





Results of a sample-to-cutoff ratio using Abbott Architect rHTLV-I/II assay allow to predict detection of HTLV-1 and HTLV-2 proviral DNA by real-time PCR

Resultados da razão sinal da amostra sobre o *cutoff* da reação no ensaio Abbott Architect rHTLV-I/II permite prever a detecção de DNA proviral por PCR em tempo real

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Received/Recebido: 24.03.2022 – Accepted/Aceito: 16.05.2022

ABSTRACT

The present study aims to correlate the sample-to-cutoff ratios (S/CO) distributions of reactive results for HTLV-1/2 antibodies with the detection of proviral DNA in a population of blood donor candidates. It was carried out a retrospective data search of 632 HTLV-1/2 reactive samples, submitted to confirmatory testing from January 2015 to December 2019. Serological screening was performed by chemiluminescent microparticle immunoassay Architect rHTLV-I/II, whereas confirmatory testing was performed by in-house real-time polymerase chain reaction method. 496 out of 632 samples (78%) had undetectable HTLV-1/2 proviral DNA and 136 (22%) had detectable proviral DNA. HTLV infection was not confirmed in any individual for whom the S/CO ratio value was <4 , and proviral DNA detection rates gradually escalated as S/CO ratio values increased. The sensitivity and predictive positive value found for the Architect rHTLV-I/II was 100% and 22%, respectively. The receiver operating characteristic (ROC) curve analysis showed that the optimal S/CO ratio value for predicting the presence of HTLV-1/2 was 18.11. High S/CO ratios were more associated with the detection of proviral DNA. The S/CO ratio value <4 suggests excluding true HTLV infection and the risk of blood transmission.

Keywords. HTLV-1 Infections, HTLV-2 Infections, Chemiluminescent Microparticle Immunoassay (CMIA), Real-Time Polymerase Chain Reaction, Blood Donors.

RESUMO

O estudo tem como objetivo correlacionar às distribuições das razões *sample-to-cutoff* (S/CO) de resultados reagentes para anticorpos HTLV-1/2 com a detecção de DNA proviral em uma população de candidatos à doação de sangue. Realizou-se uma busca retrospectiva de dados de 632 amostras reagentes para HTLV-1/2 submetidas à testagem confirmatória entre janeiro de 2015 a dezembro de 2019. A triagem sorológica foi realizada pelo imunoensaio quimioluminescente de micropartículas Architect rHTLV-I/II, enquanto o teste confirmatório foi realizado pelo método de PCR em tempo real *in-house*. 496 de 632 amostras (78%) apresentaram DNA proviral indetectável e 136 (22%) apresentaram DNA proviral detectável. A infecção por HTLV não foi confirmada em nenhum indivíduo com valor de S/CO <4 e as taxas de detecção de DNA proviral escalonaram gradualmente à medida que as razões S/CO aumentaram. A sensibilidade e valor preditivo positivo encontrados para o Architect rHTLV-I/II foram 100% e 22%, respectivamente. Utilizando análise de curva ROC, o valor de razão S/CO ideal para prever a presença de DNA proviral foi de 18,11. Razões S/CO elevadas foram mais associadas à detecção de DNA proviral. Em suma, o valor de S/CO <4 sugere a exclusão de infecção por HTLV e o risco de transmissão pelo sangue.

Palavras-chave. Infecções por HTLV-1, Infecções por HTLV-2, Imunoensaio Quimioluminescente de Micropartículas (CMIA), Reação em Cadeia da Polimerase em Tempo Real, Doadores de Sangue.

INTRODUCTION

The human T-lymphotropic virus (HTLV) is a member of the family *Retroviridae*, genus *Deltaretrovirus*¹. It was the first human retrovirus to be discovered and associated with cancer, and it is currently classified into types 1, 2, 3 and 4, although both HTLV-3 and HTLV-4 have not been consistently linked to diseases^{2,3}. Conversely, HTLV-1 and 2 infection is considered pathogenic, with HTLV-1 primarily infecting CD4+ T lymphocytes, which can lead to fatal and/or inflammatory and neurodegenerative diseases such as adult T-cell leukemia (ATL) and HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP), respectively. HTLV-2 infects primarily CD8+ T lymphocytes and its pathogenicity is less certain since it is rarely associated with other diseases^{4,5}.

