Implications of Human T-Lymphotropic Virus Type-I and Type-II Testing in Donors and Patients*†

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ABSTRACT

The retroviruses known as Human T-Lymphotropic Virus Types I and II (HTLV-I and -II) were recognized before the human immunodeficiency virus (HIV-1). Associated diseases of HTLV-I infection, including a particular kind of leukemia or the development of a specific demyelinating disease, have also been observed. Screening of blood donors for antibodies to HTLV was mandated in November of 1988. This paper examines the biology of HTLV-I and HTLV-II and reviews the testing methods for HTLV-I/II. Data from 39,908 blood donations of volunteer donors at The University of Texas M. D. Anderson Cancer Center (UTMDACC), Division of Laboratory Medicine, Section of Transfusion Medicine are presented. Initially reactive specimens for HTLV antibodies were 158 (0.4 percent). Of these 0.26 percent or 105 of 39,908 were repeatedly reactive. Eight hundred and sixty-seven cancer patients were also tested for HTLV antibodies. Eight or 0.9 percent were repeatedly reactive for HTLV antibodies by enzyme immunoassays (EIA), but only one could be confirmed as positive. HTLV-I/II has a very low incidence in the ambulatory population. The relationship of clinical sequelae and the rate of transmission of these viruses remain unclear. A readily applicable confirmatory test is not yet available. Even significant improvements in the sensitivity and specificity of testing will present ongoing problems for identification of true HTLV carriers. The clinical decision-making process related to the meaning of these results continues to be difficult.

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Introduction

Although the human T-Lymphotropic viruses type I (HTLV-I) and II (HTLV-II) had been isolated and known several years before the human immunodeficiency virus (HIV-I), less attention was
given to these viruses and their transmission by blood products. The delay in blood donor testing might have been due in part to the lack of convincing evidence that the transmission of HTLV-I would invariably cause disease, as had been observed with HIV. Furthermore, the incubation time for the manifestation of a presumed associated type of lymphoma/leukemia may require more than 20 years and occurs in less than 2 to 4 percent of the infected individuals. The development of a demyelinating disease (tropical spastic paraparesis) has been reported in only a few cases and is even more difficult to link epidemiologically when compared to the relationship of HIV to AIDS. An association of HTLV-II infection with any distinct disease is less proven than for HTLV-I infection, although initially a few cases of a special type of T-cell hairy cell leukemia were reported. Mandated screening of blood donors for antibodies to HTLV began in November, 1988, more than three years later than for HIV screening which had been introduced in the Spring of 1985.

Both HTLV-I and HTLV-II are human single-stranded ribonucleic acid (RNA) viruses, so-called C-type retroviruses, which had been described by Gallo’s group at the National Cancer Institute in 1980 and 1982, respectively. Both viruses were isolated from patients with T-cell leukemias/lymphomas and have been shown to infect human CD4 and CD8 positive lymphocytes. They do not contain any oncogene but can transform human lymphocytes in vitro. They differ from all known animal retroviruses in their nucleic acid composition, hybridization characteristics, and structural proteins. The viruses consist of essentially four genes: gag, pol, env, and px, which code for structural and regulatory proteins. They are enveloped viruses and have the capability to integrate their genome into the human deoxyribonucleic acid (DNA) via the transcription of RNA into DNA by the enzyme reverse transcriptase. Both viruses are closely related and share about 60 percent homology in their nucleotide sequence. They show great similarity in their structural and envelope proteins as well as the immune responses induced in humans. Both HTLV-I and II demonstrate a similarity of antigens and cross-reactivity of the generated antibodies. The best method to discriminate between them is testing by polymerase chain reaction (PCR). Attempts have also been made to use the relative strength of the antibody reactivity of p19 and p24 bands of the Western Blot test or synthetic peptide antigens for the respective antibody detection for the differentiation of each virus, but these have not proved to be reliable.

