.

* Marital status: married included all types of stable couples; unmarried: included also divorced and widow individuals who did not report a stable partner.

TABLE 2. Results from confirmatory tests among CUE-users and non-CUE-users who had at least one positive test in the serologic screening

	CUE-donor		Non-CUE			
	Screening *(n)*	Confirmatory (n, %)	Screening *(n)*	Confirmatory (n, %)	OR (95% CI)	*p*-value
HIV 1/2	25	16 (0.74)	1,423	350 (0.12)	*6.09 (3.68–10.07)*	<0.001
HBV/anti-HBs	64	20 (0.93)	6,717	2,277 (0.10)	*1.16 (0.74–1.81)*	0.279
HBV/anti-HBc	47	28 (1.29)	4,528	2,056 (0.72)	*1.81 (1.24–2.64)*	0.004
HBV/HBsAg	6	3 (0.14)	576	70 (0.02)	*5.68 (1.78–18.07)*	0.017
HCV	6	4 (0.19)	936	104 (0.04)	*5.10 (1.87–13.86)*	0.090
Syphilis	26	14 (0.65)	2,194	1,040 (0.36)	*1.78 (1.05–3.04)*	0.030
HTLV-I/II	3	1 (0.05)	329	288 (0.10)	*0.45 (0.06–3.27)*	0.362

%: relative frequency; p-value < 0.05; OR: odds ratio with 95% confidence interval (95% CI).

HIV: human immunodeficiency virus type 1 and 2; HBV: hepatitis B virus, anti-HBc: antibodies to the total hepatitis B core antigen; HBsAg: hepatitis B surface protein antigen; HCV: hepatitis C virus; HTLV: human lymphotropic virus type I/II. Except for HIV, HCV, and syphilis, donors with any positive result in the screening tests were recruited to return to the blood bank to have another blood sample collected which was tested with the same kit used for screening and the data bank reported repeat-positive results as "confirmatory" tests.

donors (OR = 6.09, 95% CI 3.68–10.07, p < 0.001) (Table 2). It is noteworthy that despite the higher rate of HIV in CUE-users, the rate of HIV-positive results among non-CUE donors was also high. Regardless of the CUE-usage, 366 blood donors were diagnosed with HIV infection during the study period (Table 2). The overall prevalence of HIV has varied among CUE-users and non-CUE donors (0.74% versus 0.12%; p < 0.001).

CUE-donors also had higher confirmed positivity for anti-HBc and HBsAg (anti-HBc OR = 1.81, 95% CI 2.24–2.64, p = 0.004; HBsAg OR = 5.68, 95% CI 1.78–13.06, p = 0.017). CUE-donors had increased confirmed seromarker for syphilis (OR = 1.78, 95% CI 1.05–3.04, p = 0.030) (Table 2). Seropositivity for HCV and HTLV-I/II was similar in CUE and non-CUE-donors (Table 2).

HIV positive donors during window period identified by NAT only

HIV-NAT was implemented at HEMOAM in June 2012 and covered part of our study period (2010–2015). During that overlap in time, 73.4% donations (211,075 out of 287,588) were also tested by NAT: 63.4% of CUE-users (1,818 out of 2,154 donors) and 73.83% of non-CUE donors (210,739 out of 285,434 donors) (data not shown). Nearly one-fourth (26.61%) of participants were tested by serological methods

TABLE 3. Characteristics of HIV-positive donors by CUE-usage option at HEMOAM, Manaus

	Total (n = 366)	CUE-donor (n = 16)	Non-CUE (n = 350)		
	n (%)	n (%)	n (%)	OR (95%CI)	*p-value*
Gender					
Female	76 (20.8)	3 (18.8)	73 (20.9)	1	
Male	290 (79.2)	13 (81.2)	277 (79.1)	1.14 (0.32–4.11)	0.889
Age (years)					
16–34	284 (77.6)	14 (87.5)	270 (77.1)	1	
35–54	77 (21.0)	2 (12.5)	75 (21.4)	0.52 (0.08–2.04)	0.408
≥ 55	5 (1.4)	---	5 (1.4)	---	---
Marital status*					
Married*	63 (17.3)	2 (12.5)	61 (17.5)	1	
Unmarried	303 (82.7)	14 (87.5)	289 (82.5)	1.47 (0.32–6.66)	0.664
Donor					
Repeat	222 (60.7)	7 (40.0)	215 (61.5)	1	
First time	144 (39.3)	9 (60.0)	135 (38.6)	2.05 (0.74–5.63)	0.173
Donation					
Voluntary	152 (41.6)	7 (40.0)	145 (41.5)	1	
Replacement	214 (58.4)	9 (60.0)	205 (58.5)	0.90 (0.33–2.49)	0.850
Education (years)					
≤ 8 years	61 (16.7)	14 (87.5)	47 (13.4)	1	
9 ≤ 11 years	208 (56.8)	---	208 (59.4)	---	---
university	97 (26.5)	2 (12.5)	95 (27.2)	0.07 (0.02—0.32)	<0.0001