It is estimated that 5 to 10 million people worldwide are infected with HTLV-1. The Southwestern part of Japan, sub-Saharan Africa, South America, the Caribbean area, and a few foci in the Middle East and Australo-Melanesia are considered HTLV-1 endemic regions⁶. In Brazil, an endemic area, approximately 800,000 to 2.5 million HTLV-1 carriers are estimated. The prevalence varies according to geographic regions of the country, and highest rates are found in the Northern and Northeastern^{7,8}. However, HTLV infection is not included on the list of notifiable diseases in most Brazilian states, thus the available epidemiological data are usually from specific population groups such as blood donors, pregnant women, injecting drug users and sex workers^{9,10}. Regarding the epidemiology among blood donors in the State of Pará, some recent HTLV seroprevalence studies, previously conducted by our group, described prevalence rates of 0.2%⁸ and 0.3%¹¹, but only 22% of such reactive samples confirmed the presence of HTLV-1/2 proviral DNA¹¹.

HTLV transmission is lymphocyte mediated and occurs mostly by direct contact between infected cells and target cells, known as cell-to-cell transmission¹². The virus is spread through unprotected sexual intercourse, from mother to child (during birth and mainly by breastfeeding), and via parenteral route (including needle sharing among intravenous drug users and transfusion/transplantation of contaminated blood/organs)^{13,14}. To mitigate the risks of transfusion transmission, HTLV-1/2 mandatory screening of blood donors has been implemented in several countries since the late-1980s¹⁵.

In Brazil, HTLV-1 and HTLV-2 screening in hemotherapy services became mandatory in 1993¹⁶. Following the Brazilian technical regulations on hemotherapeutic procedures determined by the Ministry of Health¹⁷, the screening consists in serological testing for the detection of antibodies to HTLV-1 and HTLV-2. Currently, highly sensitive enzyme-linked immunosorbent (ELISA), chemiluminescence (CLIA) and electrochemiluminescence (ECLIA) assays are widely used as screening tests¹⁸.

Blood donor samples repeatedly reactive on anti-HTLV-I/II screening tests require further confirmatory testing to define a final donor status. Although serological immunoassays, Western blotting (WB) and line immunoassay (LIA) are the most commonly used tests for confirmation worldwide, the molecular assay polymerase chain reaction method (PCR) can also be performed. It offers higher sensitivity and more accurate discrimination between HTLV-1 and HTLV-2 infections. In addition, PCR tests have been increasingly used for confirmation given the cost reduction of molecular biology reagents and the introduction of real-time PCR methods (qPCR)¹⁹⁻²¹. It is important to note that in Brazil, confirmatory tests are not mandatory in blood banks, and blood bags are discarded without confirming HTLV-1/2 infections.

The need for confirmatory testing is partly due to the high biologic false reactive (BFR) rates of the commercial screening tests. False reactivity in blood donation screening tests is a well-known

concern as a number of reports have shown that most repeatedly reactive results are not confirmed by further testing. Furthermore, the signal sample-to-cutoff ratio (S/CO) of screening immunoassays appears to play an important role in distinguishing between BFR and confirmed-positive results²²⁻²⁴. The comparative analysis between HTLV-1 and 2 screening and confirmatory tests results should help to define the serological and molecular profile of these deferred blood donors and determine the S/CO ratios of HTLV truly infection. Therefore, the purpose of this study was to correlate the S/CO ratio values distributions of reactive results for HTLV-1 and HTLV-2 antibodies with the detection of proviral DNA in a population of blood donor candidates.

MATERIAL AND METHODS

The present study was reviewed and approved by the Integrated School Brazil Amazon (FIBRA) Research Ethics Committee, under protocol number CAAE 19-004-819.7.0000.8187 and was conducted at the Foundation Center for Hemotherapy and Hematology of Pará (HEMOPA), Northern Brazil. The HEMOPA Foundation encompasses 11 blood donation centers located throughout the Pará State, in the cities of Belém, Ananindeua, Castanhal, Santarém, Marabá, Abaetetuba, Altamira, Tucuruí, Redenção and Capanema.

This was a retrospective, cross-sectional, descriptive and quantitative study that included serological and molecular data on blood donations from January 2015 to December 2019. As inclusion criteria, subjects over the age of 18 who had samples that were reactive on serological screening and were sent to confirmatory detection by molecular biology were chosen. Subjects under the age of 18 and samples that did not provide results for one of the tests were used as exclusion criteria in the research.

