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Seronegative human T-cell lymphotropic virus 1 carriers in blood banks: A potential viral source for silent transmission?

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Abstract

Background and Objectives: Transfusion-transmitted viruses count among the greatest threats to blood safety. In Argentina, current laws oblige testing all donated blood for the presence of antibodies against human T-cell lymphotropic viruses 1 and 2 (HTLV-1/2). In endemic zones of the country, a high rate of seronegative HTLV-1 individuals with clear evidence of infection because of symptoms and/or presence of *tax* sequences of HTLV-1 and/or IgG anti-Tax antibodies has been recently described. Migration from endemic to nonendemic zones of Argentina is very frequent.

Materials and Methods: During a 1-year period, in the blood bank of Córdoba city, we performed molecular screening of all donors who were born in or arose from endemic zones for HTLV-1/2 in Argentina and neighbouring countries.

Results: By screening 219 bp of HTLV-1/2 *tax* gene, 0.6% (2/317) of the blood donors proved to be positive for HTLV-1 *tax* sequence. One of the donors presented anti-Tax antibodies, demonstrating the transcriptional activity of the *tax* gene, and the other donor was also positive for LTR and *pol* gene sequences. The HTLV-1 genetic analysis of the LTR sequence determined that it belonged to the Cosmopolitan subtype HTLV-1aA.

Conclusion: These findings suggest potential limitations of some currently approved screening assays for HTLV-1 detection applied in some donor populations and the possibility of an HTLV-1 seronegative carrier state with the potential for silent transmission by blood.

KEYWORDS
Argentina, blood safety, nonendemic HTLV-1 zone, seronegative HTLV-1 carriers

Highlights

- Seronegative human T-cell lymphotropic virus 1 (HTLV-1) carriers in the blood banks of Argentina were detected.
- Possible limitations of some current pre-transfusions-screening assays for HTLV-1 detection.
- Current pretransfusion-screening assays for HTLV-1 detection have limitations.
- It is uncertain if seronegative HTLV-1 carriers represent a threat to transfusion safety.

María C. Frutos and Sandra Gallego are scientific members of CONICET.

INTRODUCTION

Human T-cell lymphotropic virus 1 (HTLV-1) is the etiological agent of adult T-cell leukaemia and tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM) [1]. However, most people infected with this virus never develop any sign or symptom of the disease, and if they do, it is usually late in life [2].

HTLV-1 infection has been reported in almost all South American countries, including Brazil, Colombia, Argentina, Peru, French Guyana and Chile [3]. Moreover, some areas of South America, such as northeast of Brazil and northwest of Argentina, are considered endemic to HTLV-1 [4]. Concerning specifically to HTLV-1 infection in Argentina, there are two different areas: one endemic zone in the Northern part of the country, where blood banks report the highest prevalence of HTLV-1/2 infection (0.6%–1.0%), and a nonendemic area in central and southern regions of the country where the prevalence of HTLV-1/2 infection in blood banks is lower than 0.1% [5, 6].

In a recent publication, we describe for the first time the existence of seronegative HTLV-1 carriers in highly endemic areas of our country [7]. In this study, 64.5% of the subjects were seronegative for HTLV-1 infection but carried proviral sequences of HTLV-1. Besides, 35.7% of these subjects presented antibodies to Tax protein of HTLV-1 [7], and the Tax antigen is not included in commercially available HTLV kits for serological testing. This issue opened the question of whether the prevalence of HTLV-1 infection in Argentina may be greater than detected by currently used serologic tests.

Earlier studies have reported the condition of seronegative HTLV-1 carriers in intravenous drug users [8], seronegative TSP/HAM patients [9, 10], subjects with mycosis fungoides [11], patients with infective dermatitis [12], and healthy blood donors as well [13, 14].

Although in Argentina, the current blood laws oblige testing every donated blood for antibodies against HTLV-1/2, the finding of seronegative HTLV-1 carriers in the general population of highly endemic areas in Argentina, which cannot be detected by conventional serological screening, warns us about a potential hazard for blood banks.

Migration from endemic to nonendemic zones of Argentina is very frequent. During a 1-year period, in the blood bank of Córdoba city (capital of a nonendemic province), we performed molecular screening to all donors born in or had grown up in the endemic zones for HTLV-1/2 in Argentina and neighbour countries.

Córdoba city, capital of Córdoba province (1.3 million inhabitants), usually receives a large influx of subjects from endemic areas.

MATERIALS AND METHODS

Samples

Blood samples were collected between August 2015 and August 2016 at Fundación Banco Central de Sangre, Córdoba, Argentina. This is a blood bank that centralizes different blood transfusion departments working all over the 165.321 km^2 of Córdoba province. Serological and molecular pre-transfusion screening of almost 50% of the blood units collected throughout the province is performed in this blood bank.