Infections of humans by HTLV are thought to be life long and appear to be world-wide, having been reported from North and South America, the Caribbean and Europe. These HTLV-I associated leukemias/lymphomas have been observed mainly in Japan and Africa. Neurological disorders such as tropical spastic paraparesis (TSP) or HTLV-I associated myelopathy (HAM) are predominately reported from Japan and the Caribbean. The route of infection is thought to be by heterosexual contact, transfusion, sharing of needles or syringes with infected individuals, and breast feeding. Transplacental transmission has also been suspected as a route of transmission. Cellular blood products are the main source of transfusion associated HTLV transmission, whereas fresh frozen plasma, cryoprecipitate, or coagulation factor concentrates appear not to be implicated in causing infection. Recently, it has been shown by increased application of polymerase chain reaction (PCR) that significant numbers of HTLV antibody positive individuals are actually infected by HTLV-II, and not, as
previously assumed, HTLV-I. This fact has been demonstrated particularly for intravenous drug users in the United States of America. Simultaneous infections by both HTLV-I and -II have been described in Japanese blood donors.

The prevalence of HTLV infection in the general USA population is not known. Current data are based on testing of volunteer whole blood donors. A seroprevalence of about 0.025 to 0.30 percent has been reported. Plasma donors from the USA had a significantly higher prevalence in one study.

Blood donor screening in the USA utilizes mainly EIA which have been approved and recommended for blood donor screening by the Food and Drug Administration (FDA). Other test methods, not FDA licensed, are agglutination assays widely used in Japan or more cumbersome indirect immunofluorescence tests. The EIA allows a fast and efficient HTLV antibody screen in approximately 2 to 3 hours. The EIA methods most often use the microtiter plate format with viral antigens either synthetic, recombinant, or from viral lysates bound to the plastic plate. Each specimen is tested first as a singlet and repeated in duplicate if initially reactive (IR) by the same test procedure. If the IR sample is reactive in the repeat test, it is designated as repeat reactive (RR) or positive. Each RR specimen is subjected to further confirmatory testing usually by Western blot and, if necessary, by additional radioimmunoprecipitation assay (RIPA) (figure 1). These confirmatory tests either identify or rule out the presence of antibodies to distinct viral antigens such as gag (p19 or p24) and envelope (gp21e[recombinant], gp 46 and gp 61/68) (table I). Other more sophisticated tests based on PCR technology or nucleic acid analysis or hybridization methods are not yet approved by the FDA and are performed only in specialized laboratories. Another method utilizes co-culturing cells suspected or harboring virus with non-infected cells in order to stimulate production and expression of HTLV. The expressed viral antigens can then be detected by immunofluorescence using an antibody such as anti-p19.

The commercially available test kits were originally designed to exclude HTLV-I antibody positive blood products from the blood supply. This was done to prevent patients from acquiring HTLV-I infections which were associated with adult T-cell leukeamias, certain lymphomas, or HTLV-associated myelopathy/tropical spastic paraparesis. Recent reports of HTLV-II transmission via blood transfusion and HTLV-II associated hematological and neuromuscular diseases have intensified the efforts to protect patients from HTLV-II contaminated blood products by improved donor screening.

Objective

This work describes methods currently used for donor HTLV-I/II screening in the USA and at UTMDACC. Data on seroprevalence for HTLV in blood donors in the USA and UTMDACC are reviewed. Seroprevalence of UTMDACC patients are examined and presented.

Methods

A total of 39,908 blood donations from volunteer donors and specimens from 867 cancer patients were screened for HTLV antibodies by a licensed EIA method at UTMDACC during the period from September 1, 1993 through August 31, 1994.

Results

Initially reactive specimens for the HTLV antibodies were 158 or 39,908 (0.4
FIGURE 1. Schema for blood screening for human T-lymphotropic virus types I and II at The University of Texas M. D. Anderson Cancer Center.

percent). One hundred and five specimens (0.26) were repeatedly reactive. Thirty-three (0.08 percent) samples were negative, and 66 specimens (0.17 percent) tested indeterminate. Only 6 (0.015 percent) were positive in the confirmatory tests utilizing Western Blot (WB) in 4 cases and WB plus RIPA in 2 cases (table II). The test specificity at UTMDACC was 99.8 percent; it compared well with estimated specificities of 99.3 percent to 99.9 percent reported by the Public Health Service Working Group (PHSWG). The predictive value is quite low as only 6/105 (6 percent) of the

<table>
<thead>
<tr>
<th>TABLE I</th>
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<tbody>
<tr>
<td>Criteria by the Center for Disease Control and the World Health Organization for Interpretation of Positive Human T–Lymphotropic Virus Western Blot Tests and Radioimmunoprecipitation Assays</td>
</tr>
<tr>
<td>HTLV I</td>
</tr>
<tr>
<td>HTLV II</td>
</tr>
</tbody>
</table>

*p21e is a recombinant antigen not originally included in the criteria.

