Increased mortality associated with HTLV-II infection in blood donors: a prospective cohort study

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Abstract

**Background:** HTLV-I is associated with adult T-cell leukemia, and both HTLV-I and -II are associated with HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Several published reports suggest that HTLV-I may lead to decreased survival, but HTLV-II has not previously been associated with mortality.

**Results:** We examined deaths among 138 HTLV-I, 358 HTLV-II, and 759 uninfected controls enrolled in a prospective cohort study of U.S. blood donors followed biannually since 1992. Proportional hazards models yielded hazard ratios (HRs) for the association between mortality and HTLV infection, controlling for sex, race/ethnicity, age, income, educational level, blood center, smoking, injection drug use history, alcohol intake, hepatitis C status and autologous donation. After a median follow-up of 8.6 years, there were 45 confirmed subject deaths. HTLV-I infection did not convey a statistically significant excess risk of mortality (unadjusted HR 1.9, 95% CI 0.8–4.4; adjusted HR 1.9, 95% CI 0.8–4.6). HTLV-II was associated with death in both the unadjusted model (HR 2.8, 95% CI 1.5–5.5) and in the adjusted model (HR 2.3, 95% CI 1.1–4.9). No single cause of death appeared responsible for the HTLV-II effect.

**Conclusions:** After adjusting for known and potential confounders, HTLV-II infection is associated with increased mortality among healthy blood donors. If replicated in other cohorts, this finding has implications for both HTLV pathogenesis and counseling of infected persons.
Background

Human T-lymphotropic viruses-I and -II (HTLV-I and -II) are human retroviruses with worldwide distributions [1]. HTLV-I is endemic to southern Japan, to certain Melanesian peoples, and to Western and Equatorial Africa, the Caribbean and Brazil. HTLV-II is endemic to indigenous peoples throughout the Americas, as well as among injection drug users (IDU) in the U.S. and Europe. HTLV-I is known to be associated with adult T-cell leukemia, uveitis, arthropathy, and Sjögren Syndrome [1]. Both HTLV-I and -II are associated with HTLV-associated myelopathy (HAM, also known as tropical spastic paraparesis or TSP) [2]. In addition, HTLV-II has been associated with an increased incidence of pneumonia, bronchitis and urinary tract infection [3-5].

Several investigators have reported an association between HTLV-I and decreased survival rates among certain unique populations; namely HTLV-I-infected leprosy patients in the Congo [6], and survivors of the atomic bomb dropped on Nagasaki [7]. In addition, there have been a small number of reports of survival rates negatively impacted by HTLV-I and viral co-infections; in particular, hepatitis C (HCV)/HTLV-I dually infected persons in Miyazaki, Japan [8]. The majority of studies of HTLV-II infected populations have focused on HTLV-II/Human Immuno-deficiency Virus (HIV) coinfection, particularly among IDU, and have reported that HTLV-II has a minimal effect on survival [9-12]. The impact of HTLV-I and -II on mortality in otherwise healthy persons such as blood donors has not been previously assessed.

Investigators with the HTLV Outcomes Study (HOST, formerly known as the Retrovirus Epidemiology Donor Study, or REDS HTLV Cohort) have performed a prospective evaluation of health outcomes in a large cohort of HTLV-I and HTLV-II infected subjects identified at the time of blood donation. For this analysis, our primary aim was to determine whether HTLV-I and -II are independently associated with an increase in mortality among infected blood donors, as compared with matched uninfected blood donors.

Results

HOST enrolled 154 HTLV-I, 387 HTLV-II, and 799 uninfected donors (total enrollment: 1340). For this analysis, subjects were excluded if their HTLV status could not be confirmed or if they failed to complete an initial interview and physical exam. The latter exclusion criterion insured that persons with pre-existing, clinically apparent conditions would not introduce bias. Mortality was ultimately assessed in 1255 subjects (94% of the cohort), including 138 HTLV-I infected subjects (10% excluded), 358 HTLV-II infected subjects (7% excluded), and 759 uninfected controls (5% excluded). The characteristics of the subjects at the baseline visits are given in Table 1. Age and gender were similar among groups, but Black race was more common in the HTLV-I group. The HTLV-II group had lower educational achievement and income, as well as higher prevalence of cigarette smoking, alcohol intake, HCV seropositivity and lifetime IDU, (although only 1 percent admitted current IDU). Median follow-up time was 8.6 years, with a range of 1.1 to 11 years.