A total of 317 healthy adults without risk factors for transfusion-transmitted infections were studied. All donors donated along 1 year at Fundación Banco Central de Sangre; they were born in or had grown up in the endemic zones for HTLV-1/2 in Argentina or other countries; all were seronegative for HTLV-1/2 antibodies when screened with Architect rHTLV-I/II assay (Abbott Laboratories Wiesbaden, Germany). Thus, 75.7% (240/317) had come from endemic zones of Argentina (Jujuy, Salta, Formosa, Chaco and Misiones) and 8.2% (26/317) from Peru, 4.1% (13/317) from Paraguay, 2.5% (8/317) from Bolivia, 2.2% (7/317) from Venezuela, 1.9% (6/317) from Chile, 1.6% (5/317) from Brazil, 1.6% (5/317) from Colombia, 1.6% (5/317) from Mexico, 0.3% (1/317) from Ecuador and 0.3% (1/317) from Guatemala. The study population included 69% (219/317) males and 31% (98/317) females aged 18–64 years. These proportions reflect the characteristics of regular blood donors in Argentina, showing that males constitute more than 60% of the blood donor population in this country [15].

The samples were codified as H followed by a number and were an aliquot of blood obtained from the same tube used for triplex nucleic acid amplification testing studies in the routine pre-transfusion screening of all blood donors. Thereby, the quality of samples for molecular analysis was guaranteed.

This study complied with the principles outlined by the Declaration of Helsinki and was approved by the Ethics Committee of OULTON Institute (10/2015) of Córdoba, Argentina. Written informed consent was signed by all the participants prior to sample collection.

Polymerase chain reaction assays

DNA was extracted from whole-blood samples of the 317 selected blood donors. Nested polymerase chain reaction (PCR) was carried out to amplify the 219-bp sequence of the *tax* gene following protocols described by Vandamme et al. [16]. The HTLV-1/2 positive samples by generic nested PCR were subsequently typed by specific nested-PCR for HTLV-1 (100 bp) and HTLV-2 (151 bp), targeting the *tax* region [16]. Also, an additional PCR was carried out to amplify 100-bp of the HTLV-1 *tax* gene following protocols previously described using primers designed for the detection of HTLV-1 strains prevalent in Argentina [17].

The PCR products were separated on a 2% agarose gel with SYBR Safe DNA gel (Invitrogen) staining and visualised under UV light.

Amplification of 1119 bp of the *tax* region from the proviral genome was performed in all *tax*-positive samples [18]. The other two sequences, 561 bp and 672 bp from the *env* gene in addition to the LTR region, were also amplified using nested-PCR [19]. The reaction to detect the *pol* gene (107 bp) of HTLV-1 was performed using real-time PCR developed by Andrade et al. [20].

Sequencing

PCR products corresponding to *tax* sequences and LTR region were purified using QIAquick Gel Extraction Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. *Tax* (100–219 bp) and LTR fragments (672 bp) were subjected to direct nucleotide sequencing reaction in both directions using the internal PCR primers by Macrogen, Inc. (Seoul, Korea). The alignment of the sequences from seronegative HTLV-1 donors was performed using the Clustal W program (Conway Institute UCD Dublin, Dublin, Ireland) and compared with Pairwise/Blast/NCBI. The sequence was deposited in GenBank (MZ687332).

The maximum likelihood tree was constructed with the PhyML 3.0 software (Université de Montpellier, Montpellier, France) [21]. The model of nucleotide substitution was selected according to the Akaike Information Criterion implemented in the ModelTest 3.7 software (Universidad de Vigo, Galicia, Spain) [22] for the data set analysed.

Molecular signatures on LTR seronegative HTLV-1 carrier sequence were analysed with VESPA software [23].

The identity matrix was calculated using the Distance Matrix tool (IVisTMSA) [24].

Serological assays

Samples that resulted positive for HTLV-1 by molecular assays were re-tested for HTLV-1/2 antibodies by PA assay (Serodia Fujirebio Inc., Tokyo, Japan) and also analysed by an "in-house" IFA on MT-2 cell line [25]. Besides, samples that were positive for HTLV-1 by molecular assays were further tested for IgG anti-Tax antibodies using anti-Tax-IgG enzyme-linked immunosorbent assay (ELISA) [26]. The sensitivity and specificity of this assay had been previously reported [7]; it was performed at the Laboratório de Virologia Básica e Aplicada, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil.

Statistical analysis

Distribution of frequencies for each variable was analysed with the Kruskal–Wallis test and Dunn's post-test. Statistical analyses for IgG anti-Tax antibodies were conducted using GraphPad Prism 8.0.1 software (GraphPad Software Inc., San Diego, CA). Analysis of unpaired *t*-test for anti-Tax IgG reactivity was performed with Mann–Whitney's post-test. Significance was assumed at $p < 0.05$.

RESULTS

Among the 317 donors analysed, 2 (0.63%) tested positive for HTLV-1 sequences in blood, detected by two PCRs targeting different sequences of the *tax* gene, both of 100 bp [16, 17]. These subjects were also HTLV-1/2 negative for antibodies by PA and IFA assays (Figure 1).

