High Seroprevalence of Anti-HTLV-I/II Antibodies Among Solid Organ Donors Necessitates Confirmatory Testing

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Background. Human T-cell lymphotrophic virus (HTLV) type I has been linked to adult T-cell leukemia/lymphoma (ATL) and HTLV-I associated myelopathy (HAM). Transmission of HTLV by blood and organ transplantation has been documented, with some infections leading to clinical disease. Organ donors are tested for anti-HTLV antibodies and donor suitability is determined primarily by results from enzyme immunoassays (EIA). Confirmatory testing is not routinely performed, and the number of false positive organ donors is unknown.

Methods. In order to investigate the contemporary seroprevalence of anti-HTLV I/II antibodies among solid organ donors and determine the number of false positive samples, we tested 1,408 specimens from prospective organ donors in 2002 and 2003. All specimens were tested for anti-HTLV antibodies by a commercial EIA. Repeatedly reactive specimens underwent confirmatory testing using a commercial Western blot.

Results. There were 22 repeatedly EIA reactive donor specimens (1.56%). Five specimens did not undergo further testing because of case shutdown or insufficient sample quantity. HTLV I/II western blot confirmed six positives, whereas five were negative and six were indeterminate. The majority of confirmed specimens were positive for antibodies to HTLV-II.

Conclusions. Our data shows that 29% of initially reactive specimens were false positives. With the increasing demand for organs, the unnecessary rejection of organs that are falsely positive for HTLV antibodies becomes of tremendous importance and stresses the need for timely confirmatory testing for HTLV.

Keywords: Human T-cell lymphotrophic virus, Organ transplantation, Viral infection.

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The human T-cell lymphotrophic virus (HTLV) type I and II are double-stranded RNA viruses that share 60% genomic homology (*1*). HTLV-I has been linked to adult T-cell leukemia/lymphoma (ATL) and HTLV-I-associated myelopathy (HAM), also known as tropical spastic paraparesis. Most carriers of HTLV-I are asymptomatic, with disease developing only rarely after a long incubation period (*1*, *2*). HTLV-II is thought to be less pathogenic and has not been definitively linked to any disease. These infections are transmitted by blood, sexual contact, and from mother to child.

HTLV-I is endemic in southern Japan, parts of Africa, the Caribbean, Middle East, and South America, where the seroprevalence of infection is as high as 30% (*1–3*). Transmissions by blood transfusion and organ transplantation have been described, with some infections leading to clinical disease (*3–6*). In U.S. blood donors, the seroprevalence has been cited at 0.035–0.046% (*2*). Surveys of HTLV seroprevalence among blood and tissue donors in the United States, however, may not be representative for organ donors (*7–10*). Based on United Network for Organ Sharing (UNOS) data from 1988–2000, Shames et al. reported that the prevalence of HTLV-I infection in organ donors was 0.027% and the prevalence of HTLV-II was 0.064% (*2*). No HTLV-related diseases were detected in patients that received the 22 infected organs after a median follow-up time of 12 months. Additionally, UNOS reported five HTLV-positive transplanted organs between 1994–2001, with no confirmed cases of malignancy (*11*). However, in the study by Shames et al., it was unknown whether these organs underwent confirmatory testing, and the UNOS data does not reflect the number of organs rejected because of falsely positive initial screening tests.

During the donor suitability evaluation the determination of donor HTLV-I/II status is based primarily on the results obtained from enzyme immunoassay (EIA) testing. HTLV confirmatory testing is not always readily available and requires additional time. Individual organ procurement organizations (OPO) may perform confirmatory tests, but results are not reported to any central registry (*11*). In a 1996 survey of the 63 OPO in the United States, only 65% routinely performed confirmatory testing for HTLV (*12*).

Current UNOS regulation states, "UNOS members shall not knowingly participate in the transplantation or sharing of organs from donors who are confirmed positive for HTLV-1-Ab by an FDA licensed screening test unless subsequent confirmation testing unequivocally indicates the original test results were false positive" (*11*). As there is a growing shortage of organs for transplantation, confirmatory testing performed in a timely manner may identify a substantial number of donors with false positive EIA results that are not confirmed by western blot. The aim of this study is to evaluate the contemporary seroprevalence of anti-HTLV I/II antibod-

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ies and the number of falsely positive samples among solid organ donors by screening and confirmatory testing.

