

Western Blot Seroindefinite Individuals for Human T-lymphotropic Virus 1/2 (HTLV-1/2) in Fortaleza (Brazil): A Serological and Molecular Diagnostic and Epidemiological Approach

Terezinha de Jesus Teixeira Santos,
 Carlos Maurício de Castro Costa,
 Patrick Goubau, Anne-Mieke Vandamme,
 Jan Desmyter, Sonia Van Dooren, Rosa M. S. Mota,
 Francine Bovy de Castro Costa, Ana C. S. Oliveira,
 Vânia Barreto A.F. Gomes, Anna B. Carneiro-Proietti,
 Veralice Meireles Sales de Bruin, Francisca C. F. de Sousa
 and Reinaldo Barreto Oriá

*Department of Physiology and Pharmacology,
 Federal University of Ceará, Fortaleza, CE, Brazil;
 Unit of Virology, Université Catholique de Louvain,
 Louvain, Belgium; Rega Institute for Medical
 Research, Katholieke Universiteit Leuven, Leuven,
 Belgium; Department of Statistics and Applied
 Mathematics, Federal University of Ceará,
 Fortaleza, CE, Brazil; HEMOCE of Fortaleza,
 Fortaleza, CE, Brazil; HEMOMINAS Foundation,
 Belo Horizonte, MG, Brazil*

How to handle Western blot (WB) seroindefinite individuals for Human T-lymphotropic Virus 1/2 (HTLV-1/2) constitutes a challenge for blood banks and families. We made a cross-sectional study of 191 enzyme linked immunoassay (EIA) reactive individuals from the hematological center (HEMOCE) of Fortaleza (Brazil), examining their serological (WB) and molecular (PCR) diagnosis, and demographic profiles, as well as a possible association of their condition with other infectious pathologies and risk factors. Ethical institutional approval and personal consent were obtained. Out of 191 EIA reactive individuals, 118 were WB seroindefinite and 73 were seropositive for HTLV-1/2. In the PCR analysis of 41 WB seroindefinite individuals, 9 (22%) were positive and 32 (78%) were negative for HTLV-1/2. The demographic analysis indicated a trend towards a predominance of males among the seroindefinite individuals and females in the seropositive ones. The seroindefinite individuals were younger than the seropositive ones. We did not find any association of these conditions with syphilis, Chagas disease or HIV or hepatitis, and with risk factors such as breast-feeding, blood transfusion, STD (syphilis) and IDU.

Key Words: Epidemiology, PCR, HTLV-1/2, seroindefinite individuals, hepatitis, syphilis, blood transfusion.

The human T-lymphotropic virus (HTLV) belongs to the family Retroviridae and the subfamily Oncovirinae. It is divided into types 1 and 2, sharing around 60% similarity. Based on molecular epidemiology HTLV-1 is

divided into the 1a (cosmopolitan), 1b (Central Africa), 1c (Melanesia), 1d, 1e and 1f (Central Africa) subtypes, and HTLV-2 into the 2a, 2b and 2d subtypes. HTLV-1/2 is variably endemic in Central and West Africa, the Caribbean, South America, Japan and Melanesia. In Brazil, its prevalence is 0.46% [1]. This retrovirus is transmitted vertically (mother-to-child) and horizontally (sexual contact, blood transfusion and intravenous drug use). Tropical Spastic Paraparesis/HTLV-1-associated Myelopathy (TSP/HAM) and Adult T-cell Leukemia/Lymphoma (ATL) are the main associated pathologies [2,3].

The laboratorial diagnosis of HTLV is performed using screening (enzyme linked immunoassay (EIA) and

Received on 09 July 2002; revised 30 September 2002.

Address for correspondence: Dr. Terezinha de Jesus Teixeira Santos – Lab. de Neurologia Experimental e Neurofisiologia – Depto. de Fisiologia e Farmacologia – Universidade Federal do Ceará – Rua Cel. Nunes de Melo, 1127 – Rodolfo Teófilo – Fortaleza-CE – 60.430-270 – Fax: +55 85 288-8333 – E-mail: mcastro@ufc.br

The Brazilian Journal of Infectious Diseases 2003;7(3):202-209
 © 2003 by The Brazilian Journal of Infectious Diseases and Contexto Publishing. All rights reserved.

particle agglutination), confirmatory (Western blot, IFA, RIPA, and more recently INNO-LIA) and molecular (PCR, NASBA and bDNA) tests. Serological tests for HTLV infection have been mandatory in some countries since 1986, and in Brazil since 1993. Screening and confirmatory results from blood banks are varied, with negative, positive and indeterminate profiles apparent.