*Marital status: married included all types of stable couples; unmarried: included also divorced and widow individuals who did not report a stable partner.

only (CUE-users: 36.6%, 788 out of 2,154; non-CUE users: 26.17%, 74,698 out of 285,434) (data not shown). Among the 16 HIV-positive CUE-users, five were confirmed by NAT while 11 donors were diagnosed before NAT (data not shown). In the 350 HIV-positive non-CUE users, 229 were confirmed by NAT. Therefore, regardless of CUE-use, a total of 334 HIV-positive donors were also confirmed by HIV-NAT (data not shown). Among these, 330 donors were positive by HIV serology and HIV-NAT. During the study period, four seronegative, NAT positive, non-CUE donors were identified (data not shown): young males (21–28 years old), first time (n = 2) and repeat donors (n = 2), reporting two and three previous donations each (data not shown). During the study period, 639 out of 2,154 repeat blood donors, who used CUE at index donation, returned for donation, but none of them re-used CUE-option. Among them, 9 out of 639 (1.4%) tested positive for HIV-1/2 during screening and one 24-year-old male donor had a positive confirmatory test (0.15%; 1 out of 639) (data not shown).

Epidemiological profiles of HIV, anti-HBc, HBsAg, and syphilis-positive donors by CUE-usage option

The socio-demographic characteristics of 366 HIV-positive donors stratified by CUE-option (Table 3) show the predominance of young males, nearly 50% within the 25–34 age range and more than 80% unmarried, 60.7% repeat donors, 58.4% coming for replacement donations, 56.8% with up to 11 years of education. Most HIV-positive CUE-users were male, within 25–34 years, single, first time, providing a replacement donation with up to 8 schooling years. Socio-demographic characteristics of HIV-positive CUE-users and non-CUE donors were similar ($p > 0.05$) except for the educational level. HIV-positive CUE-users were concentrated in the lower educational status (≤ 8 years: 87.5%), while the majority of HIV-positive non-CUE user (86.6%) had higher educational level (OR = 0.07, 95% CI 0.02–0.32, $p < 0.0001$).

Among 2,084 anti-HBc positive blood donors (Table 4), most were male, within 16–34 years of age, unmarried, first-time donors coming for replacement donations with ≤ 11 years of education. The socio-demographic features of anti-HBc-positive CUE and non-CUE donors differed by the lower rate of positivity among repeat non-CUE donors (Table 4), (OR = 0.10 95% CI 0.03–0.26, $p < 0.001$). Apart from the CUE usage, the 73 HBsAg-positive donors (Table 5) were mostly males, within 16–34 years of age, married, repeat donors coming for replacement donations, nearly half of them with 9 to 11 years of education (Table 5).

Despite the CUE-option, there were 1,054 syphilis-positive donors (Table 6) that were mostly males, within 16–34 years, unmarried, first-time donors coming for replacement donations, having from 9 to 11 schooling years. Between CUE and non-CUE syphilis-positive donors, a lower rate of positivity was seen in CUE-users with up to 11 schooling years ($p = 0.026$).

DISCUSSION

This study conducted at a major public blood bank in the Brazilian Amazon showed that the CUE-usage was associated with high rates of HIV, HBV (anti-HBc/HBsAg), and syphilis. Out of nearly 300,000 blood donations, only 0.75% had used the CUE option; nevertheless, CUE-users

TABLE 4. Characteristics of 2,084 a-HBc-positive donors stratified by CUE-usage option at HEMOAM, Manaus – AM (2010–2015)