This table is adapted from Morbidity Mortality Weekly Report 1988;37(34):736–47.
TABLE II
Seroprevalence of Human T–Lymphotropic Virus in Blood Donors at M. D. Anderson Cancer Center*

<table>
<thead>
<tr>
<th>Electroimmunoassay</th>
<th>No. Samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initially reactive (IR)</td>
<td>158 (0.4%)</td>
</tr>
<tr>
<td>Repeatedly reactive (RR)</td>
<td>105 (0.25%)</td>
</tr>
</tbody>
</table>

Confirmatory Test Results in RR Specimens**

<table>
<thead>
<tr>
<th></th>
<th>No. Samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>33 (0.08%)</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>66 (0.17%)</td>
</tr>
<tr>
<td>Positive</td>
<td>6 (0.015%)</td>
</tr>
</tbody>
</table>

*39,908 blood donations made between 9/1/93 and 8/31/94 were screened by enzyme–immunoassay.

**105 repeatedly reactive specimens were subjected to confirmatory testing (Western blot and/or by radioimmunoprecipitation assay [RIPA]). Of the six specimens positive by Western blot, two were also confirmed positive by RIPA.

Repeatedly reactive donor specimens could be confirmed. The PHSWG found that only 10/68 (15 percent) tested positive by confirmatory testing among more than 5,000 normal blood donors from nonendemic areas.34

From 9/1/93 to 8/31/94, a total of 867 cancer patients were tested for HTLV antibodies. Eight (0.9 percent) patients were repeatedly reactive for HTLV antibodies. Further confirmatory testing showed that only 1 (0.1 percent) patient could be confirmed for previous HTLV exposure or infection (table III). Five (0.58 percent) patients had indeterminate and two (0.23 percent) patients had negative Western Blot results. Three of these patients had hematological malignancies, and two had breast cancer. One patient with mycosis fungoides showed antibodies against the recombinant envelope antigen gp21e only. Because the results were reviewed retrospectively, risk factors could not be assessed. No further DNA based testing was performed on the specimens of the patients with indeterminate results.

TABLE III
Results of Confirmatory Testing of Patients Seropositive for Human T–Lymphotropic Virus at M. D. Anderson Cancer Center

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Sex</th>
<th>Age (Yrs)</th>
<th>Western Blot Result</th>
<th>Bands Present</th>
<th>RIPA Result</th>
<th>Final Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>F</td>
<td>63</td>
<td>Ind</td>
<td>p19, p24</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>F</td>
<td>40</td>
<td>Ind</td>
<td>p19, p28, p53</td>
<td>ND</td>
<td>Ind</td>
</tr>
<tr>
<td>B–CLL</td>
<td>M</td>
<td>50</td>
<td>Neg</td>
<td>None</td>
<td>ND</td>
<td>Neg</td>
</tr>
<tr>
<td>T–cell malignancy</td>
<td>M</td>
<td>79</td>
<td>Ind</td>
<td>gp21e</td>
<td>ND</td>
<td>Ind</td>
</tr>
<tr>
<td>(Mycosis fungoides)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>F</td>
<td>35</td>
<td>Ind</td>
<td>p19, p28</td>
<td>ND</td>
<td>Ind</td>
</tr>
<tr>
<td>Cervical carcinoma</td>
<td>F</td>
<td>36</td>
<td>Ind</td>
<td>p19, p26, p28</td>
<td>ND</td>
<td>Ind</td>
</tr>
<tr>
<td>Squamous carcinoma</td>
<td>F</td>
<td>41</td>
<td>Neg</td>
<td></td>
<td>ND</td>
<td>Ind</td>
</tr>
<tr>
<td>tongue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>M</td>
<td>60</td>
<td>Ind</td>
<td>p19</td>
<td>ND</td>
<td>Ind</td>
</tr>
</tbody>
</table>