MATERIALS AND METHODS

In this report, we present HTLV-I/II EIA and Western blot data accumulated during testing of all prospective cadaveric organ donors between 2002 and 2003 sent to our laboratory. We tested serum specimens from 1408 donors for anti-HTLV antibodies by a commercial EIA (Abbott Laboratories, Abbott Park, IL). Repeatedly reactive specimens were tested using a commercial western blot (Genelabs Diagnostics, Singapore). The HTLV Western blot 2.4 incorporates MTA-1, a unique HTLV-1 envelope recombinant protein (rgp46-I); K55, a unique HTLV-II envelope recombinant protein (rgp46-II); and GD21. The differentiation between HTLV-I and HTLV-II was made by the combination of reactivities to rpg46-I, rpg46-II, and GD21 recombinant proteins.

RESULTS

In Tables 1 and 2, we provide results obtained from testing 1,408 tested donors. There were 22 repeatedly reactive donors by HTLV-I/II EIA (1.56%). Based on available epidemiological data for 20 donors, 40% of HTLV EIA reactive donors were African-American, 20% were Hispanics, and 40% were of Caucasian origin. The majority of donors were male (70%). Five specimens underwent no further testing due to case shutdown or insufficient sample quantity. The HTLV-I/II western blot confirmed six samples, while five were negative and another six were indeterminate. One of the Western blot negative specimens was from an African American donor, one from a Hispanic donor and three were from Caucasian donors. The overall percentage of not confirmed HTLV EIA reactive specimens was 29% over the period of two years. Most of the confirmed anti-HTLV positive donors were positive for HTLV-II specific antibodies.

DISCUSSION

HTLV-I infection can lead to ATL, a non-Hodgkin's lymphoma that is not curable with standard chemotherapy. Median survival is less than 12 months (*1*, *3*). HTLV-I infection can also lead to HAM, which manifests as lower extremity weakness, sensory disturbances and incontinence. The lifetime risk of ATL is 3–5%, while the risk of developing HAM is less than 2% (*1*).

Transmission of HTLV-I by blood transfusion and organ transplantation has been documented. In a report by Gout et al., a 41 year-old male underwent a heart transplant and blood transfusions and later developed a subacute myelopathy. It was discovered that he had been infected with HTLV-I from one of the blood transfusions (*4*). Remesar et al., who described the transmission of HTLV-I from a 35-year-old mother to her daughter, reported the first incident of HTLV-I transmission by organ transplantation (*5*). The infected recipient remained asymptomatic after four years. In contrast, Toro et al. in 2003 reported the transmission of HTLV-I to three organ transplant recipients from a single donor. In less than two years after transplantation, all three recipients developed a subacute myelopathy (*6*). The donor had been infected by vertical transmission from his mother, who was originally from Venezuela, where HTLV-I seroprevalence is 0.39% among Mestizos (*3*).

HTLV-I transmission is dangerous because there is no effective treatment for ATL or HAM. It is also unknown how immunosuppression affects the course and manifestations of infection. In the case report by Gout et al., the patient developed a subacute myelopathy just four weeks after seroconversion (*4*). This shortened time frame may be secondary to immunosuppression and the high transmitted viral load, which is associated with development of both ATL and HAM (*1*). On the other hand, Tanabe et al. followed 16 HTLV-1 positive patients who underwent kidney transplantation and immunosuppression. After a mean follow-up time of eight years, none of these patients had manifested clinical symptoms (*13*).

Our data show an unexpectedly high number of repeatedly EIA-reactive anti-HTLV specimens among prospective organ donors evaluated by our laboratory. However, approximately 29% of EIA reactive donors were not confirmed by the Western blot and another 35% gave indeterminate results. This raises the question of the EIA specificity and sensitivity, and its ability to detect a true anti-HTLV positive donor. Additionally, the University of Wisconsin reported four falsely positive donors between January 2000 and July 2001, which resulted in the discard of 14 potential organs (*2*). Based on informal questioning during one of the organ procurement organization meetings, approximately 50% of transplantation centers declared that they would use organs from HTLV EIA-positive, HTLV Western blot–negative donors (personal communication, Thoracic Medical Affairs Meeting, March 1, 2005, San Francisco, CA).