Blood banks worldwide are thus faced with blood donor candidates exhibiting a seroindeterminate Western blot (WB) profile. This is mainly reported in tropical and in some temperate regions endemic for HTLV-1/2 infection, and less frequently in northern countries, where prevalence of HTLV-1/2 is very low [4,5].

Individuals seroindeterminate for HTLV-1/2 represent a challenge for blood banks, as well as in familial contexts, due to the risk they represent in spreading infections through blood donation and sexual contact, if truly infected, and because of the psychological burden it presents for the person with this undefined condition.

With the aim of delineating a profile of Western Blot (WB) seroindeterminate individuals for HTLV-1/2 from the hematological center (HEMOCE) of Fortaleza (Brazil), we carried out a cross-sectional study, taking into consideration their serological and molecular diagnosis, and demographic characteristics, as well as the possible association of this condition with other infectious pathologies and risk factors.

Materials and Methods

One hundred and ninety one EIA reactive candidates among blood donors at the HEMOCE were tested with confirmatory HTLV WB version 2.4 (WB2.4) from Genelabs Diagnostic. Seropositivity was interpreted according to the stringent criteria indicated by Genelabs Diagnostics. A WB test was scored as HTLV-1 positive only if bands for the gag proteins (p19 with or without p24) and two env proteins (GD21 and rgp46-1) were present. It was scored as HTLV-2 positive if bands for the gag proteins (p24 with or without p19) and two env proteins (GD21 and rgp46-2) were found. The test was considered as indeterminate if

specific bands for HTLV that did not fulfill the criteria of positivity for HTLV-1 and 2 were present. Those that did not exhibit bands specific for HTLV were considered negative.

For HTLV-1/2 molecular analysis, 41 out of the 118 samples from WB seroindeterminate individuals were tested with PCR at the Rega Institute for Medical Research and University Hospitals of the Katholieke Universiteit Leuven (Belgium).

For provirus detection in patient samples, 10^6 peripheral blood mononuclear cells were pelleted and lysed in PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl Perkin-Elmer), containing 2 mM $MgCl_2$, 0.5% Tween-20, 0.5% NP40, and 100 μ g/ml proteinase K (Boehringer Mannheim, stabilized proteinase K solution), for 1 hr at 56°C. The DNA was extracted using phenol/chloroform (Life Technologies), precipitated with ethanol, and dissolved in Milli-Q water (Millipore system). The Qiagen blood kit was used (Westburg) for extracting the DNA of the control cell lines.

DNA from 10^5 cells was used for amplification in a 50 μ l reaction volume containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 200 μ M nucleotide triphosphates, 0.2 μ M outer or 0.5 μ M inner primers and 0.025 U/ μ l AmpliTaq (Perkin-Elmer), with 1.5 mM $MgCl_2$ for the generic primers and 2 mM for the discriminatory inner primers (Table 1). Cycling conditions on a GeneAmp PCR System 9600 (Perkin-Elmer) were 30 sec 94°C, 15 sec 50°C, 45 sec 72°C for the outer generic PCR; on a Triothermobloc (Biometra) they were 30 sec 95°C, 15 sec 52°C, 1 min 72°C for the inner generic PCR; and 30 sec 95°C, 15 sec 50°C, 1 min 72°C for the inner discriminatory PCR. The outer fragment was amplified for 45 cycles; 2 μ l was transferred to the inner PCR, and amplified for 25 cycles. A globin PCR was performed using primer pair PC03/KM38 to assure the quality of the DNA [6]. Amplification products were separated on a 6% polyacrylamide gel and visualized by ethidium bromide staining. The images were processed on a video imager (ImageMaster VDS, Pharmacia Biotech, Roosendaal, The Netherlands). Positive controls for the PCR were MT-2 cells harboring