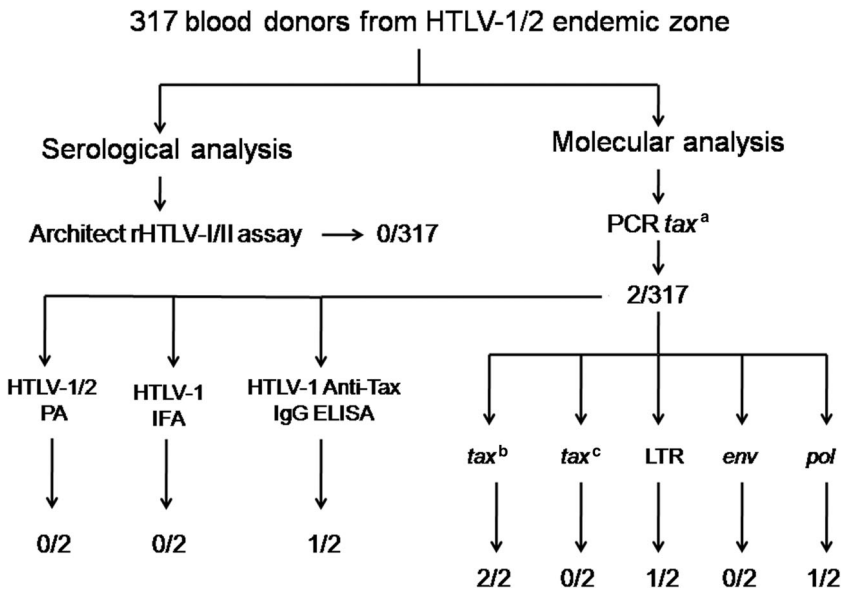

FIGURE 1 Human T-cell lymphotropic virus (HTLV) serology and polymerase chain reaction (PCR) analysis of samples from blood donors coming from HTLV-1/2 endemic areas. Blood samples from donors who were negative for HTLV-1/2 antibody screening by Architect rHTLV-I/II assay at the blood bank were further analysed at InViV with a nested-PCR for the HTLV *tax* gene. Blood samples that resulted positive for a sequence of *tax* were further tested for another *tax* region by PCR, *env* gene and LTR regions by nested-PCR, and *pol* gene by qPCR. Also, plasma samples of *tax* positive donors were subsequently retested serologically for antibodies against structural antigens by HTLV-1/2 PA (Serodia Fujirebio Inc., Tokyo, Japan), HTLV-1 IFA (in house) and tested by HTLV-1 non-structural antibodies anti-Tax IgG enzyme-linked immunosorbent assay (in house). [a]215 bp and 100 bp [16]. [b]100 bp [17]. [c]1100 bp [18]

In one of the blood samples, sequences from the LTR region (672 bp) and *pol* gene (107 bp) were also amplified. This donor was a 48-year-old male (H94) born in Jujuy province (Argentina). The other *tax*-positive donor was a 28-year-old female (H256) native from the Chaco province (Argentina); IgG anti-Tax antibody was also detected in her blood sample (Figure 2).

The HTLV-1 *tax* sequence detected in both donors (100 bp) was highly homologous to prototypic ATK-1 HTLV-1 *tax*, also showing high homology with other isolates from the endemic zone of Argentina (>97%) and strains from neighbour countries (Table 1).

The genetic analysis of the HTLV-1 LTR region showed that the sequence from the donor H94 belonged to the Cosmopolitan subtype HTLV-1a Transcontinental subgroup A within the Latin American and Jujuy subclusters (Figure 3).

The VESPA analysis of HTLV-1 LTR sequences showed that, as compared to the reference strain ATK-1, the seronegative HTLV-1 carrier sequence contained not only the polymorphisms typical for the Transcontinental HTLV-1aA subgroup (T246C, C306G, T479C, A529G and G675A) and deletion at position A209 but also a singular position T188C (data not shown). These polymorphisms were identical to those previously described in infected HTLV-1 seropositive subjects from Jujuy.

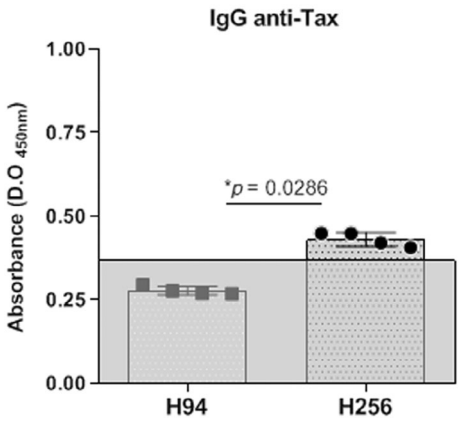

FIGURE 2 IgG anti-Tax antibodies against Tax protein of human T-cell lymphotropic virus 1 detected by enzyme-linked immunosorbent assay in seronegative blood donors. The grey area represents the cutoff point optical density (OD = 0.371) of the assay

DISCUSSION

Herein, we describe for the first time the existence of seronegative HTLV-1 carriers in the blood banks of Argentina. Among the 317 blood donors born in or arose from endemic zones for HTLV-1/2 of Argentina or other countries recognized as endemic for the virus, we detected 2 donors (0.63%) harbouring *tax* sequences with the absence of antibodies evidenced by commercially available CE-marked or FDA-approved HTLV-1/2 assays.