	Total (n = 2,084)	CUE-donor (n = 28)	Non CUE (n = 2,056)		
	n (%)	n (%)	n (%)	OR (95%CI)	*p*-value
Gender					
Female	702 (33.7)	11 (39.2)	691(33.7)	1	
Male	1,382 (66.3)	17 (60.8)	1,365 (66.3)	0.79 (0.36–1.68)	0.528
Age (years)					
16–34	1,016 (48.6)	18 (64.3)	998 (48.4)	1	
35–54	949 (45.5)	10 (35.7)	939 (45.6)	0.59 (0.26–1.28)	0.186
≥55	119 (5.9)	---	---	---	
Marital status *					
Married*	976 (46.9)	8 (28.6)	968 (47.1)	1	
Unmarried	1,108 (53.1)	20 (71.4)	1,088 (52.9)	2.22 (0.97–5.07)	0.052
Donor					
First time	1,408 (67.5)	5 (17.9)	1,403 (68.2)	1	
Repeat	676 (32.5)	23 (82.1)	653 (31.8)	0.10 (0.03–0.26)	<0.001
Donation					
Replacement	1,432 (68.7)	19 (67.8)	1,413 (68.7)	0.96 (0.43–2.13)	
Voluntary	652 (31.3)	9 (32.2)	643 (31.3)	1	0.53
Education (years)					
≤ 8 years	487 (23.3)	5 (17.8)	482 (23.4)	1	
9 ≤ 11 years	1,324 (63.5)	20 (71.4)	1,304 (63.4)	1.48 (0.55–3.96)	0.452
university	273 (13.2)	3 (10.8)	270 (13.2)	1.08 (0.25–4.52)	0.908

*Marital status: married included all types of stable couples; unmarried: included also divorced and widow individuals who did not report a stable partner.

TABLE 5. Characteristics of 73 HBV/HBsAg-positive donors by CUE-usage option at HEMOAM. Manaus – AM (2010–2015)

	Total (n = 73)	CUE-donor (n = 3)	Non-CUE (n = 70)		
	n (%)	n (%)	n (%)	OR (95% CI)	*p*-value
Gender					
Female	26 (35.6)	---	26 (37.2)	1	
Male	47 (64.4)	3 (100.0)	44 (62.8)	---	0.160
Age (years)					
16–34	34 (46.4)	1 (33.3)	33 (47.0)	1	
35–54	33 (45.1)	2 (66.7)	31 (44.2)	2.11 (0.15–64.58)	0.603
≥ 55	6 (8.5)	---	6 (8.8)	---	0.333
Marital status *					
Married*	39 (53.5)	1 (33.3)	38 (54.2)	1	
Unmarried	34 (46.5)	2 (66.7)	32 (45.8)	2.35 (0.17–71.77)	0.544
Donor					
Repeat	40 (54.7)	3 (100.0)	37 (52.8)	1	
First time	33 (45.2)	---	33 (63.6)	---	0.465
Donation					
Replacement	47 (64.3)	3 (100.0)	44 (62.8)	1	
Voluntary	26 (35.7)	---	26 (37.2)	---	0.704
Education (years)					
≤ 8 years	13 (17.8)	---	13 (18.7)	1	
9 ≤ 11 years	37 (50.6)	3 (100.0)	34 (48.5)	---	0.273
university	23 (31.6)	---	23 (32.8)	---	0.632

*Marital status: married included all types of stable couples; unmarried: included also divorced and widow individuals who did not report a stable partner.

concentrated 4.4% of all HIV-infected donors with six times higher odds of having an HIV-positive result. On the other hand, HIV positivity was also elevated in non-CUE users. These results are consistent with other studies.[6,15] During the study period, four window period donations detected by HIV-NAT only were from non-CUE users indicating a good performance of the Brazilian HIV-NAT test. Previous studies have shown both low sensitivity and predictive value of CUE to identify window period donations.[7–9] These non-CUE users window period HIV cases suggest that the donors might had been concerned in using the CUE-option and not having their blood tested, overcoming all the blood bank screening barriers in order to donate blood and getting tested. Two of the window period were repeat donors with

TABLE 6. Characteristics of syphilis-positive donors by CUE-usage option (n = 1,054) at HEMOAM, Manaus AM (2010–2016)