There were a total of 45 deaths in the cohort, including 8 (5.8%) HTLV-I, 19 (5.3%) HTLV-II and 18 (2.4%) HTLV seronegative subjects. Crude survival was lower in both the HTLV-I and HTLV-II groups than in the seronegative subjects (Figure 1). HTLV-II infection conveyed a significant independent risk of death (unadjusted HR 2.8, 95%CI 1.5–5.5; adjusted HR 2.3, 95%CI 1.1–4.9), but we did not find a statistically significant association of HTLV-I with mortality (unadjusted HR 1.9, 95%CI 0.8–4.4; adjusted HR 1.9, 95%CI 0.8–4.6). No single cause of death appeared responsible for the HTLV-II excess mortality, but numbers in all categories were small (Table 2).

Four (9%, 3 HTLV-negative, 1 HTLV-I) of 45 deaths in the cohort were due to accidents or violence. Unadjusted and adjusted hazard ratios for HTLV were calculated both by censoring accidental and violent deaths, and by including them among all causes of mortality. No significant differences in hazard ratios for any included variable resulted, and these deaths are included among the total in the final adjusted model (Table 3). Ten of the 45 deaths (22%) were of subjects whose donations were autologous (those who are donating for their own personal use, usually prior to a planned surgery). Half of these were HTLV seronegative subjects, two were HTLV-I infected, and three were HTLV-II infected. Because of the possibility that autologous donors might be sicker than allogeneic donors, we calculated the unadjusted hazard ratio (HR 0.9, 95%CI 0.4–1.9) for autologous donors and found that donation type was not significantly associated with death. Inclusion of a donation type variable did not have a significant effect on the results of our adjusted model.

Because of the mortality risk inherent in IDU and the well-established association between IDU and HTLV-II even in blood donors [13-15], we assessed IDU status as a potential confounding variable. One percent of both the uninfected subjects and those with HTLV-I reported a lifetime history of IDU, compared to 20% of those with HTLV-II infection. Although IDU was a significant predictor of mortality in the unadjusted model (OR = 3.5, 95% CI 1.5–8.0), the adjusted model indicated that a lifetime history of IDU was not significantly associated with mortality (HR 2.0, 95% CI 0.7 – 6.3). Because of the high prevalence of HCV co-infection in our HTLV-II group presumably due to past IDU (see Table 1), we considered HCV infec-
tion as a potential explanation for the increased mortality rate we found among those with HTLV-II. Among the 45 deaths, six (13%) were HCV positive; all of the subjects with missing HCV data (n = 53; 4.2% of the entire cohort) were alive at the time of our analysis. HCV infection was not associated with mortality in our adjusted model (HR = 1.1, 95% CI, 0.4 – 3.5).

We examined race as a potential confounder because of the recognized association between Black race and increased mortality and minor imbalances in race by HTLV status. Black race was significantly associated with death (adjusted HR 2.2, 95%CI 1.2–4.1). Alcohol intake was the only other factor significantly associated with mortality (p = 0.0069). Subjects who did not provide information on their quantity of alcohol consumption