To our knowledge, there is only one published article regarding HTLV-1 carriers in blood banks, which reports cases from a blood bank in the United States [14]. The researchers found a higher prevalence of seronegative HTLV-1/2 carriers (8.6%) detected by molecular screening of 250 plasma samples from healthy blood donors. In contrast with this study, in which only sequences of *tax* genes were found, sequences of other genes were detected (LTR and *pol*) in one donor of our study as well. In addition, other investigators sought donors who were seronegative HTLV-1/2 carriers in a blood bank, but they did not find any [27].

TABLE 1 Matrix identity of human T-cell lymphotropic virus 1 (HTLV-1) *tax* gene sequences in blood samples and comparison with isolates from different sources

	ATK-1	H94	H256	ArJ03-06	ArJ13-01	HN1	B1033	HAM16	BRSP65679	LC210018	ArJ54-2	ArJ17-2
ATK-1	100											
H94	97.2	100										
H256	98.3	96.6	100									
ArJ03-06	100	97.2	98.3	100								
ArJ13-01	100	97.2	98.3	100	100							
HN1	100	97.2	98.3	100	100	100						
B1033	99.4	96.6	97.7	97.7	99.4	99.4	100					
HAM16	100	97.2	98.3	100	100	100	99.4	100				
BRSP65679	99.4	96.6	97.7	99.4	99.4	99.4	99.4	99.4	100			
TT0021	100	97.2	98.3	100	100	100	99.4	100	99.4	100		
ArJ54-2	100	97.2	98.3	100	100	100	99.4	100	99.4	100	100	
ArJ17-2	100	97.2	98.3	100	100	100	99.4	100	99.4	100	100	100

Note: ATK-1 (J02029): pattern sequence of HTLV-1; ArJ03-06 (MK63897) and ArJ13-01 (MK638974): seronegative HTLV-1 carriers from Jujuy (endemic zone of Argentina); ArJ54-2 (DQ227188) and ArJ17-2 (DQ227165): seropositive HTLV-1 subjects from Jujuy. HN1 (KC807984): HTLV-1 infected patients from China. TT0021 (LC210018): HTLV-1 infected blood donors from Japan. HAM16 (KY007274): HAM patient from Brazil. B1033 (AB513134): HTLV-1 patient with adult T-cell leukaemia from Japan. BRSP65679 (KY928595): sequence from patient coinfected with HTLV-1 and HIV-1 from Brazil.