	Total (n = 1,054)	CUE-donor (n = 14)	Non-CUE (n = 1,040)		
	n (%)	n (%)	n (%)	OR (95% CI)	*p*-value
Gender					
Female	289 (27.4)	2 (14.3)	287 (27.6)	1	
Male	765 (72.6)	12 (85.7)	753 (72.4)	2.28 (0.57–15.13)	0.285
Age (years)					
16–34	613 (58.2)	9 (64.3)	604 (58.1)	1	
35–54	397 (37.7)	5 (35.7)	392 (37.7)	0.86 (0.26—2.58)	0.803
≥ 55	44 (4.2)	---	44 (4.2)	---	
Marital status *					
Married	394 (37.4)	4 (28.6)	390 (37.5)	1	
Unmarried	660 (62.6)	10 (71.4)	650 (62.5)	1.50 (0.48–5.55)	0.518
Donor					
Repeat	439 (41.6)	6 (42.9)	433 (41.7)	1	
First time	615 (58.4)	8 (57.1)	607 (58.3)	0.95 (0.32–2.95)	0.919
Donation					
Replacement	618 (58.6)	8 (57.1)	610 (58.6)	1	
Voluntary	436 (41.4)	6 (42.9)	430 (41.4)	1.06 (0.34–3.16)	0.902
Education (years)					
≤ 8 years	154 (14.6)	5 (35.7)	149 (14.3)	1	
9 ≤ 11 years	684 (64.9)	5 (35.7)	679 (65.2)	0.22 (0.06–0.83)	0.026
university	216 (20.5)	4 (28.6)	212 (20.5)	0.56 (0.13–2.26)	0.415

*Marital status: married included all types of stable couples; unmarried: included also divorced and widow individuals who did not report a stable partner; n = absolute frequency; p-value < 0.05.

2 and 3 previous donations; one used the CUE option. A previous Brazilian study suggested that NAT alone would not be sufficient to reduce transmission as observed in the US or Europe.[16] During the study period (66 months, 5.5 years), 366 HIV-infected blood donors were detected, representing nearly 67 HIV-positive blood donors diagnosed per year. Official data show that the Amazonas State ranks third in the number of new AIDS cases detected in Brazil, which are characterized by late diagnosis and high mortality.[1] Our results of HIV among HEMOAM blood donors reflect the high endemicity for HIV/AIDS in Manaus, Amazonas, and potentially a high-risk, test-seeking behavior.

Previous Brazilian studies on CUE use[17–19] have shown rates of CUE use ranging from 0.62% to 3.3% while in our study 0.75% donors used CUE. CUE usage rates vary according to different socio-educational and cultural factors. While CUE-users from two of these studies were mostly first-time donors, in our study CUE-users were repeat donors, suggesting a possible change in the CUE-user profile. In the context of a highly endemic area for HIV, as the Amazonas State, the rate of seroconversion in future donations of those repeat donors who once used CUE should be evaluated.

After the implementation of NAT for HIV, HBV, and HCV by blood banks, CUE usage is no longer useful to prevent donations of blood contaminated with these pathogens. However, our results of CUE association with seromarkers, which are not covered by NAT, as syphilis and HBsAg/anti-HBc, indicate that CUE usage at HEMOAM indeed adds an additional layer for blood safety. However, over 95% discarded blood units associated with the CUE-option was seronegative for all pathogens, but for HEMOAM the evaluation of the cost–benefit of discarding safe blood units associated with CUE-usage, prioritizes the benefit of higher safety.

In a highly endemic area for HIV, as Manaus, /Amazonas, one could speculate that high-risk test seekers might use the blood bank due to the lack of, or due to difficulties in accessing, voluntary testing centers. However, Manaus has 73 widespread basic public health units ("Unidade Básica de Saúde/UBS/SUS") that perform nearly 2,500 rapid tests monthly (HIV, syphilis, HBV, HCV).[20] Additionally, since 2014, once a month, a fluvial health unit ("Barco Catuira") offers rapid tests for riverine communities, given that the Amazonas State represents a vast territorial area in which the rivers are often the only transportation route available.[20] In all these public health units, pre and post-testing counseling are available and results are offered within 30 minutes.[20] In addition, in Manaus, eight large reference volunteer testing centers (VTC) perform rapid tests (HIV, HCV, HBV, syphilis), three large public health hospitals offer free-of-charge post-exposure prophylaxis (PEP) and a large reference hospital offers free pre-exposure prophylaxis (PrEP).[20] Therefore, lack of testing sites is certainly not the explanation for why high-risk blood donors from Manaus use the blood bank. Nevertheless, studies have shown the blood bank "magnet effect" by which donors believe that HIV tests performed at the blood banks are more reliable than those performed elsewhere. As the HIV-NAT testing is only performed at the blood banks, this probably corroborates this perception. At HEMOAM the implementation of HIV-NAT was not publicized, as this

information could reinforce this notion by the general population attracting potential test-seeking donors. Also, more recently, as an attempt to avoid test-seekers, HEMOAM only provides serologic results 30 days after the donation.