| Table 1: Characteristics of HTLV mortality cohort study population at baseline showing number (percent) in each category, except as indicated. |
|-------------------------------------------------|
| Characteristics                                | HTLV-I (n = 138) | HTLV-II (n = 358) | HTLV negative (n = 759) |
|------------------------------------------------|
| Age in years (mean (range))                    | 46 (19–78)       | 42 (18–78)        | 44 (18–79)              |
| Sex                                            |                   |                   |                         |
| Female                                         | 98 (71.0)        | 266 (74.3)        | 516 (68.0)              |
| Male                                           | 40 (29.0)        | 92 (25.7)         | 243 (32.0)              |
| Race/Ethnicity                                 |                   |                   |                         |
| Black                                          | 56 (40.6)        | 115 (32.1)        | 229 (30.2)              |
| White                                          | 53 (38.4)        | 134 (37.4)        | 299 (39.4)              |
| Other                                          | 29 (21.0)        | 109 (30.4)        | 231 (30.4)              |
| Education                                      |                   |                   |                         |
| ≤ high school                                  | 44 (31.9)        | 139 (38.8)        | 137 (18.1)              |
| Some college                                   | 61 (44.2)        | 167 (46.6)        | 342 (45.1)              |
| ≥ college                                      | 33 (23.9)        | 52 (14.5)         | 280 (36.9)              |
| Income                                         |                   |                   |                         |
| <$40 k                                         | 70 (50.7)        | 222 (62.0)        | 322 (42.4)              |
| >$40 k                                         | 68 (49.3)        | 136 (38.0)        | 437 (57.6)              |
| Donation type                                  |                   |                   |                         |
| Autologous                                     | 24 (17.4)        | 34 (9.5)          | 103 (13.6)              |
| Allogeneic                                     | 114 (82.6)       | 324 (90.5)        | 656 (86.4)              |
| IDU                                            |                   |                   |                         |
| Lifetime (past)                                | 2 (1.5)          | 73 (20.4)         | 8 (1.1)                 |
| Never                                          | 132 (95.7)       | 260 (72.6)        | 730 (96.2)              |
| Current                                        | 0 (0.0)          | 3 (0.8)           | 1 (0.1)                 |
| Missing                                        | 4 (2.9)          | 22 (6.1)          | 20 (2.6)                |
| Smoking history (pack/year)                    |                   |                   |                         |
| Non-smoker                                     | 67 (48.6)        | 122 (34.1)        | 403 (53.1)              |
| 1–15                                           | 29 (21.0)        | 120 (33.5)        | 190 (25.0)              |
| >15                                            | 38 (27.5)        | 91 (25.4)         | 146 (19.2)              |
| Missing                                        | 4 (2.9)          | 25 (7.0)          | 20 (2.6)                |
| Alcohol intake (avg # drinks/week)             |                   |                   |                         |
| Mean (S.D.)                                    | 4.8 (12.2)       | 10.7 (30.4)       | 4.7 (12.9)              |
| Non-drinker                                    | 17 (12.3)        | 19 (5.3)          | 67 (8.8)                |
| 1–14                                          | 109 (79.0)       | 268 (74.9)        | 620 (81.7)              |
| >14                                           | 9 (6.5)          | 50 (14.0)         | 52 (6.9)                |
| Missing                                        | 3 (2.2)          | 21 (5.9)          | 20 (2.6)                |
| HCV Serology                                   |                   |                   |                         |
| Positive                                       | 6 (4.3)          | 68 (19.0)         | 8 (1.1)                 |
| Negative                                       | 120 (87.0)       | 253 (70.7)        | 747 (98.4)              |
| Not available                                  | 12 (8.7)         | 37 (10.3)         | 4 (0.5)                 |
| Blood center region                            |                   |                   |                         |
| Baltimore/Washington                           | 28 (20.3)        | 49 (13.7)         | 118 (15.5)              |
| Detroit                                        | 25 (18.1)        | 32 (8.9)          | 98 (12.9)               |
| Southern California                            | 43 (31.2)        | 193 (53.9)        | 325 (42.8)              |
| San Francisco                                  | 28 (20.3)        | 62 (17.3)         | 145 (19.1)              |
| Oklahoma                                       | 14 (10.1)        | 22 (6.1)          | 73 (9.6)                |

Note: Percentages may not sum to 100 due to rounding.
had significantly higher mortality than those with moderate alcohol consumption (HR = 3.5, 95% CI 1.4–8.9).

Our calculations of standardized mortality rates and ratios demonstrated that the age-adjusted mortality rate of our HTLV seronegative control donors was half that of the general population (mortality rate = 354 per 100,000 person-years, SMR = 0.6, 95% CI 0.3–0.9). Although both the HTLV-I and -II infected former blood donors had almost twice the mortality of the HTLV seronegative donors enrolled and followed as an internal control group (rates = 727 and 545 per 100,000 person years, respectively), their standardized mortality ratios (SMR = 0.9, 95% CI 0.4–1.7 and SMR = 0.9, 95% CI 0.6–1.5, respectively) were not significantly different from those of the general U.S. population in the year 2000.

**Discussion**

We found that HTLV-II increased the risk of death in infected blood donors relative to uninfected blood donors, while HTLV-I infection had an adverse, but not statistically significant, effect on mortality. No particular cause of death was increased among the HTLV-II group, although numbers were small in all cause of death categories. The association of HTLV-II with increased mortality persisted after adjustment for multiple potential confounding factors, including race, socioeconomic status, alcohol intake, cigarette smoking, HCV infection and IDU. Although an etiologic basis for the mortality excess cannot be identified from current information, the pathogenic effects of chronic HTLV infection include tax protein toxicity and HTLV-induced autoimmune responses.