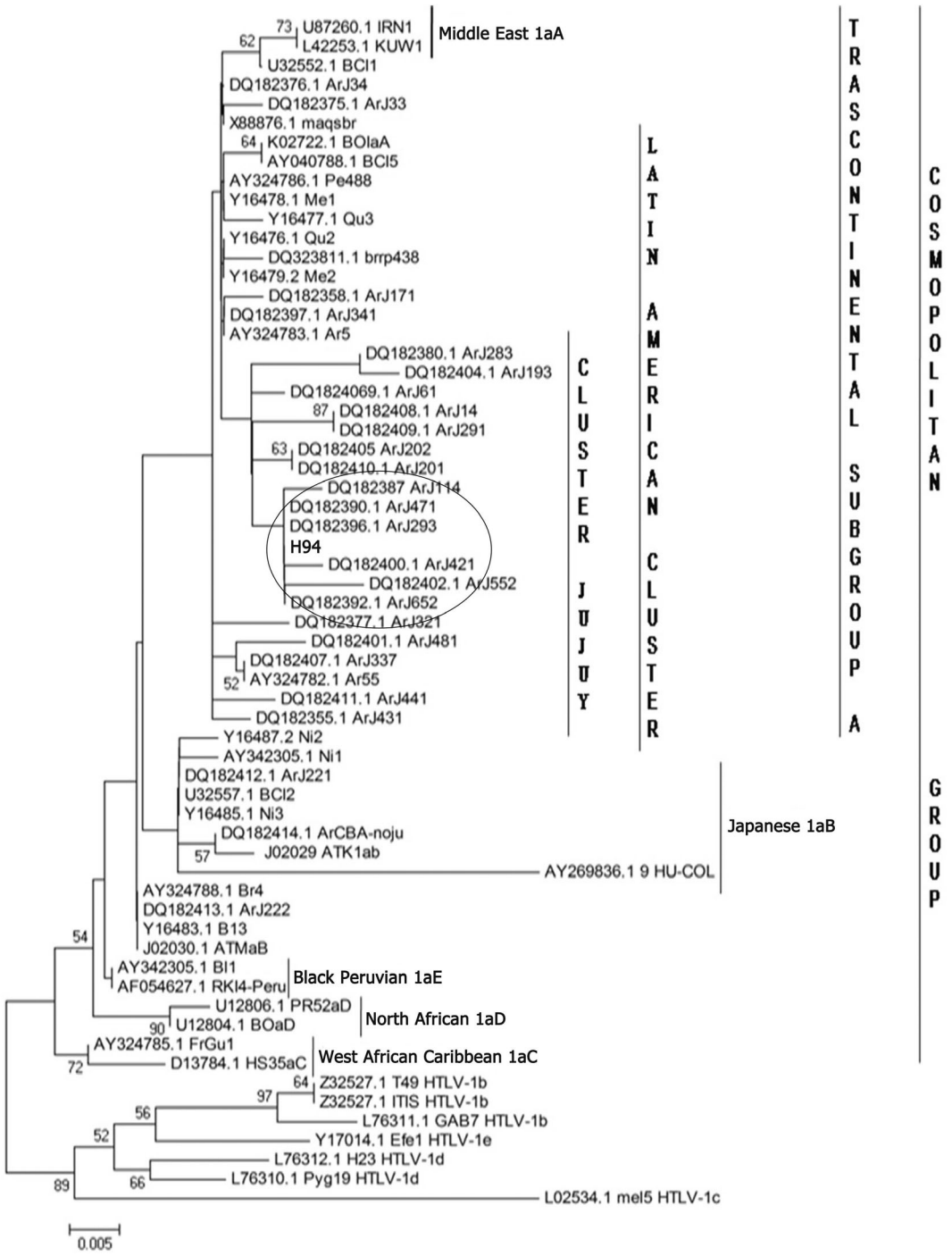

FIGURE 3 Maximum likelihood dendrogram for the human T-cell lymphotropic virus 1 (HTLV-1) LTR sequences. HTLV-1 LTR (630 bp) genetic tree comparing sequences from seronegative HTLV-1 blood donors and worldwide sequences, including HTLV-1 reference. It was constructed using TIM2 + G as a model of nucleotide substitution with parameters suggested by ModelTest 3.7 (PhyML software). The strain that belongs to this study begins with H and is written in bold. Numbers above branches: bootstrap values over 1000 bootstrap pseudoreplicates. Only bootstrap values >50% are shown at nodes

It has been suggested that the lack of LTR sequences may explain the replication incompetence and inexpression of HTLV-1 antigens and the consequence of the absence of immune response. Thus, the authors propose that TSP/HAM patients carry a defective HTLV-1 provirus, probably as a consequence of a vigorous immune response early in the infection, which successfully eradicates the infected cells, leaving only those with defective sequences [28]. Despite the case of our donor, in whom sequences from three different viral genes were detected (LTR, *pol* and *tax*), the possibility of a defective HTLV-1 provirus cannot be discarded in the face of the absence of immune response.

Furthermore, it is not surprising that the *tax* gene is always found in these cases, and in many cases, this is the only one. The genetic stability of the HTLV-1 *tax* gene has been determined through different studies [29]; this is why several PCRs targeting *tax* sequences have been developed and largely used for molecular diagnosis and investigation of HTLV infection [17, 20, 30]. However, the genetic versatility of HTLV-1 also reaches *tax* sequences, as we have recently demonstrated in the case of infected people with missed *tax* genes [31].

The specificity of the HTLV-1 *tax* gene sequences amplified from the two seronegative blood donors by different PCRs was confirmed by nucleotide sequencing (100 bp). HTLV-1 is genetically very stable; a low degree of genetic variation (0.5%–3%) has been described for HTLV-1 strains from Africa, Japan, the Caribbean basin, and the Americas [32, 33]. The *tax* sequences from the two donors in this study showed 97.2%–98.3% homology to the ATK-1 sequence, also demonstrating high homology with other isolates from endemic zones of Argentina. Thus, the high homology found between the strains corroborated that the amplified sequences corresponded to HTLV-1.

HTLV-1aA is the prevalent subgroup in South American countries, such as Colombia, Peru, Chile and Brazil [32, 34]. In our study, the analysis of the amplified LTR sequence from one of the seronegative donors identified it as HTLV-1, Cosmopolitan Group (a), and Transcontinental subgroup (A). This sequence was very similar to that found in previous studies regarding HTLV-1 in Jujuy province and grouped in a particular cluster within the Latin American/Transcontinental subgroup, named Jujuy subcluster [35]. In accordance with this finding, the blood donor was a native of the Jujuy province.

Although we did not demonstrate the transmission of HTLV-1 from these carriers to blood recipients, this possibility cannot be excluded. In this sense, Zucker-Franklin showed transmission of *tax* to rabbits by transfusion of PBMC from *tax only* HTLV-1 seronegative blood donors [36]. Moreover, a seronegative status with stable HTLV-1 infection has been established in an animal model [37]. The major finding of this study was that the persistent presence of HTLV-1 without antibody response was successfully established, experimentally, in syngeneic rats inoculated with an HTLV-1-infected cell-line scarcely expressing major HTLV-1 structural proteins but preferentially expressing Tax.

Undoubtedly, seronegative carriers of HTLV-1 from which some proviral sequences are deleted exist, and this state may be associated with disease [9–12]. Moreover, we have recently described high rates of seronegative symptomatic and asymptomatic HTLV-1 carriers in Argentina, harbouring only tax sequences [7].