During the study period, the HEMOPA Foundation performed HTLV serological screening using the chemiluminescent microparticle immunoassay (CMIA) Architect rHTLV-I/II (Abbott Laboratories) for the qualitative detection and quantification of antibodies to HTLV-1 and HTLV-2. The resulting chemiluminescent reaction was detected by the Architect System optics as relative light units (RLUs), which have a direct relationship with the amount of anti-HTLV-1/2 antibodies in the sample. The results were calculated based on the ratio of the sample RLUs signal to the cutoff RLU signal (S/CO) for each specimen and control, and defined by the manufacturer as positive when $S/CO \geq 1.00$. According to the product insert, the sensitivity of the Architect rHTLV-I/II is 100% and the specificity among blood donors is 99.95%.

In the study period, Abbott Architect reactive samples were subsequently submitted to confirmatory tests performed by the in-house real-time PCR (qPCR) method, using the TaqMan[®] (Applied Biosystems) system, as previously described²⁵. This assay consists of searching for three targets: the albumin gene as an endogenous control and the non-homologous regions of the HTLV-1 and HTLV-2 *pol* genes it has a detection limit of 215 copies/mL²⁵.

The data was obtained from the HEMOPA Foundation systems databases (SBS Web and Progress) and included the results of serological screening (CMIA) and qPCR. Serological results were collated and stratified by the S/CO ratio values into four strata: 1-4, 4.01-20, 20.01-100 and >100; and were given a final status according to the qPCR results as HTLV detectable or HTLV undetectable. The sensitivities and positive predictive values (PPVs) of the initial Abbott Architect S/CO ratio results were calculated for each

S/CO range, and receiver operating characteristic curve (ROC Curve) analysis was conducted to define an optimized threshold based on diagnostic accuracy in the software Statistical Package for Social Sciences (SPSS), version 20.0 (SPSS Inc., Chicago, USA).

RESULTS

A total of 453,626 blood donation samples were screened serologically in the state of Pará between January 2015 and December 2019. Overall, 1,476 (0.3%) of these donations were found to be inapt due to the serological detection of HTLV, and 747 (51%) of these samples were submitted to a confirmatory test for HTLV-1 and 2 proviral DNA detection by qPCR. A total of 115 samples were omitted from the analyses due to the fact that they did not satisfy the selection criteria for the study. The data on 632 samples was thus analyzed, of which 496 (78%) had undetectable proviral DNA and 136 (22%) had detectable HTLV. HTLV-1 was detected in 106 out of 136 detectable samples (77.94%) and HTLV-2 was detected in 30 (22.06%).

HTLV infection was not confirmed in any individual for whom the initial S/CO ratio was <4 . When the S/CO ratio in the initial sample was in the range of 4.01–20, 4.26% of the subjects were confirmed to have HTLV infection. For S/CO ratios range 20.01–100, HTLV infection was confirmed in 85.07% of the subjects, whereas for S/CO ratios of >100 , HTLV infection was confirmed in 95.06%. In comparison to HTLV-1, HTLV-2 infection was not confirmed in any sample in the 4.01–20 range and was detected less frequently in all subsequent ranges. The range of S/CO ratios according to detection of proviral DNA are presented in [Table](#).

Table. S/CO ratios from the Abbott Architect rHTLV-I/II according to the final status of HTLV infection

Architect rHTLV-I/II S/CO range	N	Undetectable HTLV	Detectable HTLV	Detectable HTLV-1	Detectable HTLV-2
1–4	437	437 (100%)	0 (0%)	0 (0%)	0 (0%)
4.01–20	47	45 (95.74%)	2 (4.26%)	2 (100%)	0 (0%)
20.01–100	67	10 (14.93%)	57 (85.07%)	39 (68.42%)	18 (31.58%)
>100	81	4 (4.94%)	77 (95.06%)	65 (84.42%)	12 (15.58%)
Total	632	496 (78.48%)	136 (21.52%)	106 (77.94%)	30 (22.06%)

Overall, the sensitivity and PPV found in this study for the Architect rHTLV-I/II were 100% and 22%, respectively. For the range of 1–4 S/CO the sensitivity of the initial Architect rHTLV-I/II results was also 100%, whereas PPV was 0%. In the range of 4.01–20, the sensitivity and PPV were 100% and 4%, respectively. In the range of 20.01–100, the sensitivity was 51% and the PPV was 85%, and for the S/CO >100 range, the sensitivity was 85.7% and PPV was 95%.

The ROC curve analysis ([Figure](#)) was performed to determine the optimal S/CO ratio of Architect rHTLV-I/II for predicting HTLV-1 and HTLV-2 proviral DNA detection by qPCR. The maximum diagnostic sensitivity (100%) and specificity (97.2%) were observed at an S/CO ratio of 18.11.