Another possibility for test-seeking behavior at HEMOAM is the lack of knowledge and understanding about HIV, TTIs, and the CUE option. In our study, regardless of the CUE-option, most blood donations came from individuals with at least 8 schooling years, similarly to other Brazilian donor populations from different geographic regions.[8,21,22] At HEMOAM, HIV-positive non-CUE users had a higher education level than HIV CUE-users, indicating that the pre-donation educational information on HIV risk factors has increased HIV knowledge; however, it has not prevented high-risk individuals from donating blood. The institutional challenge remains to find a strategy to benefit from the CUE process to prevent high-risk donors from providing donations. Our results suggest that the more donors learn, the more they seem capable of overcoming the screening barriers to donate blood without complying with the blood donation requirements and regulations. In our study, higher education did not prevent donations of high-risk individuals, but lower education did lead to higher levels of TTIs as HIV. Also, syphilis-positive CUE-users were highly concentrated in the lower education group.

The Brazilian Ministry of Health/Viral Hepatitis and Sexually Transmitted Diseases Department promotes several campaigns yearly to increase awareness about HIV/AIDS, acquired/congenital syphilis, and viral hepatitis.[23] Short-duration TV educational videos and social media are released especially during special events as Carnival, AIDS World Day, and National Prevention Day for Syphilis. Also, informative folders are placed at strategic locations targeting youngsters, pregnant women, homo-affective couples, and transgender women.[23]

Our study has limitations inherent to the use of a large institutional databank and we cannot exclude potential flaws during the CUE process either in the explanation, its purpose, or its comprehension. We also recognize that the grammatical format of the CUE option may lead to unclear answers. The higher rate of TTI in CUE-users suggests test-seeker behavior, however, the potential reasons for test-seeking are not necessarily associated with high-risk behavior. Some blood donors may be healthy, "worried-well" individuals, with no risk behavior that just want to be sure about their good health status benefiting from the free and reliable tests provided by the donation at a blood bank. There is no way to predict if these "worried-well" donors could have used the option "My blood can be used/ transfused" or the alternative: "My blood cannot be used/transfused." In contrast, at-risk individuals may have chosen the option "My blood can be used/transfused" for fear of not being tested if they used the other option. Therefore, regardless of the grammatical format used, or the level of comprehension about the CUE aim, the motivation for selecting the CUE-option can only be supposed and the interpretation of results should take these limitations into account.

Syphilis transmission by blood transfusion is very rare, however, syphilis seromarkers in blood donors suggest association with high-risk sexual behavior and HIV infection.[24] Despite the availability of simple diagnostic tests and effective single dose long-acting penicillin treatment, syphilis currently represents a re-emerging global public health problem.[25] Syphilis prevalence in Brazil is 0.85% with the highest incidence of congenital syphilis in Latin America and a steady increase in pregnant women, congenital, and acquired syphilis.[25,26] In our study, syphilis prevalence of 0.65% and 0.36% was reported in CUE and non-CUE-users respectively. Compared to the national rates, the Brazilian Amazon registered higher levels of active syphilis, HBV/HBsAg, and HIV in vulnerable population from the triple Brazil-Colombia-Venezuela border.[27] A recent rapid test screening of indigenous populations in remote Brazilian Amazon areas showed an overall prevalence of 0.13% for HIV and 1.82% for syphilis.[28]

In our study, the prevalence of seromarkers for HBV infection (HBsAg, anti-HBc) was higher in CUE-users. Despite its low population density, the Brazilian Amazon has long been considered highly endemic for HBV, where it may be associated with hepatitis delta virus and worse prognosis.[29,30] The north region is responsible for 14.2% of official HBV cases and has continually shown rates above the national level, Manaus ranking sixth in the among capitals[31] Our data from HEMOAM are compatible with an HBV endemic area where the HBV-NAT, an additional blood safety tool for donations, was implemented in December 2014 and results were included in the HEMOSys in September 2015.

CONCLUSIONS

A high rate of TTIs was observed at HEMOAM especially in CUE-users, however CUE-usage was not associated with HIV window period donations. Despite the loss of seronegative units associated with CUE-option, the CUE usage offered an additional layer of blood safety locally by its association with HBsAg/anti-HBc and syphilis that are not covered by NAT. One of the biggest challenges for blood banks, especially the ones located in highly endemic areas for HIV and TTI as HEMOAM, remains how to identify at-risk donors that deny risky behavior and how to orient these individuals to get tested at proper sites.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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