AML = acute myelogenous leukemia.
ND = not done.
Neg = negative.
F = female.
B–CLL = B–cell chronic lymphocytic leukemia.
Ind = indeterminate.
Pos = positive.
M = male.
Discussion

The seroprevalence of 0.015 percent among UTMDACC blood donors is slightly lower than the 0.025 percent reported by Williams et al for about 40,000 US blood donors from eight different blood centers. Similar results were obtained from 267,650 blood donors of the Armed Forces which showed 72 donors (0.027 percent) as confirmed positives for HTLV antibodies. Somewhat higher seroprevalence of 0.08 percent was reported for 18,257 whole blood donors from the greater New York area. Higher seroprevalence rates compared to the aforementioned studies were also observed in paid plasma donors from 5 different parts of the US with 19 of 6,286 (0.3 percent) donors being positive. However, when 154 French and 25 US hemophiliacs who regularly received noninactivated plasma products were tested, no HTLV seropositive patient was found.

Recently, Hjelle et al reported that of 61,752 donations, 17 tested HTLV EIA positive which were confirmed by Western Blot. However, using 218 samples which had an absorbance reading of greater than 50 percent of the EIA cutoff, they found by testing 178 samples that 11 specimens were confirmed by both Western Blot and PCR. This result suggests that the current EIA based testing might miss up to 40 percent of HTLV positive donors.

Busch and co-workers found that out of 994 HTLV repeatedly-reactive specimens, only 410 samples could be confirmed. Subsequently, 407 of the 410 specimens could be shown by PCR to be infected with HTLV-I/II; three were false positive results on samples obtained in the first year of testing. Six of 426 (1.4 percent) indeterminate specimens proved to be positive by PCR; of these, 5 were positive for HTLV-II and 1 positive for HTLV-I.

Lee et al tested 480,000 blood donors in 5 different regions of the U.S. and investigated the relative seroprevalence of HTLV-I versus HTLV-II infections. Among 207 HTLV-I/II seropositive donors, 65 were further investigated by PCR; 34 specimens (52 percent) showed HTLV-II infection, 28 (43 percent) HTLV-I infections, and 3 samples were uninformative. Further interviews of donors demonstrated that the risk for HTLV-I infection was the donor geographic origin, i.e., coming from endemic areas, whereas the main risk factor for HTLV-II infection was the use of intravenous drugs.

Summary and Conclusion

The introduction of blood donor screening for HTLV antibodies in November 1988 has significantly decreased the transmission of HTLV I & II by cellular blood components and should, as a consequence, protect patients from developing HTLV-associated T-cell leukemia/lymphoma and neurodegenerative disease owing to transfusion. The prevention of HTLV infection by blood donor screening has already been shown in Japan. In the USA, the low HTLV seroprevalence in the blood donor population with 2.5/10,000 donors infected by HTLV, the limited survival (10 to 14 days) of the provirus in stored red blood cell concentrates, and the requirement of a sufficient number of infective and viable lymphocytes might account for the low incidence of post transfusion HTLV infections. The absence of lymphocytes in the coagulation factor concentrates or cryoprecipitate used for treatment of hemophiliacs has spared these patients from becoming infected. It is becoming clear that there is currently a significant rate of HTLV-II infection in the population of intravenous drug users. This is of concern because the current serologic
testing is more effective in detecting HTLV-I than HTLV-II seropositive blood. Further improvement of the methods for the detection of HTLV-II infected donors is needed. A simpler and faster assay for HTLV-II confirmation is also necessary in order to provide more effective donor counseling.

The preliminary data on the seroprevalence in 867 UTMDACC patients are a caution signal. Patients with indeterminate results require retesting. Although the seroprevalence in UTMDACC patients is 0.1 percent, it is approximately 8 times higher than in our ambulatory blood donor population. The limited numbers do not allow a conclusive evaluation. Risk factors such as transfusion history, place of origin, and intravenous drug use were not fully assessed. Testing of patients is indicated whenever risk factors are known and HTLV-associated diseases are suspected. Interpretation of results must take into account methodological limitations and varying aspects of the natural history of HTLV-associated diseases.

References


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