HTLV-1 MT2 (kindly provided by Luc Montaigner, Institut Pasteur, Paris, France), SI-5 cells harboring HTLV-1 Me15 (kindly provided by Richard Yanagihara, NIH, Bethesda, MD), 729 cells harboring HTLV-2 Mo (kindly provided by Helen Lee, Abbott Laboratories, North Chicago, IL) clone 19 cells harboring HTLV-2 (kindly provided by Dana Gallo, Viral and Rickettsial Disease Laboratory, Berkeley, CA), Cu cells harboring HTLV-2, Gu, PH969 cells harboring STLV-PH969, and PP1664 cells harboring STLV-PP1664. Negative controls for the PCR were Hut-78 cells (kindly provided by Institut Pasteur, Brussels, Belgium) and ACH-2 cells (kindly provided through the AIDS reagent project of the Medical Research Council, UK).

The PCR primers were designed using the tax gene alignment of all available HTLV and STLV strains. The primer sequences for the generic nested PCR and the four discriminatory inner PCRs are given in Table 1. Primers were developed and analyzed using the Oligo software (Medprobe, Oslo, Norway). Synthesis was carried out by Pharmacia Biotech and Perkin-Elmer/Applied Biosystems.

For the demographic study, age and gender were considered. Thus, 118 seroindefinite and 73 seropositive individuals previously diagnosed by WB, and 41 out of 118 WB seroindefinite individuals diagnosed by PCR, were included.

The analysis of co-infection and risk factors was based on previous information obtained from some of the individuals of our series. This explains the different numbers shown below.

The analysis of co-infection included laboratorial evidence for infections such as syphilis, Chagas disease, HIV, and hepatitis. Thus, 83 WB seroindefinite and 27 seropositive individuals for HTLV-1/2, as well as 31 WB seroindefinite individuals, diagnosed by PCR, were studied.

The risk factors, breast-feeding, blood transfusion, sexually transmitted diseases (STD) (syphilis) and intravenous drug use (IDU) were analyzed in 95 seroindefinite and 65 seropositive individuals diagnosed by WB, and in 36 seroindefinite ones diagnosed by PCR.

This research project was approved by the Committee for Ethics in Research of the Faculty of Medicine of the Federal University of Cear (Brazil), with the participating individuals each signing a consent document.

Descriptive statistics and association tests for contingency tables (Fischer's exact test) were used in the analysis. Levene's and Student's t-tests were used for analysis of variance and to determine if the means differed [7]. A level of significance of $p \leq 0.05$ was considered.

Results

The WB confirmatory test of our sample of 191 EIA reactive individuals showed that 73 were seropositive and 118 were seroindefinite for HTLV-1/2.

The PCR analysis of the 41 WB seroindefinite individuals revealed that 32 (78%) were negative and 9 (22%) were positive (7 for HTLV-1 and 2 for HTLV-2).

The demographic analysis of the 118 WB seroindefinite and 73 seropositive individuals showed that there was a significant ($p=0.0420$) association between sex and WB results, from which one could depict a trend for female predominance in the group of individuals seropositive for HTLV-1/2, and for male predominance in the group of seroindefinite individuals (Table 2). The mean age of HTLV-1/2 seroindefinite and seropositive individuals was 34.5 years ($SD=9.6$; Median=32) and 40.4 years ($SD = 14.7$; Median = 41.5), respectively. The mean age of seroindefinite individuals was significantly lower ($p=0.0029$) than that of HTLV-1/2 seropositive individuals.

When we examined associated diseases, there was no significant correlation between the results of WB and PCR tests and co-infections (syphilis, Chagas disease, HIV, and hepatitis) (Tables 3 and 4).

There was also no significant association of WB HTLV-1/2 seroindefinite and seropositive individuals with risk factors such as breast-feeding, blood transfusion, STD (syphilis) and IDU, based on the WB and PCR/WB results (Table 5).

