

# HTLV-III/LAV Infection in Hemodialysis Patients

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• **Twenty-five (4.8%) of 520 hemodialysis patients were seropositive for antibody to human T-cell lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) by enzyme immunoassay. Four had high reactivity on enzyme immunoassay and positive results of Western blot tests, and one of the four had a positive culture. The remaining 21 seropositive patients had low reactivity on enzyme immunoassay, negative results of Western blot tests, and negative cultures. All had received blood transfusions and 19 had antibodies to antigens associated with the H9 cell line used to propagate HTLV-III for serological tests. We found that HTLV-III/LAV was not transmitted in the dialysis centers. Frequent blood transfusion places dialysis patients at risk for HTLV-III/LAV infection, but may more commonly lead to false-positive results of enzyme immunoassay tests.**

(*JAMA* 1986;255:2324-2326)

SOON after the epidemic of acquired immunodeficiency syndrome (AIDS) began, researchers recognized that the risk factors for AIDS were similar to risk factors for hepatitis B virus (HBV) infection.<sup>1</sup> The epidemiology of HBV infection was used as a model to develop infection-control guidelines for AIDS even before the virus was identified.<sup>2,3</sup> The HBV model has recently been used in recommending precautions for hemodialysis centers caring for patients infected with the human T-cell lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/

LAV), the virus that causes AIDS.<sup>4</sup> Although transmission of HTLV-III/LAV has not been documented in a dialysis center and HTLV-III/LAV appears to be less infectious than HBV,<sup>5,7</sup> it has been recommended that dialysis centers use precautions similar to those used for HBV carriers when treating patients with HTLV-III/LAV antibody. Suggested infection-control strategies for HTLV-III/LAV range from conservative (separate room and separate machine, as for HBV) to less stringent (separate machine only).<sup>8</sup> These recommendations might imply that screening for antibody to HTLV-III/LAV in dialysis centers could help prevent transmission of infection.

The HTLV-III enzyme immunoassay (EIA) was used to screen all hemodialysis patients and staff members at eight Chicago-area chronic dialysis centers in the summer of 1985 after it was learned that a patient

had received a blood transfusion from a donor who developed AIDS. Initial EIA testing identified two (1.2%) of 161 staff members and 25 (4.8%) of 520 patients with positive test results. We report herein our investigation of this high rate of EIA seropositivity in these dialysis centers.

### Methods

The 25 patients and two staff members reported to have positive HTLV-III EIA test results were interviewed to see if they had risk factors for AIDS or symptoms of an HTLV-III/LAV infection. Dialysis center records were reviewed for each seropositive patient and for reportedly seronegative controls who were matched for dialysis center and selected from the center's patient list using a random-number table. During the record review we recorded the patient's age, sex, race, diagnoses, medications, length of time on dialysis, dialysis appointments missed because of hospitalization, number of renal transplants, and units of blood received in the dialysis center. The appointment times and machines used for dialysis were recorded for all seropositive patients and controls for the preceding three years.

The initial HTLV-III EIA screening had been performed at a commercial laboratory using the Abbott EIA kits. New blood specimens were sent by overnight mail at room temperature for blinded repeated Abbott EIA testing at the Centers for Disease Control (CDC). Enzyme-immunoassay reactivity ratios (defined as the specimen optical density/cutoff optical density) were calculated for all Abbott EIA tests.<sup>8</sup> Reactivity ratios greater than or equal to 6.0 times the cutoff value were considered high while reactivity ratios 0

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1.0 to 5.9 times the cutoff value were considered low.<sup>8</sup> The specimens that were positive by Abbott EIA were tested using the Western blot method.<sup>9</sup> The results of the Western blot test were considered positive if antibodies to either p24 or gp41 were detected. T-cell subset analysis was done for specimens from seropositive patients and seronegative controls.<sup>10</sup> Culture of peripheral blood lymphocytes for HTLV-III was performed only if the patient was seropositive by the Abbott EIA.<sup>11</sup>