Since most carriers of deleted HTLV-1 sequences seem to retain the tax sequence and/or its gene product, p40tax [7, 9–11, 14], and considering that tax is the transcriptional transactivator of HTLV-1 and has a role in the upregulation of innumerable cellular growth factors, cytokines, and oncogenes [38–40], the transmission of tax is an important question that requires attention.

In our study, a second donor resulted positive for *tax* sequences and also positive for IgG anti-Tax antibodies. The presence of antibodies anti-Tax in the same individual may help to alleviate concern about the possibility of PCR contamination. This last possibility was dismissed by the repeated collection of samples in the donor without anti-Tax antibody and analysis of specimens obtained from the same donors at different times and handled by different personnel in a blind manner.

The evidence of IgG anti-Tax in this donor also confirmed that despite a short sequence of *tax* genes detected in the absence of other gene sequences, this individual probably had an active infection at some point in life. It has been suggested that anti-Tax antibodies are involved in TSP/HAM pathogenesis [41], and researchers suggest that the presence of anti-Tax antibodies contributes to the aggravation of HTLV-1 infection and is a marker of disease evolution [41, 42].

In Argentina, many efforts are being made for the implementation of nucleic acid techniques for viral screening in blood banks and highly sensitive tests for the detection of antigens and antibodies, which are efficient tools that reduce residual risks of infection-transmission through blood transfusions. In this sense, screening of antibodies against HTLV-1/2 is nowadays mandatory all over the country (Law 22990) since Argentina has some areas well known as endemic for HTLV-1 and HTLV-2 infection, and also because these viruses have been detected in different blood banks across the country [6]. The decision to screen blood donations for a particular pathogen should be based on the risk assessment of transfusion-transmitted infections determined by the prevalence of such pathogens in the donor population, susceptibility of the recipients, and the reported number of transfusion-transmitted cases in each region. Thus, the question raised in our study is if seronegative HTLV-1 carriers are capable of transmitting the infection by blood transfusion and, as a consequence, if it is necessary to implement further techniques for serological screening or incorporate molecular screening specific for this virus. In anti-HTLV routine screenings, positive tests can occur, as well as non-reactive results, taking into account the use of reagents containing only viral envelope proteins. This sensitivity can be augmented using chimeric antigen from *env*, *gag* and *pX* HTLV-1/2 regions [43].

In Argentina, leukoreduction of blood products is not mandatory, and in consequence, it is not performed routinely in local blood banks. It is reasonable to think that leukoreduction substantially reduces the risk of HTLV-1 transmission, and since the provirus is integrated into the CD4+ lymphocytes, there are few cell-free viruses. In this sense, a look-back study of blood transfusions in the United Kingdom found that filter-leukoreduced or buffy coat reduced blood product transmission of HTLV-1 by 93% [44]. Other studies demonstrated that leukoreduced blood products, although safer, still carry a theoretical risk of HTLV-1 transmission from donors with high proviral load [45]. Thus, in countries with a high prevalence of HTLV, like Argentina and Brazil [46], where universal leukoreduction is not recommended, it is important to be aware of any residual risk for HTLV transmission.

The findings suggest potential limitations of some currently approved screening assays for HTLV-1 detection applied in some donor populations and the possibility of an HTLV-1 seronegative carrier state with the potential for silent transmission by blood. Therefore continuous epidemiological surveillance in blood banks, including follow-up of positive blood donors and recipients of positive blood, should be

performed. This is mainly worrying in Argentina and neighbouring countries with endemic areas for this virus. Assessment of the epidemiological risk through investigation and surveillance of agents with potential for blood transmission is critical to determine the infectious risk and implement newer interventions to ensure safe blood supplies [47].

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CONFLICT OF INTEREST

There are no conflicts identified.