Table 1. Primers in the tax/rex gene used for the generic and discriminatory HTLV PCRs

PCR	Primers	Sequence 5'-3'	Orientation
Generic outer	AV45	GGACGCGTT(A/G)TC(A/G)GCTC	Sense
	AV46	(G/T)GG(A/G)GAIAG(C/T)TGGTA(G/T)AGGTA	Antisense
Generic inner	AV42	CTCCCCTCCTTCCCCAC	Sense
	AV43	CCA(G/C)(A/G)(G/T)GGTGTATAIGTTTTGG	Antisense
HTLV-1 inner	AV49	CCCTCCTTCCTCCAGGCCAT	Sense
	AV80	GGTCTGGAAAAGACAGGGTTG	Antisense
HTLV-2 inner	AV50	TCAATCAATGCGAAAGCACACC	Sense
	AV81	TAGGTATAGGCATACTACGGTT	Antisense
STLV-PH969 inner	AV51	ACAATTGCCTCGAGCTCACCC	Sense
	AV82	GAGGCACACGACGGAGCT	Antisense
STVL-PP1664 inner	AV52	ACGGGTGCCTATACCCAATC	Sense
	AV83	GGTACAAGCAAACACTACGGTTC	Antisense

Table 2. Distribution of WB seroindefinite (n = 118) and seropositive (n = 73) individuals by gender

WB Test	Sex					
	Female		Male		Total	
	n	%	n	%	n	%
Seroindefinite (n = 118)	33	28.0	85	72.0	118	100.0
Seropositive (n = 73)	31	42.5	42	57.5	73	100.0
Fischer's exact test	p = 0.0420					

Discussion

We found that 9 (22%) and 32 (78%) of the 41 WB seroindefinite individuals were positive and negative for HTLV-1/2, respectively, as determined by PCR; consequently, this method could be useful for defining the condition of the WB seroindefinite individuals for HTLV-1/2.

These findings are coherent with previous investigations. For example, Medrano et al. [8] studied 16 seroindefinite individuals, and found HTLV-1/2 with PCR in 4 (25%). In addition, Garin et al. [9] studied 67 blood samples of seroindefinite individuals in Zaire, and he found HTLV-1 (with PCR) in only 10 (15%) of them, whilst none were positive for HTLV-2. On the other hand, Caterino-de-Araújo

et al. [10] analyzed 553 samples from HIV positive individuals from São Paulo (Brazil), of which 24 (4.3%) were seroindefinite for HTLV-1/2, 25% of them being positive for HTLV-2 by PCR. Delaporte et al. [11] carried out two studies in Gabon and found positivity by PCR in 16% and 31% of the WB seroindefinite samples. However, Lipka et al. [12] analyzed 67 seroindefinite samples and did not find positivity for HTLV-1/2 by PCR in any of them. Nerurkar et al. [13] studied 27 seroindefinite individuals from Papua New Guinea and the Solomon Islands, and viral sequences of HTLV-1 were not found in any of them. Gessain et al. [14] reported a high prevalence of seroindefinite patterns in the tropics, with PCR negative results. This may mean that there are additional etiologies for this condition.

Table 3. Distribution of WB seroindefinite (n = 83) and seropositive (n = 27) individuals for HTLV-1/2 as a function of the Co-Infection test results

WB Test	Co-Infection Test Results											
	Chagas disease				Syphilis				HIV			
	Negative		Positive		Negative		Positive		Negative		Positive	
	n	%	n	%	n	%	n	%	n	%	n	%
Seroindefinite	83	76.1	–	–	83	75.5	–	–	81	75.0	2	100.0
Seropositive	26	23.9	1	100.0	27	24.5	–	–	27	25.0	–	–
Total	109	100.0	1	100.0	110	100.0	–	–	108	100.0	2	100.0

WB Test	Co-Infection Test Results											
	Hepatitis B (HBsAg)				Hepatitis B (HBc)				Hepatitis C (HCV)			
	Negative		Positive		Negative		Positive		Negative		Positive	
	n	%	n	%	n	%	n	%	n	%	n	%
Seroindefinite	78	75.7	5	71.4	71	78.9	12	57.1	78	77.2	5	50.0
Seropositive	25	24.3	2	28.6	19	21.1	9	42.9	23	22.8	5	50.0
Total	103	100.0	7	100.0	90	100.0	21	100.0	101	100.0	10	100.0