The HTLV-II association with increased mortality was robust after consideration of bias or confounding by covariates that were not balanced between the groups in this observational prospective cohort study. Black race and alcohol intake were significantly associated with mortality, and there was a strong trend toward an effect by lifetime IDU, all plausible associations, which diminished but did not nullify the HTLV-II effect in our multivariate model. Nor did three other potential confounders, namely educational attainment, HCV infection and autologous blood donation have an effect on mortality. The groups were initially stratified by donation status because autologous blood donors are a less healthy group than all-eneic donors, with increased prevalence of several infectious disease markers. Although HTLV-II subjects had lower educational attainment compared to seronegatives, this imbalance did not outweigh HTLV-II effects in our multivariate model. Nor did three other potential confounders, namely educational attainment, HCV infection and autologous blood donation have an effect on mortality. The groups were initially stratified by donation status because autologous blood donors are a less healthy group than all-eneic donors, with increased prevalence of several infectious disease markers. Although HTLV-II subjects had lower educational attainment compared to seronegatives, this imbalance did not outweigh HTLV-II effects in this or previous analyses of this cohort [4,5]. Finally, the prevalence of HCV infection was increased in the HTLV-II group. Although some hospital-based studies suggest that HCV frequently causes end-stage cirrhosis and hepatoma, prospective studies of otherwise healthy HCV seropositives have not demonstrated increased overall mortality [16,17].

There have been very few publications examining the effect of HTLV-II on mortality [10-12], and all but one of these [12] analyzed only HTLV-II/ HIV co-infection. None found a significant effect of HTLV-II on either the course of HIV disease or death. Goedert et al. [12] examined HTLV-II among IDUs with and without HIV co-infection, comparing mortality rates in these groups to that found in uninfected IDUs and in the general population. They found that IDU itself, in the absence of retroviral infection, was associated with a mortality rate over five times that of the general population. While this rate was further increased in the presence of HIV, these authors found no

| Cause of death          | HTLV-I (n = 138) | HTLV-II (n = 358) | HTLV negative (n = 759) | All Subjects (n = 1255) |
|------------------------|-----------------|------------------|------------------------|------------------------|
| Accident/Trauma        | 1               | 0                | 3                      | 4                      |
| Cancer                 | 1               | 5                | 7                      | 13                     |
| Cardiac                | 2               | 4                | 2                      | 8                      |
| Cerebrovascular        | 2               | 2                | 1                      | 5                      |
| Diabetes               | 0               | 1                | 0                      | 1                      |
| Drug-related           | 1               | 1                | 0                      | 2                      |
| Hepatic                | 0               | 1                | 0                      | 1                      |
| Infection              | 0               | 1                | 3                      | 4                      |
| Pulmonary              | 0               | 1                | 0                      | 1                      |
| Other                  | 0               | 0                | 1                      | 1                      |
| Unknown                | 1               | 3                | 1                      | 5                      |
| Total deaths           | 8               | 19               | 18                     | 45                     |
| Deaths per group       | 5.8%            | 5.3%             | 2.4%                   | 3.6%                   |
| Proportion of all deaths| 17.8%          | 42.2%            | 40.0%                  | 100%                   |
Kaplan-Meier curves showing unadjusted probability of survival at a given age for HTLV-I infected subjects (top) and HTLV-II infected subjects (bottom), both relative to HTLV seronegative controls.
What could explain the discrepancies between our estimates of the effects of IDU and HTLV-II infection, and those of studies such as Goedert et al? We believe that the answer lies in a difference in study populations. Goedert et al. studied current and chronic IDU, a population subject to high levels of competing mortality. Despite pre-donation screening intended to exclude IDU, blood donors who are deferred due to the discovery of a blood-borne viral infection often reveal a past history of injection drug use in subsequent interviews [15,18]. We also believe that only one percent of our subjects were still actively injecting drugs because blood donors with IDU experience tend to have remote and limited injecting histories [15]. The dramatic mortality risk conveyed by IDU in the Goedert et al. cohort is likely due to long-term and ongoing IDU in the population studied. Although not, to our knowledge, proven, it seems reasonable to surmise that, once IDU behavior ceases, the risk that stems from it recedes toward the individual's baseline risk, perhaps explaining why we saw a small and non-significant effect of IDU on mortality. Finally, competing mortality due to the large effects of IDU and/or HIV may have obscured the relatively small effect of HTLV-II in the Goedert et al. cohort. Conversely, since we had less active IDU and no HIV co-infection in our cohort, we were able to detect the weaker association between HTLV-II and mortality.