The H9 lymphoid cell line is used to propagate the HTLV-III used in EIA tests and H9-cell-associated antigens (particularly HLA antigens) may contaminate purified HTLV-III.<sup>12</sup> We looked for antibodies to H9-cell-associated antigens using two methods. The CDC H9 assay tested serum samples at three dilutions (neat, 1:5, 1:20) for antibodies to H9-cell-surface antigens by indirect immunofluorescence and fluorescence-activated cell-sorter analysis.<sup>10</sup> With normal serum samples, up to 11% of cells registered fluorescence intensities greater than those of reagent controls. Positive serum samples in this study registered greater than 20% of cells positive at all dilutions tested. Coded specimens were also tested in the Electro-Nucleonics laboratory using the Electro-Nucleonics HTLV-III EIA. Only those specimens that tested positive on this HTLV-III EIA were then tested for reactivity to an Electro-Nucleonics H9-cell control EIA that was prepared using H9 cells that had not been infected with HTLV-III.<sup>13</sup> Positive HTLV-III EIA reactivity was attributed to H9-cell-associated antigens if the ratio of the HTLV-III EIA optical density ( $\sim 0.1$ ) to the H9-cell control EIA optical density was less than 9.

Informed consent was obtained from the seropositive patients and seronegative controls. Three seronegative controls refused or were unavailable for blood tests and were replaced by three other randomly selected controls.

Findings were tested for statistical significance using Student's *t* test when comparing means and Fisher's exact test when comparing proportions.

## Results

The two seropositive staff members had risk factors for AIDS and had had no contact with any of the seropositive patients. No further studies involving these two individuals were done.

The 25 patients who had a positive EIA test result in the commercial laboratory also had a positive Abbott EIA test result at the CDC. However, only four patients had a high EIA reactivity ratio ( $\geq 6$  times the cutoff

Table 1.—Clinical Characteristics of Dialysis Patients\*

Characteristic	CDC Abbott EIA Ratio $\geq 6.0$ (n=4)	CDC Abbott EIA Ratio 1.0-5.9 (n=21)	CDC Abbott EIA Negative (n=25)
Age, yr†	49.8	47.6	54.6
% Males	50	67	48
% White	50	33	38
Years on dialysis†	4	6.3	4.4
Hospitalizations†	5.5	2.1	2.0
History of renal transplant, %	0	48	20
Units of blood at center†	16.5	11.0	4.4

\*CDC indicates Centers for Disease Control; EIA, enzyme immunoassay.

†Expressed as the mean.

Table 2.—Laboratory Results\*

Test	CDC Abbott HTLV-III EIA Ratio $\geq 6.0$ , No. Positive/ No. Tested (%)	CDC Abbott HTLV-III EIA Ratio 1.0-5.9, No. Positive/ No. Tested (%)	CDC Abbott HTLV-III EIA Negative, No. Positive/ No. Tested (%)
Western blot	4/4 (100)	0/21 (0)	Not done
T4/T8 $< 1.0$	4/4 (100)	3/20 (15)	4/18 (22)
HTLV-III/LAV culture	1/4 (25)	0/21 (0)	Not done
CDC H9-cell antibody	1/4 (25)	16/20 (80)	4/10 (40)
ENI HTLV-III EIA	4/4 (100)	16/19 (84)	3/17 (18)
ENI H9-cell EIA†	4/4 (100)	16/16 (100)	3/3 (100)
ENI HTLV-III/H9-cell ratio	4/4 (100)	0/19 (0)	0/17 (0)

\*CDC indicates Centers for Disease Control; EIA, enzyme immunoassay; HTLV-III, human T-cell lymphotropic virus type III; LAV, lymphadenopathy-associated virus; ENI, Electro-Nucleonics Inc.

†The ENI H9 EIA was used only if the ENI HTLV-III EIA test result was positive. The ENI HTLV-III/H9 ratio is considered positive if the reactivity is not attributable to H9 reactivity (see text).

value), while 21 had a low EIA reactivity ratio.

All patients with positive EIA test results had received at least one blood transfusion. One patient with a high EIA reactivity ratio had another risk factor for AIDS and had had a positive EIA test result before beginning dialysis. None of the patients had any symptoms suggesting HTLV-III/LAV infection. Compared with the seronegative controls, patients with low EIA reactivity had received significantly more blood transfusions at the dialysis center ( $P < .05$ ) and were more likely to have had a renal transplant ( $P = .05$ ) (Table 1). The seropositive patients were otherwise similar to controls in regard to age, race, sex, medications, diagnoses, length of time on dialysis, and dialysis appointments missed because of hospitalization.