Fischer's exact test p = 1.0000 p = 0.05159 p = 0.1183

Table 4. Distribution of PCR negative (n = 25) and PCR/WB positive (n = 33) individuals for HTLV-1/2 as a function of the Co-Infection test results

PCR/WB Test	Co-Infection Test Results											
	Chagas Disease				Syphilis				HIV			
	Negative		Positive		Negative		Positive		Negative		Positive	
	n	%	n	%	n	%	n	%	n	%	n	%
Negative (PCR)	25	43.9	–	–	25	43.1	–	–	24	42.1	1	100.0
Positive HTLV-1 (PCR/WB)	19	33.3	–	–	19	32.8	–	–	19	33.3	–	–
Positive HTLV-2 (PCR/WB)	13	22.8	1	100.0	14	24.1	–	–	14	24.6	–	–
Total	57	100.0	1	100.0	58	100.0	–	–	57	100.0	1	100.0

PCR/WB Test	Co-infection Test Results											
	Hepatitis B (HBsAg)				Hepatitis B (HBc)				Hepatitis C (HCV)			
	Negative		Positive		Negative		Positive		Negative		Positive	
	n	%	n	%	n	%	n	%	n	%	n	%
Negative (PCR)	23	42.6	2	50.0	21	47.7	4	26.7	22	44.9	3	30.0
Positive HTLV-1 (PCR/WB)	18	33.3	1	25.0	13	29.5	6	40.0	13	26.5	6	60.0
Positive HTLV-2 (PCR/WB)	13	24.1	1	25.0	10	22.7	5	33.3	14	28.6	1	10.0
Total	54	100.0	4	100.0	44	100.0	15	100.0	49	100.0	10	100.0

According to data (unpublished) from the hematological center of Fortaleza (HEMOCE-Ceará) from 1997 to 2000, the prevalence of seroindefinite individuals was 0.16% in the general population of blood donors and 21.99% among the population of EIA reactive individuals. In another study, also in Ceará (Brazil), De Castro-Costa et al. [15] analyzed 1042 blood samples of non-neurological patients from Fortaleza (n=593) and Crato (n=449), of which 6 (1.0%) in Fortaleza and 2 (0.4%) in Crato exhibited a seroindefinite profile.

There is however variation of prevalence depending on the geographic area and the population studied. While in Europe and USA the prevalence is low, in tropical areas it reaches higher values [4,5]. Lal et al. [16] analyzed 267,650 samples from blood donors, among which 379 (0.14%) were WB seroindefinite. Césaire et al. [17] carried out an analysis of 9,759 blood donors from the West Indies and found 39 (0.4%) seropositive and 49 (0.5%) seroindefinite individuals for HTLV-1. Maucière et al. [18] studied 3783 individuals from the rural population of Cameroon and found a prevalence of 1.6% seroindefinites. Garin et al. [9] studied the frequency of positivity for HTLV-1/2 in 98 samples of blood from individuals from Zaire and found that 28 (28.6%) were seropositive, 3 (3.0%) were seronegative and 67 (68.4%) were seroindefinite. In Brazil, Caterino-de-Araújo et al. [10] investigated HTLV-1/2 infection in 553 samples of serum from HIV patients in São Paulo, of which 24 (4.3%) had an indeterminate profile.

Demographically, young men predominated among the seroindefinite individuals, while older women predominated among the ones seropositive for HTLV-1/2. The difference in age between seroindefinite and seropositive individuals could be due to tardive seroconversion; while the trend of women predominating among the seropositive individuals could be due to their greater exposure to infection with HTLV-1/2 [18,19]. Césaire et al. [17], however, did not find significant gender differences between groups seroindefinite and seropositive for HTLV-1/2,

although consistent with our findings, they found that the seroindefinite and seronegative individuals were younger than the seropositives.

Our investigation of HTLV-1/2 seroindefinite and seropositive individuals with co-infections revealed seropositive individuals with anti-HBc (hepatitis B) and HCV (hepatitis C) antibodies, which is also shown in supported by previous reports from Brazil [20,21] and from other countries [17,19].