HTLV-I was the first virus shown to cause cancer in humans [19]. In addition, there is a well-established relationship between the virus and HAM/TSP, a chronic degenerative neurologic disease, as well as a smaller body of literature asserting the association between HTLV-I and a number of autoimmune conditions [20,21]. Several studies have also found an association between HTLV-I and mortality [6-8]. Although the increased mortality in our HTLV-I group was not statistically significant, these other studies provide inferential support for our significant association between HTLV-II and mortality. We recognize however that the existing literature on HTLV-I and mortality is scant, and methodological problems and unusual study populations make generalization particularly difficult.

Proven links between HTLV-I, T-cell malignancy, and the neurological disorder HAM/TSP involve putative pathogenetic mechanisms that may also be relevant to our mortality findings. One hypothetical mechanism of pathogenicity is via direct effects of the HTLV-I tax viral protein leading to either lymphocytic proliferation or neurotoxicity [22,23]. Our own and other reports of high proviral loads in most HTLV-I HAM/TSP patients support this hypothesis [24-26]. A recent study has also linked higher HTLV-I proviral load with mortality [27]. Another hypothesis proposes that HTLV-I, and presumably HTLV-II as well, causes an autoimmune phenomenon reflecting an HTLV virus-induced host response against host antigens in the central nervous system and other tissues [22].

Table 3: Factors associated with death in the HOST cohort: Hazard ratios (HRs) adjusted only for age, and adjusted for multiple covariates, are given for each variable.

| Variable          | Unadjusted HR (95% CI) | Adjusted HR (95% CI) |
|-------------------|------------------------|----------------------|
| HTLV status       | HTLV-negative 1.0 ------ | 1.0 ------ |
|                   | HTLV-I 1.9 (0.8–4.4)   | 1.9 (0.8–4.6) |
|                   | HTLV-II 2.8 (1.5–5.5)  | 2.3 (1.1–4.9) |
| Sex               | Female 1.0 ------      | 1.0 ------ |
|                   | Male 1.4 (0.7–2.6)    | 1.6 (0.8–3.0) |
| Race/Ethnicity    | Non-Black 1.0 ------  | 1.0 ------ |
|                   | Black 2.2 (1.2–4.0)   | 2.2 (1.2–4.1) |
| Donation type     | Allogeneic 1.0 ------ | 1.0 ------ |
|                   | Autologous 0.9 (0.4–1.9)| 0.6 (0.3–1.4) |
| HCV status        | HCV negative 1.0 ------ | 1.0 ------ |
|                   | HCV positive 2.3 (1.0–5.6)| 1.1 (0.4–3.5) |
| IDU history       | Never 1.0 ------      | 1.0 ------ |
|                   | Ever 3.5 (1.5–8.0)    | 2.0 (0.7–6.3) |
| Alcohol use       | 1–14 drinks/week 1.0 ------ | 1.0 ------ |
|                   | None 0.6 (0.2–1.9)    | 0.6 (0.2–2.0) |
|                   | >14 drinks/week 0.5 (0.2–1.8)| 0.4 (0.1–1.2) |
|                   | Missing 4.3 (1.8–10.5)| 3.5 (1.4–8.9) |

Adjusted for HTLV status, age, gender, race, donation type, HCV status, IDU and drinking.
This autoimmune response underlies the incidence of HAM/TSP, as well as arthritis, uveitis and polymyositis, in a minority of persons with HTLV-I or -II. However, as the dead included no HAM/TSP patients and only one ATL patient, and no other predominant cause of death emerged, the relevance of HTLV tax protein toxicity or virus-induced immune response to mortality remains speculative.

A major strength of our study is the absence of subjects with HIV and low numbers of those with active IDU, thus eliminating competing conditions with large impacts on mortality, which could have obscured the more subtle effect of HTLV. We excluded subjects without baseline questionnaire or exam and there was stratified enrollment of our HTLV and seronegative subjects on age, sex, race, blood center and donation type to improve comparability of the groups. Our analysis controlled for other potential confounders such as socioeconomic status and IDU. Finally, we used a prospective study design, our follow-up time was long and our ascertainment of deaths was active and complete in contrast to studies which relied upon death registries.

On the other hand, weaknesses of the study include the unusually healthy nature of the uninfected blood donor control population, which may have caused an overestimation of the effect of HTLV on mortality. In designing the study, we considered and rejected using population controls because our blood donor sampling frame was the same for both HTLV and seronegative donors. We still believe that comparison to the general population, as shown in our SMR calculations presented above, is not a priori more valid than our use of the internal blood donor control group. Finally, this was an observational and not a randomized study, so unrecognized confounding either by socioeconomic status, for which we attempted to control, or by other variables for which we could not control, may have biased our estimate of the HTLV-II effect on mortality.