The patients with high EIA reactivity had received dialysis at separate dialysis centers. The index case had been infected for 15 months. The duration of infection for the other three could not be determined, although one began dialysis shortly before the screening was done. There

was no clustering of patients with positive test results, although four of the patients with low EIA reactivity had chronic HBV infections and had been receiving dialysis in the HBV isolation unit.

Results of Western blot tests were positive for the four patients with high EIA reactivity (Table 2). The T4/T8 ratio was abnormal for all four of the patients with high EIA reactivity, for four (19%) of 21 with low EIA reactivity, and for four (22%) of 18 seronegative controls. Cultures for HTLV-III/LAV were positive for one of the four patients with high EIA reactivity and negative for all 21 with low EIA reactivity.

The CDC assay identified antibodies to H9-cell-associated antigens for 16 of 20 patients with low EIA reactivity and for four of ten Abbott EIA-negative controls ( $P = .04$ ). Positive results from Electro-Nucleonics HTLV-III EIA tests, all of which were attributed to antibodies to H9-cell-associated antigens, were seen for 16 (84%) of 19 patients with low Abbott EIA reactivity, but for only three (18%) of 17 Abbott EIA-negative controls ( $P < .001$ ). All but two of the

21 with low EIA reactivity had antibody to H9-cell-associated antigens by either the Electro-Nucleonics or CDC H9-cell tests. The four patients with high reactivity on EIA tests and positive results from Western blot tests were the only ones with positive results from HTLV-III antibody tests using the Electro-Nucleonics HTLV-III/H9 ratio.

#### Comment

Results of HTLV-III EIA tests were positive for 25 (4.8%) of 520 dialysis patients, but 21 of the 25 had low reactivity. In a blood donor population, positive Abbott test results with low reactivity are likely to be false-positive test results since they correlate poorly with risk factors, Western blot tests, and HTLV-III/LAV cultures.<sup>8</sup> Before this investigation, it was not clear if the low EIA reactivity among the dialysis patients also represented false-positive test results, or if it reflected the impaired ability of infected individuals to produce antibodies. The high degree of reactivity to the H9 cell line suggests that the dialysis patients were able to produce antibodies and that the antibodies to H9-cell-associated antigens

were responsible for the low HTLV-III EIA reactivity. Western blot assay and T-cell subset analyses also suggest that only the patients with the high EIA reactivity were infected with HTLV-III/LAV. Although cultures were positive for only one of these patients, this is consistent with culture results in other seropositive groups<sup>14</sup> and may reflect the relative insensitivity of the HTLV-III/LAV culture technique.

The rate of false-positive HTLV-III EIA test results in these dialysis centers (4.00%) is much higher than that for blood donors (0.17%).<sup>15</sup> This difference is probably due to exposure to H9-cell-associated antigens during blood transfusions to the patients undergoing hemodialysis. Patients with low EIA reactivity received more transfusions in the dialysis centers than seronegative controls from the same centers. Similar sensitization to H9-cell-associated antigens might also be seen in other transfusion recipients.

We found no evidence for transmission of HTLV-III/LAV infection in the dialysis centers. Four patients had probably acquired infection via previously described routes.<sup>16</sup> The in-

dex patient was infected during a blood transfusion. A second patient had a risk factor other than blood transfusions. The route of transmission to the other two patients is not known, but both were probably also infected by one of their blood transfusions—a finding that might be expected with the high number of transfusions given to the 520 patients.

Although we found no evidence of HTLV-III/LAV transmission in the dialysis centers, we cannot conclude from this study that transmission could not occur. Until more information is available, it is prudent to continue using precautions to prevent transmission of HTLV-III/LAV when dialyzing infected patients.<sup>7</sup> However, on the basis of the results of this limited study, we find little justification for screening dialysis patients for antibody to HTLV-III/LAV. Physicians considering screening should be aware of the association of a history of multiple blood transfusions and false-positive results from HTLV-III EIA antibody tests.

The authors would like to thank J. Steven McDougal, MD, Bonnie M. Jones, and Janet Nicholson, PhD, for performing the CDC H9-cell assay and for valuable advice on the laboratory investigation and manuscript.

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