Timely performance of a confirmatory assay for HTLV may save organ donations rejected because of a falsely positive screening test or due to long delays in confirmatory testing performed by commercial laboratories. At our institution, confirmatory western blot takes five hours of additional time. False-positive HTLV EIA results with negative confirmatory Western blot test results can be caused by many factors, such as influenza

TABLE 1. Anti-HTLV EIA-reactive organ donors and results of confirmatory testing

Year	Samples tested by EIA	EIA reactive	QNS/case shutdown	HTLV I/II Western blot result			HTLV subtype		
				Confirmed	Indeterminate	Not confirmed	HTLV-I	HTLV-II	Untypable
2002	681	13	3	3	3	4	1	2	3
2003	727	9	2	3	3	1	0	3	3
Total	1408	22	5	6 (35%)	6 (35%)	5 (29%)	1	5	6

QNS, quantity not sufficient; EIA, enzyme immunoassay.

TABLE 2. Specificities of anti-HTLV reactivity among donors repeatedly HTLV EIA reactive tested by Western Blot

Patient	Year	Age	Sex	Ethnicity	rgp46-I	rgp46-II	p53	gp46	p36	p32	p28	p26	p24	gp21	p19	GD21	HTLV western blot result	HTLV type
1	2002	38	F	AA					+		+	+		+	+		Indeterminate	Untypable
2	2002	43	M	H													Negative	
3	2002	53	M	AA	+		+	+	+	+	+	+	+	+	+	+	Positive	HTLV-I
4	2002	44	F	C													Negative	
5	2002	49	M	AA									+				Indeterminate	Untypable
6	2002	52	M	AA													Case shutdown	
7	2002	59	M	AA									+	+			Positive	Untypable
8	2002	18	M	C													Negative	
9	2002	32	F	C													Negative	
10	2002	45	F	H		+							+	+	+	+	Positive	HTLV-II
11	2002	55	M	C		+		+	+				+	+	+	+	Positive	HTLV-II
12	2002	47	M	C													QNS	
13	2002	57	M	C													QNS	
14	2003	46	M	AA		+							+			+	Positive	HTLV-II
15	2003	31	M	C					+		+	+			+		Indeterminate	Untypable
16	2003	54	M	AA		+	+		+				+			+	Positive	HTLV-II
17	2003	55	M	AA													Negative	
18	2003	60	U	U		+			+				+			+	Positive	HTLV-II
19	2003	26	U	U							+			+	+		Indeterminate	Untypable
20	2003	46	M	H													QNS	
21	2003	47	F	C													QNS	
22	2003	50	F	H											+		Indeterminate	Untypable

QNS, quantity not sufficient; AA, African American; C, Caucasian; H, Hispanic; U, unknown.

vaccine, some bacterial infections, autoimmune disorders and multiple pregnancies. It was suggested that the majority of nonspecific enzyme immunoassay reactions (59%) were probably caused by reactivity to HTLV viral envelope glycoprotein GD21 (*14*). Polymerase chain reaction, nucleic acid sequence-based amplification testing (NAT) or transcription mediated amplification-based studies could potentially be much more specific and revealing in identifying false-positive donors. However, at this time there is no HTLV NAT option commercially available with a short enough turnaround time to be a viable option in resolving the question of HTLV EIA reactive donors. Additionally, a relative infrequency of such donors makes it very difficult even for a large organ donor screening laboratory to develop and maintain a HTLV NAT test. A potential solution is to support the development of a commercial NAT multiplex assay that simultaneously detects hepatitis B and C viruses, human immunodeficiency virus, and HTLV (*15*).

More than 7,000 donors were accepted for organ donation in 2004 in the United States (*16*). Extrapolating our data to the nationwide donor population seems to suggest that approximately 30–50 donors were lost due to likely false positive EIA results and delays in HTLV confirmatory testing. Based on our evaluation of the contemporary seroprevalence of anti-HTLV I/II antibodies among solid organ donors from a U.S. West Coast organ procurement organization, and specifically the results of confirmatory testing, we suggest a critical re-evaluation of the current policies regarding the testing of organ donors. After confirmatory testing, we found 29% or more of initially reactive specimens by EIA were false positives. This raises the concern of unnecessarily rejecting healthy organs and stresses the importance of timely confirmatory testing.

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