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Interferon-γ Secretion Enzyme-Linked Immunospot Assay Determined Among Human T Cell Lymphotropic Virus Type 1-Infected Subjects: A Potential Laboratory Marker for Early HTLV-1–Associated Myelopathy/Tropical Spastic Paraparesis Diagnosis

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EDITOR: Approximately 5–10 million people worldwide live with the human T cell lymphotropic virus type 1 (HTLV-1), with 5%–10% developing HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP). This disabling neurodegenerative disease results from inflammation in the central nervous system,[1] mediated through inflammatory cytokines secreted by immune system cells, a process in which interferon (IFN)-γ plays a key role.[2] Some preceding HAM/TSP clinical symptoms, which do not fulfill the specific classical clinical conditions needed to that diagnosis, may in fact indicate early HAM/TSP, and have been described as intermediate syndrome/stage.[3] Previous studies showed that the IFN-γ enzyme-linked immunospot assay (ELISPOT) was able to identify HAM/TSP patients.[4] ELISPOT is a highly sensitive method used to monitor immune responses through quantification of cytokine-secreting spot-forming cells at a single cell level. We invited volunteers from the IIER HTLV clinic who remained in active follow-up from June 2016 to May 2017; they were subdivided according to their neurological status into three groups ($n = 89$ subjects); I, 31 asymptomatic HTLV-1 carriers; II, 27 intermediate stages HTLV-1 carriers; and III, 31 patients with HAM/TSP. As controls, we enrolled 34 healthy HTLV-1 negative subjects. The ELISPOT 96-well plates (Millipore) were washed once with 35% alcohol and then five times with phosphate-buffered saline (PBS) at $1\times$, and then coated with human IFN-γ capture antibodies (Mab 1d1k Mabtech AB), at a concentration of 10 μg/mL diluted in $1\times$PBS; after these procedures, plates remained in the refrigerator overnight (at 4°C) for 18–24 h. We calculated diagnostic parameters, such as sensitivity, specificity, predictive values, accuracy, area under the curve, and the Kappa index, by using the receiver operating characteristic (ROC) curve analysis. The ratio of females/males was 20/14 for the controls, 24/7 for asymptomatic subjects, 24/3 for HTLV-1 intermediate stage, and 20/11 for HAM/TSP patients; mean age was $\sim$50 years. HAM/TSP patients had a mean of 14 years since diagnosis and an Osame scale score of five points, at the moment of data collection. There was a strong correlation of spot forming cells with the clinical status ($p < .0001$). Sensitivity values reached 60% for the asymptomatic carriers, 100% for patients with the intermediate syndrome, and 96.7% for HAM/TSP patients when the cutoff value of our method was settled at 154 spot-forming cells (SFC). Mean spot values were 2726 SFC for intermediate syndrome patients and 2074 SFC for HAM/TSP patients; SFC numbers of the symptomatic patients were three to four times those of the asymptomatic subjects (645 SFC) (Table 1). In this report, *in vitro* IFN-γ secretion was a marker for predicting HAM/TSP and it seemed able to identify the clinical status of HTLV-1 disease, including the intermediate phase of the disease. The test we made can be considered confirmatory, since it had a general sensitivity of 81.2% and a high positive predictive value, the positive likelihood ratio >10 confirming this fact. Thus, the ELISPOT method can be useful to evaluate and monitor the spontaneous IFN-γ FSC, and with potential to identify patients at higher risk for HAM/TSP development.

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Table 1. Univariate Analysis of Sociodemographic and Laboratory Variables of Human T Cell Lymphotropic Virus Type 1 Volunteers and Negative Controls

	Groups				
Variables	*Controls (n = 34)*	*Asymptomatic (n = 31)*	*Intermediate stage (n = 27)*	*HAM/TSP (n = 31)*	p
Age, mean ± SD	38 ± 13	50 ± 11	47 ± 15	51 ± 11	NS
Gender, *n* (%)					
Female	20 (70)	24 (77)	24 (89)	20 (65)	NS
Male	14 (30)	7 (23)	3 (11)	11 (35)	
HTLV-1 proviral load (copies/10^4 PBMC), mean ± SD		78 ± 215	171 ± 439	276 ± 600	.0022
Lymphoproliferation (LPA) (CPM), mean ± SD	324 ± 195	1939 ± 3129	2770 ± 2569	4974 ± 9339	<.0001
Spot-forming cells BASAL, mean ± SD	46 ± 80	587 ± 705	2239 ± 2295	2010 ± 2053	<.0001

BASAL, baseline, healthy volunteers; CPM, count per minute; HAM/TSP, HTLV-1 -associated myelopathy/tropical spastic paraparesis; HTLV-1, human T cell lymphotropic virus type 1; LPA, lymphocytes spontaneous proliferation assay; NS, nonsignificant; PBMC, peripheral blood mononuclear cells; SD, standard deviation.

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