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REFERENCES

1. Tagaya Y, Matsuoka M, Gallo R. 40 years of the human T-cell leukemia virus: past, present, and future. F1000Res. 2019;8:F1000 Faculty Rev-228.
2. Bangham CR. The immune response to HTLV-I. Curr Opin Immunol. 2000;12:397–402.
3. Gessain A, Cassar O. Epidemiological aspects and world distribution of HTLV-1 infection. Front Microbiol. 2012;3:388.
4. Eusebio-Ponce E, Candel FJ, Anguita E. Human T-cell lymphotropic virus type 1 and associated diseases in Latin America. Trop Med Int Health. 2019;24:934–53.
5. Gastaldello R, Hall WW, Gallego S. Seroepidemiology of HTLV-1/II in Argentina: an overview. J Acquir Immune Defic Syndr. 2004;35:301–8.
6. Biglione MM, Astarloa L, Salomón HE, Referent HTLV-I/II Argentina Group. High prevalence of HTLV-I and HTLV-II among blood donors in Argentina: a South American health concern. AIDS Res Hum Retroviruses. 2005;21:1–4.
7. Gallego S, Frutos MC, Blanco S, Castro G, Balangero M, Elías Panigo D, et al. First description of seronegative HTLV-1 carriers in Argentina. Am J Trop Med Hyg. 2020;102:889–95.
8. Ehrlich GD, Glaser JB, Abbott MA, Slamon DJ, Keith D, Sliwkowski M, et al. Detection of anti-HTLV-I tax antibodies in HTLV-I enzyme-linked immunosorbent assay-negative individuals. Blood. 1989;74:1066–72.
9. Ramirez E, Cartier L, Rios M, Fernandez J. Defective human T-cell lymphotropic virus type I (HTLV-I) provirus in 10 Chilean seronegative patients with tropical spastic paraparesis or HTLV-I-associated myelopathy. J Clin Microbiol. 1998;36:1811–3.
10. Ramirez E, Fernandez J, Cartier L, Villota C, Rios M. Defective human T-cell lymphotropic virus type I (HTLV-I) provirus in seronegative tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM) patients. Virus Res. 2003;91:231–9.
11. Morozov VA, Syrtsev AV, Ellerbrok H, Nikolaeva EV, Bavykin AS, Pauli G. Mycosis fungoides in European Russia: no antibodies to human T cell leukemia virus type I structural proteins, but virus-like sequences in blood and saliva. Intervirology. 2005;48:362–71.
12. de Oliveira M d F, Fatal PL, Primo JR, da Silva JL, Batista Eda S, Farré L, et al. Infective dermatitis associated with human T-cell lymphotropic virus type 1: evaluation of 42 cases observed in Bahia, Brazil. Clin Infect Dis. 2012;54:1714–9.
13. Zucker-Franklin D, Pancake BA, Marmor M, Legler PM. Reexamination of human T cell lymphotropic virus (HTLV-I/II) prevalence. Proc Natl Acad Sci U S A. 1997;94:6403–7.
14. Zucker-Franklin D, Pancake BA. Human T-cell lymphotropic virus type 1 Tax among American blood donors. Clin Diagn Lab Immunol. 1998;5:831–5.
15. Blanco S, Carrizo LH, Moyano RW, Mangeaud A, Gallego SV. Gender-neutral donor deferral policies: experience in Argentina implementing individual risk-assessment policies. Vox Sang. 2020;115:548–54.
16. Vandamme AM, Van Laethem K, Liu HF, Van Brussel M, Delaporte E, de Castro Costa CM, et al. Use of a generic polymerase chain reaction assay detecting human T-lymphotropic virus (HTLV) types I, II and divergent simian strains in the evaluation of individuals with indeterminate HTLV serology. J Med Virol. 1997;52:1–7.
17. Castro GM, Balangero MC, Maturano E, Mangeaud A, Gallego SV. Development and validation of a real-time PCR assay for a novel HTLV-1 tax sequence detection and proviral load quantitation. J Virol Methods. 2013;189:383–7.
18. Furukawa Y, Yamashita M, Usuku K, Izumo S, Nakagawa M, Osame M. Phylogenetic subgroups of human T cell lymphotropic virus (HTLV) type I in the tax gene and their association with different risks for HTLV-I-associated myelopathy/tropical spastic paraparesis. J Infect Dis. 2000;182:1343–9.
19. Liu HF, Goubau P, Van Brussel M, Van Laethem K, Chen YC, Desmyter J, et al. The three human T-lymphotropic virus type I subtypes arose from three geographically distinct simian reservoirs. J Gen Virol. 1996;77:359–68.
20. Andrade RG, Ribeiro MA, Namen-Lopes MS, Silva SM, Basques FV, Ribas JG, et al. Evaluation of the use of real-time PCR for human T cell lymphotropic virus 1 and 2 as a confirmatory test in screening for blood donors. Rev Soc Bras Med Trop. 2010;43:111–5.
21. Guindon S, Gascuel O. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol. 2003;52:696–704.
22. Posada D, Crandall KA. MODELTEST: testing the model of DNA substitution. Bioinformatics. 1998;14:817–8.
23. Korber B, Myers G. Signature pattern analysis: a method for assessing viral sequence relatedness. AIDS Res Hum Retroviruses. 1992;8:1549–60.
24. Pervez MT, Babar ME, Nadeem A, Aslam N, Naveed N, Ahmad S, et al. IVisTMSA: interactive visual tools for multiple sequence alignments. Evol Bioinform Online. 2015;11:35–42.
25. Gallego S, Recalde A, Gastaldello R, Isa M, Nates S, Medeot S. Kinetics study of human retrovirus antigens expression in T lymphocytic cell lines by indirect immunofluorescence assay. Viral Immunol. 1997;10:149–57.
26. De Souza JG, Fonseca FG, Martins-Filho OA, Teixeira-Carvalho A, Martins CP, Carvalho LD, et al. Diagnostic tool based on an HTLV-1 Tax expression system in eukaryotic cells using a poxvirus vector. J Virol Methods. 2010;166:65–71.
27. Dezzutti CS, Guenthner PC, Daniel S, Utz U, Cabrera T, Marshall JH, et al. Detection of human T-lymphotropic virus (HTLV) tax sequences in New York City blood donors seronegative for HTLV types 1 and 2. Clin Diagn Lab Immunol. 2003;10:715–7.
28. Daenke S, Parker CE, Niewiesk S, Newsom-Davis J, Nightingale S, Bangham CR. Spastic paraparesis in a patient carrying defective human T-cell leukemia virus type I (HTLV-I) provirus sequences but lacking a