Other presumptive associations of indeterminate profiles have been reported with *Plasmodium falciparum* [22-27], varicella zoster virus or herpes simplex [28-31], and dengue virus [32]. Moreover, recent seroconversion to positivity for HTLV-1/2 [17], incomplete reactions to molecularly distinct variants of HTLV-1/2 or eventually to another exogenous or endogenous retrovirus [30], and transient infection by HTLV-1 [14], have also been reported. Other conditions are also supposedly correlated with a seroindefinite profile. For example, Haynes et al. [33] and Palker et al. [34] analyzed the antigenic relationship between p19 and the thymic epithelium, possibly linked to immune conditions in HTLV-1 infected individuals. Seroindefinite patterns have been found in Europe and the USA in a series of patients with multiple sclerosis, although at a very low frequency [35]. A seroindefinite profile may also be associated with hematological abnormalities compatible with viral infection [36] or with a possible, as yet unidentified, genetic or environmental factor [37].

These different possibilities offer an exciting path of research towards the etiology and significance of the seroindefinite profile for HTLV-1/2 infection, especially in tropical and in some temperate regions. Longitudinal follow-up of these seroindefinite individuals, defined initially as positive or negative by PCR, is suggested. This follow-up should include other sophisticated tests for defining HTLV-1/2 infection, such as for example, INNO-LIA and real time PCR, as well as tests for defining parasitic, bacterial or viral infections, endemic in the related environmental setting. This may stimulate multicentric studies to define the real status of WB seroindefinite individuals in these different contexts.

Table 5. Distribution of WB seroindefinite (n = 95) and seropositive (n = 69) individuals for HTLV-1/2 as a function of the Risk Factors

WB Test	Risk Factors															
	Breast-feeding				Blood transfusion				STD (Syphilis)				IDU			
	No		Yes		No		Yes		No		Yes		No		Yes	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Seroindefinite (n = 95)	3	42.9	92	60.1	82	62.1	13	46.4	75	63.0	20	48.8	94	59.9	1	50.0
Seropositive HTLV-1/2 (n = 65)	4	57.1	61	39.9	50	37.9	15	53.6	44	37.0	21	51.2	64	40.1	1	50.0
Total	7	100.0	153	100.0	132	100.0	28	100.0	119	100.0	41	100.0	158	100.0	2	100.0
Fischer's exact test	p = 0.4433				p = 0.1416				p = 0.1402				p = 1.0000			

Acknowledgments

We thank the team of researchers and technicians of the Rega Institute of the Katholieke Universiteit Leuven (Belgium), the hematological centers of Fortaleza, CE (HEMOCE) and of Belo Horizonte, MG (HEMOMINAS), the Central Laboratory of the University Hospital of Fortaleza, CE (Brazil) for their collaboration, Dr. David Neil Criddle for the review of English style and Sílvia A Costa for the accurate electronic typewriting of this manuscript.