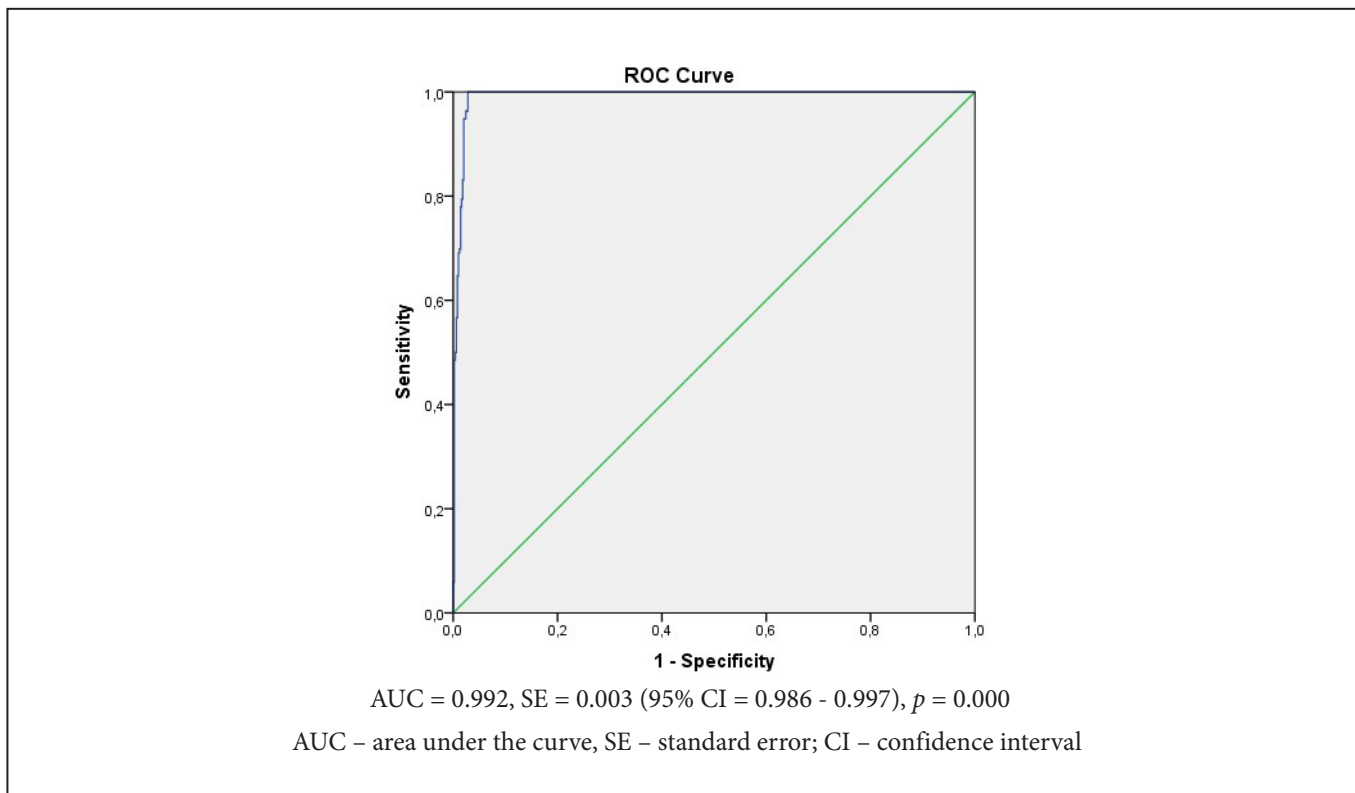


Figure. ROC curve for Architect rHTLV-I/II

DISCUSSION

In the present study, the S/CO ratios of reactive results for anti-HTLV-1/2 antibodies and the detection of proviral DNA in Brazilian blood donors were examined. It has been shown previously in blood donor populations that high reactivity in serological screening tests for transfusion transmissible infections (TTI) is predictive of confirmed-positive results, whereas low reactivity is frequently associated with BFR results^{26,27}.

The results of S/CO ratios according to detection of proviral DNA observed in this study demonstrated that although the highest frequency of reactive results occurred in the 1–4 S/CO range (437/632; 69.14%), none of the samples had confirmed HTLV infection. Moreover, a correlation between high S/CO ratios and confirmed-positive results was found, since proviral DNA detection rates gradually increased as S/CO ratios increased. This result is in agreement with the study by Tosswill and Taylor²⁴, in which no HTLV infection was confirmed when the S/CO ratio value was below 4, and higher S/CO ratios were more predictive of confirmed-positive results. Thus, blood bags that had S/CO <4 may have been discarded unnecessarily.

