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Commentary and point of view

Utility of HTLV proviral load quantification in diagnosis of HTLV-1-associated myelopathy requires international standardization

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The geographic distribution of Human T-cell Lymphotropic Virus Type 1 (HTLV-1) infection makes one thing clear: except Japan, most of the estimated 20 million infected individuals are clustered within communities with limited health care access [1].

Given that the majority of infected persons remain disease-free, one of the challenges of the clinical management of HTLV-1-infected patients with myelopathic symptoms is to establish the definite diagnosis of HTLV-Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP). Asymptomatic carriers may complain of a range of symptoms that cannot be excluded from an association with HTLV-1, such as dry eyes, urinary incontinence and constipation. Current HAM/TSP diagnostic procedures are based on criteria established by the World Health Organization (WHO), which consists of a list of neurological signs and symptoms in HTLV-1 seropositive subjects. In addition, imaging of the central nervous system is essential to exclude other neurological diseases with similar clinical features, whilst isolation of HTLV-1 proviral in the cerebrospinal fluid (CSF) [2] is a positive finding, especially when the viral load in CSF lymphocytes is greater than in PBMCs [3]. However, these complementary tests are not readily available in many communities. In our experience, patients rarely present with all the essential features of HAM/TSP to meet the diagnostic requirements of the complete syndrome. To complement WHO criteria, a new classification strategy, the Belem Criteria, based on three diagnostic ascertainment levels was proposed [4]. HTLV-1-infected patients with neurological defects are categorized as: (i) *Definite HAM/TSP:* patients who meet the established WHO criteria with a complete clinical presentation; (ii) *Probable HAM/TSP:* patients with a myelopathic mono-symptomatic presentation, in which other diseases resembling HAM/TSP have been excluded; (iii) *Possible HAM/TSP:* patients who present with a complete or incomplete clinical picture; however, other disorders resembling HAM/TSP cannot be excluded.

In recent years, several studies have demonstrated a clear association between HAM/TSP and HTLV-1 proviral load [5–11]. Moreover, compelling evidence indicates that patients with other

Abbreviations: HTLV, human T-cell lymphotropic virus; HAM/TSP, HTLV-1-associated myelopathy/tropical spastic paraparesis; PBMC, peripheral blood mononuclear cells; PVL, proviral load.

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Table 1
Methodological characteristics of HTLV-1 proviral load measurements using PCR method from asymptomatic carriers and HAM/TSP patients in endemic countries for HTLV-1-infection.

Author, year	Country	PCR method	Region amplified	% infected cells asymptomatic	Number of asymptomatic carriers	% infected cells HAM/TSP	Number of HAM/TSP patients
Nagai et al., 1998 [8]	Japan	TaqMan	pX	0.3[a]/3.2[a]	200	5.4	202
Manns et al., 1999 [7]	Jamaica	TaqMan	pX	1.1	50	4.9	27
Olindo et al., 2005 [7]	Martinique	TaqMan	pol	0.8	34	8.1	100
Montanheiro et al., 2005 [10]	Brazil	TaqMan	pol	2.7	45	6.8	44
Best et al., 2006 [5]	Peru	SYBR green	pX	5.6	33	18	35
Silva et al., 2007 [11]	Brazil	TaqMan	pX	1.0	93	6.3	197
Grassi et al., 2011 [14]	Brazil	TaqMan	pol	0.7	189	11.6	47
Furtado et al., 2012 [15]	Brazil	SYBR[a] Green	pol	0.5	75	3.4	78
Demontis et al., 2012 [3]	United Kingdom	SYBR Green	Tax	1.8	211	14.7	85

#-Number of evaluated patients, % infected cells: data represents median.
[a] DNA sample from whole blood. All other DNA samples were from peripheral blood mononuclear cells.

HTLV-1-associated inflammatory conditions, such as infective dermatitis [12] or keratoconjunctivitis sicca [13], display significantly higher levels of proviral load compared to asymptomatic carriers.

In a published study conducted in Bahia, Brazil, we found that a proviral load cut-off value of 50,000 copies/10^6 PBMCs (5%), differentiated asymptomatic carriers from HAM/TSP patients with 87% sensitivity and 81% specificity [14]. Using the Belem criteria, only 22% of probable and 17% of definite HAM/TSP patients' HTLV-1 proviral loads fell below this cut-off value. If this threshold had been included as an additional criterion to diagnose HAM/TSP, 73% of patients from the probable group would be reclassified as definite.

Furtado et al. [15] also attempted to establish a proviral load cut-off value to distinguish asymptomatic carriers from HAM/TSP patients in Minas Gerais (Brazil). Using whole blood, a cut-off of 114 HTLV-1 copies/10^4 white blood cells (1.14%), offered 78.2% sensitivity and 28% specificity in patients with HAM/TSP. This value is considerably lower than what was observed in Bahia. As members of the HAM/TSP Clinical Trial Study Group (HAM/TSP-CTSG) we performed a literature review of proviral loads reported in asymptomatic carriers and HAM/TSP patients (Table 1). The percentage of infected cells was approximately 6× higher in HAM/TSP patients (median 6.8%, 5.4–18%) than in asymptomatic carriers (median 1.1%, 0.3–5.6%). Despite overlapping ranges, the trends are consistent across studies. However, study groups used diverse methods to measure proviral load, including different regions of the targeted HTLV-1 genome, the DNA sample source, e.g. whole blood cells or isolated PBMCs and cells quantified from patient samples. In personal consultation with HAM/TSP CTSG members we established that 7.04% and 10% of patients with definite HAM/TSP, living in Bahia, Brazil and Japan respectively, have a proviral load of <1% in PBMCs. These patients have only mild disease. In contrast, all definite HAM/TSP patients from UK or USA had a proviral load of >1% [3]. Based on the reviewed data and our own observations across four centres (Bahia/Japan/UK/USA) a single cut-off for asymptomatic versus HAM/TSP or for definite versus probable HAM/TSP cannot be recommended.

We therefore propose an international pilot study in an attempt to achieve consistent results in order to evaluate HTLV-1 proviral load in PBMCs of asymptomatic carriers as well as patients with probable, possible and definite HAM/TSP living in HTLV-1 endemic and non-endemic areas. By testing these patients at several chosen established laboratories blinded to samples' origins, we hope to take initial steps towards the standardization of proviral load quantification technique, with the ultimate goal of determining a relevant proviral load cutoff value to distinguish asymptomatic carriers from HAM/TSP patients. At the same time, we recognize that, due to variation from assay to assay, there are limitations with respect to viral load quantification [16,17]. In the future, the HTLV research community might propose the incorporation of a WHO international standard to aid in clinical applications by soliciting assistance from such groups as Standardization of Genome Amplification Techniques or National Institute for Biological Standards and Control.

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Competing interests

None declared.

Ethical approval

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