References

- Galvão-Castro B., Loures L., Rodrigues L.G., et al. Distribution of human T-lymphotropic virus type 1 among blood donors: a nationwide Brazilian study. *Transfusion* **1997**;37(2):242-3.
- Gessain A., Barin F., Vernant J.C., et al. Antibodies to human T-lymphotropic virus type-1 in patients with tropical spastic paraparesis. *Lancet* **1985**;2:407-10.
- Osame M., Izuma S., Igata A., et al. Blood transfusion and HTLV-1 associated myelopathy. *Lancet* **1986**;2:104-5.
- Lal R.B., Lipka J.J., Fong S.K., et al. Human T lymphotropic virus type 1/2 in Lake Lindu Valley, Central Sulawesi, Indonesia. *J Acquir Immune Defic Syndr Hum Retrovirol* **1993**;6:1067-8.
- Mahieux R., Horal P., Maucière P., et al. Human T-cell lymphotropic virus type 1 gag indeterminate Western blot patterns in Central Africa: relationship to *Plasmodium falciparum* infection. *J Clin Microbiol* **2000**;38:4049-57.
- Vandamme A.M., Fransen K., Debaisieux L., et al. Standardization of primers and an algorithm for HIV-1 diagnostic PCR evaluated in patients harbouring strains of diverse geographical origin. The Belgian AIDS Reference Laboratories. *J Virol Methods* **1995**;51:305-16.
- Freeman J., Daniel H. Applied categorical data analysis. Marcel Dekker, **1987**.
- Medrano F.J., Soriano V., Calderón E.J., et al. Significance of indeterminate reactivity to human T-cell lymphotropic virus in Western blot analysis of individuals at risk. *Eur J Clin Microbiol Infect Dis* **1997**;16:249-52.
- Garin B., Gosselin S., De Thé G., Gessain A. HTLV-1/2 infection in a high viral endemic area of Zaire, Central Africa: comparative evaluation of serology, PCR, and significance of indeterminate Western blot pattern. *J Med Virol* **1994**;44:104-9.
- Caterino-de-Araújo A., de los Santos-Fortuna E., Meleiro M.C., et al. Sensitivity of two enzyme-linked immunosorbent assay tests in relation to Western blot in detecting human T-cell lymphotropic virus type 1 and 2 infection among HIV-1 infected patients from São Paulo, Brazil. *Diagn Microbiol Infect Dis* **1998**;30:173-82.
- Delaporte E., Monplaisir N., Louwagie J., et al. Prevalence of HTLV-1 and HTLV-2 infection in Gabon, Africa: comparison of the serological and PCR results. *Int J Cancer* **1991**;49:373-6.
- Lipka J.J., Young K.K., Kwok S.Y., et al. Significance of human T-lymphotropic virus type 1 indeterminate serological findings among healthy individuals. *Vox Sang* **1991**;61:171-6.
- Nerurkar V.R., Miller M.A., Leon-Monzon M.E., et al. Failure to isolate human T cell lymphotropic virus type 1 and to detect variant-specific genomic sequences by polymerase chain reaction in Melanesians with indeterminate Western immunoblot. *J Gen Virol* **1992**;73:1805-10.
- Gessain A., Mahieux R., De Thé G. HTLV-1 "indeterminate" Western blot patterns observed in sera from tropical regions: the situation revisited (Letter). *J Acquir Immune Defic Syndr Hum Retrovirol* **1995**;9(3):316-8.
- De Castro-Costa C.M., Goubau P., Liu H.-F., et al. HTLV-negative and HTLV-positive tropical spastic paraparesis in Northeastern Brazil. *AIDS Res Hum Retroviruses* **1995**;11:315-18.