**Conclusion**

We have demonstrated a significant, independent association between HTLV-II and increased mortality among healthy blood donors. This finding requires replication in other prospective studies of HTLV-II, preferably without HIV or IDU, and in a population other than blood donors. A prospective cohort study among HTLV-II endemic Amerindians would be the ideal setting. Nevertheless, the majority of HTLV infections in the United States are diagnosed in the setting of blood donation. If confirmed, the results of this study will enable us to better inform HTLV infected blood donors of the long-term implications of their infections. These findings may also stimulate further investigation into the causes of death among HTLV-infected persons, and into the pathogenic mechanisms which may underlie increased mortality.

**Methods**

**Subjects and study design**

This was a prospective cohort study. Blood centers in five United States regions (Baltimore/Washington, Detroit, Oklahoma City, San Francisco, and Los Angeles) participated in HOST (see Appendix). We asked several non-HOST blood centers to refer HTLV seropositive patients to the study to increase the sample of infected donors. Study personnel contacted all donors with confirmed HTLV serology since the initiation of HTLV-I testing in 1988 through July 1992 and offered enrollment in a general health study of HTLV-I and HTLV-II. We selected HTLV-seronegative controls from among all those persons who donated blood at the five HOST blood centers between 1988 and July 1992. The study design called for an HTLV negative-to-positive matching ratio of 2:1 within each stratum based on age, sex, race/ethnicity, blood center, and type of blood donation (allogeneic, autologous, or directed). All subjects were HIV-seronegative at baseline. The human subjects committees of the American Red Cross, the Oklahoma Blood Institute, and the University of California, San Francisco approved the study protocol.

Subjects were enrolled in the study based upon HTLV-I or -II seropositivity as measured by licensed enzyme immunoassay screening and supplemental testing at the participating blood centers. Additional confirmatory testing and HTLV-I versus HTLV-II typing consisted of a combination of serologic and polymerase chain reaction (PCR) assays as previously reported by Busch et al [28]. Subjects were followed biannually through the fifth study visit in February 2000 through July 2001 with assessments and examinations. If a subject did not respond to routine contact attempts, we searched credit bureau records, U.S. Postal Service change of address files, and other internet resources. Eventually, if tracing attempts by study staff were unsuccessful, a professional tracing specialist was assigned to the subject. Forty-one of 45 (91%) subject deaths and cause of death were confirmed by death certificate, the remainder were confirmed by the Social Security Death Index and/or discussion with family members. Causes of death were grouped into eleven categories (accidental/trauma, cancer, cardiac, cerebrovascular, diabetes, drug related, hepatic, infectious, pulmonary (non-infectious), other and unknown). These categories did not overlap.

**Statistical analysis**

Mortality rates were calculated separately for HTLV-I and HTLV-II infected donors and HTLV negative donors. For each group, the mortality rate was calculated as the number of deaths per 100,000 person-years of observa-
tion. To determine how the mortality within the cohort compared with the U.S. population as a whole, we computed a standardized mortality ratio (SMR) for each HTLV group using age-specific mortality rates for the U.S. population [29]. For each HTLV group and seronegatives, the age-specific mortality rates were applied to person years of follow-up accrued within each age category to calculate the expected age specific deaths, which were then summed across age categories to obtain the expected number of deaths in each group. Within each HTLV and seronegative group, we then calculated the SMR as the actual number of deaths divided by the expected number of deaths. Confidence intervals (CI) for SMRs were computed assuming a Poisson distribution.

The probability of survival in the HTLV-I, HTLV-II, and HTLV negative groups was calculated using the Kaplan-Meier method. For the analysis of predictors of mortality, we used Cox proportional hazard models to obtain hazard ratios (HR) for each HTLV group compared to the HTLV negative group and for other covariates, in both unadjusted and adjusted analyses. Unadjusted hazard ratios were derived from models including only the variable and age. The adjusted hazard ratios were determined using a backward-selection procedure. Death was the dependent variable, HTLV status was the primary independent variable, and the following 11 variables were the potential covariates: age, gender, race, education, income, blood center, donation type, hepatitis C virus (HCV) infection status, IDU, alcohol intake and smoking history. Initially, we entered HTLV status and all 11 covariates into the model. Variables were then sequentially removed, starting with the least statistically significant. We forced four covariates (gender, donation type, IDU, and HCV) into the final model because of their reported association with mortality, although they were not statistically significant in our adjusted model.