humoral or cytotoxic T-cell response to HTLV-I. J Infect Dis. 1994;169:941–3.
29. Kubota R, Hanada K, Furukawa Y, Arimura K, Osame M, Gojobori T, et al. Genetic stability of human T lymphotropic virus type I despite antiviral pressures by CTLs. J Immunol. 2007;178:5966–72.
30. Gonçalves DU, Proietti FA, Ribas JG, Araújo MG, Pinheiro SR, Guedes AC, et al. Epidemiology, treatment, and prevention of human T-cell leukemia virus type 1-associated diseases. Clin Microbiol Rev. 2010;23:577–89.
31. Blanco S, Frutos MC, Balangero M, Gallego SV. Human T-lymphotropic virus type 1 infection in absence of *tax* gene: a challenge for molecular diagnosis. Infect Genet Evol. 2021;90:104765.
32. Gessain A, Gallo C, Franchini G. Low degree of human T-cell leukemia/lymphoma virus type I genetic drift in vivo as a means of monitoring viral transmission and movement of ancient human populations. J Virol. 1992;66:2288–95.
33. Nobre AFS, Almeida DS, Ferreira LC, Ferreira DL, Júnior ECS, Viana MNDSA, et al. Low genetic diversity of the human T-cell lymphotropic virus (HTLV-1) in an endemic area of the Brazilian Amazon basin. PLoS One. 2018;13:e0194184.
34. Carneiro-Proietti AB, Catalan-Soares BC, Castro-Costa CM, Murphy EL, Sabino EC, Hisada M, et al. HTLV in the Americas: challenges and perspectives. Rev Panam Salud Publica. 2006;19:44–53.
35. Gastaldello R, Iñiguez AM, Otsuki K, Lamas G, Balangero M, Barbas MG, et al. HTLV type 1 genetic types among native descendants in Argentina. AIDS Res Hum Retroviruses. 2008;24:1139–46.
36. Zucker-Franklin D, Pancake BA, Lalezari P, Khorshidi M. Transmission of human T-cell lymphotropic virus type 1 tax to rabbits by tax-only-positive human cells. Clin Diagn Lab Immunol. 2000;7:274–8.
37. Koya Y, Ohashi T, Kato H, Hanabuchi S, Tsukahara T, Takemura F, et al. Establishment of a seronegative HTLV-I carrier state in rats inoculated with a HTLV-I-immortalized T-cell line preferentially expressing Tax. J Virol. 1999;73:6436–43.
38. Buckle GJ, Hafler DA, Hollsberg P. HTLV-I-induced T-cell activation. J Acquir Immune Defic Syndr Hum Retrovirol. 1996;13:107–13.
39. Sodroski J. The human T-cell leukemia virus (HTLV) transactivator (Tax) protein. Biochim Biophys Acta. 1992;1114:19–29.
40. Zucker-Franklin D, Gorman JG. HTLV-I provirus in seronegative healthy blood donors. Lancet. 1997;349:999.
41. De Souza JG, da Fonseca FG, Martins ML, Martins CP, de Carvalho LD, Coelho-dos-Reis JG, et al. Anti-Tax antibody levels in asymptomatic carriers, oligosymptomatic carriers, patients with rheumatologic disease or with HAM/TSP do not correlate with HTLV-1 proviral load. J Clin Virol. 2011;50:13–8.
42. Levin MC, Lee SM, Kalume F, Morcos Y, Dohan FC Jr, Hasty KA, et al. Autoimmunity due to molecular mimicry as a cause of neurological disease. Nat Med. 2002;8:509–13.
43. Caterino-de-Araujo A, Barbosa-Stancioli EF, Alonso Neto JB, Aragón MG, Galvão-Castro B, Ishak R, et al. Laboratory diagnosis of human T-lymphotropic virus in Brazil: assays, flowcharts, challenges, and perspectives. Rev Soc Bras Med Trop. 2021;54:e01752021.
44. Hewitt PE, Davison K, Howell DR, Taylor GP. Human T-lymphotropic virus lookback in NHS Blood and Transplant (England) reveals the efficacy of leukoreduction. Transfusion. 2013;53:2168–75.
45. Sobata R, Matsumoto C, Uchida S, Suzuki Y, Satake M, Tadokoro K. Estimation of the infectious viral load required for transfusion-transmitted human T-lymphotropic virus type 1 infection (TT-HTLV-1) and of the effectiveness of leukocyte reduction in preventing TT-HTLV-1. Vox Sang. 2015;109:122–8.
46. Mendrone A, Fabron A, Langhi D, Covas DT, Dinardo CL, Amorim Ubiali E, et al. Is there justification for universal leukoreduction? Rev Bras Hematol Hemoter. 2014;36:237.
47. Stramer SL, Hollinger FB, Katz LM, Kleinman S, Metzel PS, Gregory KR, et al. Emerging infectious disease agents and their potential threat to transfusion safety. Transfusion. 2009;49:1–29.

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