16. Lal R.B., Rudolph D.L., Coligan J.E., et al. Failure to detect evidence of human T-lymphotropic virus (HTLV) type 1 and type 2 in blood donor with isolated gag antibodies to HTLV-1/2. *Blood* **1992**;80:544-50.
17. Césaire R., Bera O., Maier H., et al. Seroindeterminate patterns and seroconversions to human T-lymphotropic virus type 1 positivity in blood donors from Martinique, French West Indies. *Transfusion* **1999**;39:1145-9.
18. Mauclère P., Le Hesran J.Y., Mahieux R., et al. Demographic, ethnic, and geographic differences between human T cell lymphotropic virus (HTLV) type-1-seropositive carriers and persons with HTLV-1 gag-indeterminate Western blots in Central Africa. *J Infect Dis* **1997**;176:505-9.
19. Rouet F., Meertens L., Courouble G., et al. Serological, epidemiological, and molecular differences between human T-cell lymphotropic virus Type 1 (HTLV-1)-seropositive healthy carriers and persons with HTLV-1 gag indeterminate Western blot patterns from the Caribbean. *J Clin Microbiol* **2001**;39:1247-53.
20. Nogueira C.M., Cavalcanti M., Schechter M., Ferreira O.C. Jr. Human T lymphotropic virus type 1 and 2 infections in healthy blood donors from Rio de Janeiro, Brazil (Letter). *Vox Sang* **1996**;70:47-8.
21. Carneiro-Proietti A.B., Lima-Martins M.V., Passos V.M., et al. Presence of human immunodeficiency virus (HIV) and T-lymphotropic virus type 1 and 2 (HTLV-1/2) in a haemophiliac population in Belo Horizonte, Brazil, and correlation with additional serological results. *Haemophilia* **1998**;4:47-50.
22. Biggar R.J., Gigase P.L., Melbye M., et al. ELISA HTLV retrovirus antibody reactivity associated with malaria and immune complexes in healthy Africans. *Lancet* **1985**;2:520-3.
23. Yanagihara R., Jenkins C.L., Alexander S.S., et al. Human T-lymphotropic virus type 1 infection in Papua New Guinea: high prevalence among the Hagahai confirmed by Western analysis. *J Infect Dis* **1990**;162:649-54.
24. Hayes C.G., Burans J.P., Oberst R.B. Antibodies to human T lymphotropic virus type 1 in a population from the Philippines: evidence for cross-reactivity with *Plasmodium falciparum*. *J Infect Dis* **1991**;163:257-62.
25. Lal R.B., Rudolph D., Alpers M.P., et al. Immunological cross-reactivity between structural protein of human T-cell lymphotropic virus type 1 and the blood stage of *Plasmodium falciparum*. *Clin Diagn Lab Immunol* **1994**;1:5-10.
26. Porter K.R., Liang L., Long G.W., et al. Evidence for anti-*Plasmodium falciparum* antibodies that cross-react with human T-lymphotropic virus type 1 proteins in a population in Irian Jaya, Indonesia. *Clin Diagn Lab Immunol* **1994**;1:11-5.
27. Porter K.R., Anthony R.L., Solihin A., Hayes C.G. Mapping of a human T-lymphotropic virus type 1 gag protein epitope that cross-reacts with anti-*Plasmodium falciparum* antibodies. *J Med Virol* **1995**;45:469-74.
28. Sato A., Isaka Y., Morita F., et al. Human sera from varicella-zoster virus (VZV) infections cross-react with human T-cell leukaemia virus type 1 (HTLV-1): common epitopes in VZV gene 22 protein and HTLV-1 p19 gag protein. *J Gen Virol* **1992**; 73:2969-73.
29. Banki K., Maceda J., Hurley E., et al. Human T-cell lymphotropic virus (HTLV)-related endogenous sequences, HRES-1, encodes a 28-kDa protein: a possible autoantigen for HTLV-1 gag-reactive autoantibodies. *Proc Natl Acad Sci USA* **1992**;89: 1939-43.
30. Miyakoshi H., Sugimoto M., Igarashi H., et al. Improvement of simultaneous detection of antibodies to gag and envelope antigens of human T-lymphotropic virus type 1 by Western immunoblot assay. *J Clin Microbiol* **1992**;30:2555-59.
31. Segurado A.A., Malaque C.M.S., Sumita L.M., et al. Laboratory characterization of human T-cell lymphotropic virus types 1 (HTLV-1) and 2 (HTLV-2) infections in blood donors from São Paulo (Brazil). *Am J Trop Med Hyg* **1997**;57(2):142-48.
32. Carvalho S.M.F., Pombo-de-Oliveira M.S., Thuler L.C.S., et al. Cross-reactivity between human T-cell leukemia/lymphoma virus indeterminate Western blot and dengue virus in individuals from Rio de Janeiro, Brazil. *J Acquir Immune Defic Syndr Hum Retrovirol* **1999**;20:4(P094).
33. Haynes B.F., Robert-Guroff M., Metzgar R.S., et al. Monoclonal antibody against human T cell leukemia virus p19 defines a human thymic epithelial antigen acquired during ontogeny. *J Exp Med* **1983**;157:907-20.
34. Palker T.J., Singer K.H., Vahlne A. Characterization of an antigen shared by human thymic epithelium and human T cell leukemia virus p19 gag protein. *J Acquir Immune Defic Syndr Hum Retrovirol* **1996**;11:10-19.
35. Soldan S.S., Graf M.D., Waziri A., et al. HTLV-1/2 seroindeterminate Western blot reactivity in a cohort of patients with neurological disease. *J Infect Dis* **1999**;180: 685-94.
36. Picchio G.R., Bare P., Savignano R., et al. HTLV-1/2 indeterminate serology and natural killer cell expansion. *J Acquir Immune Defic Syndr Hum Retrovirol* **1996**; 12:428-31.
37. Tuppin P., Makuwa M., Guerma T., et al. Low HTLV-1/2 seroprevalence in pregnant women in Congo and a geographic cluster of an HTLV-like indeterminate Western Blot pattern. *J Acquir Immune Defic Syndr Hum Retrovirol* **1996**;11:105-7.