Importantly, HTLV-2 infection was detected in only 30 samples (22.06%), and was not confirmed in any individual when the S/CO ratio value was below 20. Also, in each S/CO range with detectable HTLV results, the proportion of HTLV-2 infection was inferior to HTLV-1. These findings reflect HTLV-2's lower prevalence compared to HTLV-1²⁸, as well as a lower mean PVL in HTLV-2 infected individuals, making it more difficult to detect using molecular methods^{29,30}.

With the exception of the 1–4 S/CO range, HTLV confirmed-positive results and BFR results overlapped in all S/CO ranges. Kiely et al²² also reported overlapping of S/CO ratios distributions of BFR and confirmed-positive results for HTLV in blood donors. Matsumoto et al³¹ reported a weakly positive relationship between HTLV-1 PVL and antibody levels in a cluster of blood donation samples with low antibody titers and high PVL. These findings reinforce the importance of HTLV confirmatory testing, especially because HTLV nucleic acid testing (NAT) is not currently used for blood donor screening and due to the fact that Brazil is considered an endemic area for HTLV, with higher prevalences in the North and Northeast^{6,11}.

It must be highlighted that it is not mandatory for blood services to conduct confirmatory tests for TTI¹⁷. In the HEMOPA Foundation, the confirmation and discrimination of HTLV-1 and HTLV-2 infections by qPCR have been performed since 2006 as part of its institutional testing algorithm. Even so, only reactive blood donors who returned for counseling and collection of a second sample were submitted for molecular confirmation (747/1,476; 51%).

The overall high sensitivity found for Architect rHTLV-I/II in the present study is in accordance with other studies conducted in blood banks worldwide. Architect rHTLV-I/II showed 100% sensitivity in Korea³², Saudi Arabia³³, Europe and Japan³⁴. Brito et al³⁵ evaluated the performance of commercially available screening tests in Brazil for HTLV diagnosis and reported that all screening tests showed 100% sensitivity. According to the World Health Organization³⁶, the minimum evaluated sensitivity and specificity levels of all assays used for blood screening should be as high as possible, and preferably not less than 99.5%. The maximized sensitivity (100%) and specificity (97.2%) at S/CO ratio 18.11 determined by ROC curve analysis means this threshold has greater capacity to discriminate individuals with detectable PVL of those with undetectable HTLV.

The high sensitivity of blood donation screening tests can lead to high proportions of BFR results, as demonstrated by the overall low PPV reported in this study for Architect rHTLV-I/II (22%). A study conducted in China found that PPVs for screening HTLV-1/2 antibodies assays used in blood centers varied from 0–26.30 with an average value of 10.17%³⁷. Our findings showed an optimized intersection of sensitivity and PPV in the highest S/CO range (S/CO>100), whereas low S/CO ratios displayed higher sensitivities but poor PPVs. BFR results have negative implications for blood services, not only in financial terms due to the costs regarding confirmatory testing and blood discard, but also have an impact on the blood supply and blood donors' quality of life^{38,39}. Nevertheless, avoiding false negative results is crucial in blood bank settings to enhance transfusion safety⁴⁰.

CONCLUSION

The results of this study demonstrated that high S/CO ratios were more associated with the detection of proviral DNA by qPCR. Low reactivity in the Architect rHTLV-I/II assay showed a poor relationship with further confirmation testing, especially in the lowest S/CO range in which no confirmed-positive results were reported, suggesting a low risk of HTLV infection in samples with an S/CO ratio of <4. However, the S/CO ratio distribution overlapped for detectable HTLV and undetectable HTLV results, reaffirming the importance of confirmatory tests. The optimal S/CO ratio for predicting the presence of proviral DNA was 18.11.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

FUNDING

This research did not receive any specific grant from funding agencies.

AUTHORS' CONTRIBUTIONS

All the authors contributed to the study conception and design. The preparation of material, data collection and analysis were all conducted by Lucas José Nascimento Cruz, Carolina de Alcântara Maneschy and Carlos Eduardo de Melo Amaral. The first draft of the manuscript was written by Lucas José Nascimento Cruz and Carolina de Alcântara Maneschy. All the other authors critically reviewed the article and commented on previous versions of the manuscript. All the authors have read and approved the final manuscript.

ACKNOWLEDGEMENT

The authors did not declare.

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