**Competing interests**
None declared.

**Authors’ contributions**
JO participated in the design, statistical analysis and manuscript writing. BW and DW performed the statistical analysis and helped write the manuscript. CN, GG, JS and BN performed subject follow-up and contributed to the manuscript. DS managed subject follow-up and data collection. EM was principal investigator and participated in the design, statistical analysis and manuscript writing. All authors read and approved the final manuscript.

**Appendix**
The HTLV Outcomes Study (HOST) is presently the responsibility of the following persons:

**Study headquarters**
University of California San Francisco; San Francisco, CA:

E.L. Murphy (Principal Investigator), J. Engstrom

**Blood centers**
American Red Cross Blood Services Greater Chesapeake and Potomac Region; Baltimore, MD:

C.C. Nass, C. Conry-Cantilena, J. Gibble

American Red Cross Blood Services Southeastern Michigan Region; Detroit, MI:

B. Newman

American Red Cross Blood Services Southern California Region; Los Angeles, CA:

G. Garratty, S. Hutching, A. Ziman

Blood Centers of the Pacific; San Francisco, CA:

M.P. Busch

Oklahoma Blood Institute; Oklahoma City, OK:

J.W. Smith, E. Moore

**Medical coordinating center**
Westat, Inc.; Rockville, MD:

G.B. Schreiber, D. Ameti, B. Wang

**Central laboratory**
Blood Centers of the Pacific; San Francisco, CA:

M.P. Busch, L.H. Tobler

**Diagnostic review panel**
E.L. Murphy, R. Sacher, J. Fridely

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**References**
1. Manns A, Hisada M, La Grenade L: Human T-lymphotropic virus type I infection. *Lancet* 1999, 353:1951-1958.
2. Murphy EL, Friddy J, Smith JW, Engstrom J, Sacher RA, Miller K, Gibble J, Stevens J, Thomson R, Hansma D, Kaplan J, Khabbaz R, Nemo G: HTLV-associated myelopathy in a cohort of HTLV-I and HTLV-II-infected blood donors. The REDS investigators. *Neurology* 1997, 48:315-320.

3. Modahl LE, Young KC, Varney KF, Khayam-Bashi H, Murphy EL: Are HTLV-I- and HTLV-II-seropositive injection drug users at increased risk of bacterial pneumonia, abscess, and lymphanphadopathy? *J Acquir Immune Defic Syndr Hum Retrovirol* 1997, 16:169-175.

4. Murphy EL, Glynn SA, Friddy J, Sacher RA, Smith JW, Wright DJ, Newman B, Gibble JW, Ameti DI, Nass CC, Schreiber GB, Nemo GJ: Increased prevalence of infectious diseases and other adverse outcomes in human T lymphotropic virus types I- and II-infected blood donors. Retrovirus Epidemiology Donor Study (REDS) Study Group. *J Infect Dis* 1997, 176:1468-1475.

5. Murphy EL, Glynn SA, Friddy J, Smith JW, Sacher RA, Nass CC, Glynn SA, Fridey J, Sacher RA, Smith JW, Sacher RA, Nass CC, Murphy EL, Fridey J, Smith JW, En gstrom J, Sacher RA, Miller K, Gibbet et al.: Immune Defic Syndr 1997, 1

6. Lechat MF, Shrager DI, Declercq E, Bertrand F, Blattner WA, Blum M, Alakoski M, Matsuo T, Akahoshi M, Nakashima E, Tomonaga M, Saito H: Human T-lymphotropic virus type I- infection, antibody titers and cause-specific mortality among atomic bomb survivors. *Jpn J Cancer Res* 1998, 89:797-805.

7. Boschi-Pinto C, Swerter S, Okamo Y, Trichopoulos D, Orav EJ, Tsubouchi H, Mueller N: A follow-up study of morbidity and mortality associated with hepatitis C virus infection and its interaction with human T lymphotropic virus type I in Miyazaki, Japan. *J Infect Dis* 2000, 181:33-41.

8. Giacomo M, Franco EG, Claudio C, Carlo C, Anna DA, Anna D, Franco F: Human T-cell leukemia virus type II infection among high risk groups and its influence on HIV-1 disease progression. *Eur J Epidemiol* 1995, 11:527-533.

9. Hershov RC, Galali N, Fukuda K, Graber J, Vlahov D, Rezza G, Klein RS, Des Jarlais DC, Vitk C, Khabbaz R, Freels S, Zuckerman R, Pez zotti P, Kaplan JE: An international collaborative study of the effects of coinfection with human T-lymphotropic virus type II on human immunodeficiency virus type I disease progression in injection drug users. *J Infect Dis* 1996, 174:309-317.

10. Vicenzon A, Visco L, Colucci R, Bassetti D, Viladomiu L, Amendola M, Ciacci D: HTLV-II/III-HIV coinfestation and risk for progression to AIDS among intravenous drug users. *J Acquir Immune Defic Syndr Hum Retrovirol* 1993, 6:1228-1237.

11. Goedert JJ, Fung MW, Felton S, Battjes R, Engels EA: Cause-specific mortality associated with HIV and HTLV-II/l infections among injection drug users in the USA. *Aids* 2001, 15:1295-1302.

12. Visco C, VanRaden M, Gibble J, Melpolder J, Shakil AO, Vladianou L, Cheung L, DiBiaseglie A, Hoofnagle J, Shih JW, et al.: Routes of infection, viremia, and liver disease in blood donors found to have hepatitis C virus infection. *N Engl J Med* 1996, 334:1691-1696.

13. Yoshida M, Miyoshi I, Hinuma Y: Isolation and characterization of retrovirus from cell lines of human adult T-cell leukemia and its implication in the disease. *Proc Natl Acad Sci U S A* 1982, 79:2031-2035.

14. Gessain A, Barin F, Vernet JC, Gout O, Maurs L, Calender A, des The G: Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. *Lancet* 1985, 1:207-410.

15. Gebreab M, Murphy EL: Counseling and medical evaluation of HTLV-I and HTLV-II-infected patients. *AIDS Clin Rev* 1993, 19:41.

16. Nagai M, Jacobson S: Immunopathogenesis of human T cell lymphotropic virus type I-associated myelopathy. *Curr Opin Neurol* 2001, 14:381-386.

17. Greten TF, Slansky JE, Kubota R, Soldan S, Jaffe EM, Leist TP, Par doll DM, Jacobson S, Schneck JP: Direct visualization of antigenspecific T cells: HTLV-1 Tax I-19- specific CD8+ T cells are activated in peripheral blood and accumulate in cerebrospinal fluid from HTLV-II/TSP patients. *Proc Natl Acad Sci U S A* 1998, 95:7568-7573.

18. Mansa A, Milly WJ, Wilks RJ, Morgan OS, Hanchard B, Wharfe G, Cranston B, Maloney E, Welles SL, Blattner WA, Waters D: Quantitative proviral DNA and antibody levels in the natural history of HTLV-I infection. *J Infect Dis* 1999, 180:1487-1493.

19. Nagai M, Usuku K, Matsumoto W, Kodama D, Takenouchi N, Mori toyo T, Hashiguchi S, Ichinoe M, Bangham CR, Izumo S, Osame M: Analysis of HTLV-I proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-I carriers: high proviral load strongly predisposes to HAM/TSP. *J Neurol 1998, 4:586-593.

20. Orland JR, Engstrom J, Friddy J, Sacher RA, Smith JW, Nass C, Garratty G, Newman B, Smith D, Wang B, Murphy EL, MTVL Outcomes Study, HTLV Outcomes Study: Prevalence and clinical features of HTLV neurologic disease in the HTLV Outcomes Study. *Neurology* 2003, 61:1588-1594.

21. Aiyoshi K, Berry N, Cham F, Jaffar S, Schim van der Loef M, Jobe O, N’Gom PT, Larsen O, Andersson S, Aaby P, Whittle H: Quantification of Human T-lymphotropic virus type I (HTLV) proviral load in a rural West African population: no enhancement of human immunodeficiency virus type 2 pathogenesis, but HTLV-I provirus load relates to mortality. *J Infect Dis* 2003, 188:1648-1651.

22. Busch MP, Laycock M, Kleinman SH, Wages J, Jr., Calabro M, Kap lan JE, Khabbaz R, Hollingsworth CG: Accuracy of supplementary serologic testing for human T lymphotropic virus types I and II in US blood donors. *Retrovirus Epidemiology Donor Study*. *Blood* 1994, 83:1143-1148.

23. Center for Disease Control and Prevention: . *National Vital Statistics Reports* 2003, 51:7.