DELAYED-TYPE HYPERSENSITIVITY SKIN TEST REACTIVITY AND SURVIVAL IN HIV-INFECTED PATIENTS IN UGANDA: SHOULD ANERGY BE A CRITERION TO START ANTIRETROVIRAL THERAPY IN LOW-INCOME COUNTRIES?


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Abstract. Access to antiretroviral (ARV) drugs is improving in sub-Saharan Africa but still constrained by several clinical and logistical obstacles. There is a need to develop affordable markers to guide initiation of treatment. We present a prospective cohort study of 779 patients participating in a TB prophylaxis trial. We performed separate analyses for anergic and nonanergic subjects. Prognostic factors for anergic and nonanergic subjects differed between groups. With anergy and constitutional symptoms were at the highest risk of death. Incident tuberculosis and CD4 < 200 cells/μL at enrollment were the strongest risk factors for death. HIV disease is associated with substantial morbidity and mortality in this population. The burden caused by tuberculosis is particularly high. Anergy is a strong and independent predictor of death. World Health Organization criteria to start ARV may be strengthened with the addition of DTH testing, an inexpensive and readily available tool in sub-Saharan Africa.

INTRODUCTION

In the early 1980s, infection with human immunodeficiency virus (HIV) was first recognized as a slim disease in western Uganda.1 Over the subsequent two decades, HIV spread rapidly southward through the African continent to infect more than 25 million people by 2003.2 Although some countries have been successful in curbing transmission through behavioral interventions and government programs,3 most countries still struggle under the medical, economic, and social burden of this devastating epidemic. Following the historic events of the 1998 World AIDS Conference, new international initiatives promise to improve access to life-preserving antiretroviral therapies in the years to come.

The World Health Organization (WHO) has recently published guidelines for initiating antiretroviral therapy in resource-limited countries.4 These recommendations emphasize the role of the WHO staging system and CD4+ T cell counts. The WHO staging system has face validity and has been validated in both resource-rich5,6 and poor countries7–11 as a useful prognostic tool. However, in developing countries it performs marginally as a diagnostic criterion1 and may require updating.8 CD4+ lymphocyte cell counts are extremely useful in defining the degree of immune suppression and hence the need for treatment, but this test is expensive and not widely available. Although still controversial, an absolute lymphocyte count (< 1,200 cells/μL) may be used as a proxy to the CD4+ count but is less useful in asymptomatic persons.4,12 It would be important to identify other clinical markers of prognosis to expand the limited options currently available to assess the need for treatment in African countries.13–15

To identify factors associated with reduced survival in Africa, we performed a prospective cohort study of HIV-infected patients in Kampala, Uganda, who were enrolled into a placebo arm of a clinical trial evaluating the efficacy of preventive therapy for tuberculosis. We measured factors associated with mortality separately for nonanergic and anergic subjects, a low-cost clinical marker that, despite its temporal instability,16–18 is strongly associated with death in HIV-infected patients.19–23

MATERIALS AND METHODS

Study design and study population. Subjects for this study participated in a large randomized clinical trial to determine the efficacy of TB preventive therapy in HIV-infected patients in Kampala, Uganda.24 Eligible individuals who were randomized to the placebo arms of this trial constitute the study population for this analysis. Two strata were defined by their reactivity to two subcutaneous antigens: purified protein derivative (PPD) and candidin. Study subjects with any induration size (≥ 1 mm) to PPD or candidin were considered to be “nonanergic”; study subjects with 0 mm induration to both PPD and candidin antigens were considered to be “anergic.” The two groups of subjects were ascertained over different time frames as enrollment of anergic individuals started 7 months after enrollment of the nonanergic subjects.

The study population for this analysis includes the 779 subjects (463 nonanergic and 316 anergic) randomized to the two placebo arms of the study. Participating subjects for this study were recruited between February 1993 and April 1995, through five HIV clinics in Kampala, Uganda. Inclusion and exclusion criteria were the same as for the parent study.24 Briefly, HIV-infected individuals were screened for active tuberculosis by history, physical examination, sputum smear and culture, and chest radiography. Exclusion criteria were determined to address the study objectives of the parent study. The exclusion criteria were the presence of active tuberculosis, previous treatment of tuberculosis, use of antiretroviral drugs, a white-cell count under 3,000 per cubic millimeter, a hemoglobin level under 80 g/L, serum aspartate ami-
We first performed a descriptive analysis. Subjects were censored at the last clinic visit when they were known to be alive or at the end of the study observation period. Cause of death was not determined. When a subject missed a scheduled visit, a team of trained home visitors would visit the home to encourage compliance and to ascertain their vital status. If the patient had died, the home health visitors used a structured questionnaire with surviving family members to determine the date of death.

Participating subjects were actively screened for tuberculosis twice a year, or during sick visits, with chest radiograph, sputum microscopy, and culture. Tuberculosis cases were identified through a standardized screening process. Pulmonary tuberculosis was defined as sputum smear-positive disease confirmed by culture (definite) or clinical and radiologic improvement after TB treatment (probable), in a patient with clinical signs and symptoms consistent with active tuberculosis. All cases of TB received daily self-administered antituberculous therapy including isoniazid, rifampicin, pyrazinamide and ethambutol for 2 months followed by 4 months of daily isoniazid and rifampicin. Antiretroviral therapy was not available in Uganda during the study period. All patients received standard management for HIV-1 infection available in Uganda during the study period. The distribution of baseline characteristics was determined by trained medical officers. Symptomatic present at enrollment were by self-report. At the time of screening, venous blood was collected for HIV-1 testing, complete and differential blood counts (Coulter T540 system), HIV infection was documented by enzyme-linked immunoabsorbent assay (ELISA; Recombigen HIV-1 Env and gag ELISA, Cambridge BioScience, Worcester, MA). CD4 measurements were determined by flow cytometry. At screening, all subjects underwent Mantoux skin testing using 5 tuberculin units of PPD (Tubersol, Connaught Laboratories, Swiftwater, Pasadena, CA) and 0.1 mL Candida antigen (Candida albicans allergic extract, 1:50 concentration, Berkeley Biologics, Berkeley, CA). Between 48 and 72 hours after application, experienced observers recorded the results of each skin test in millimeters. Anergy was defined as absence of reactivity (0 mm of induration) to both PPD and Candida antigens. Interobserver and intraobserver reliability coefficients for these measurements were high. Subjects had positive or negative skin test results, or no reaction, and were categorized as anergic or nonanergic.

Measurements. Demographic and clinical information was obtained through standardized interviews and physical examination performed by trained Ugandan medical officers. Symptomatic present at enrollment were by self-report. At the time of screening, venous blood was collected for HIV-1 testing, complete and differential blood counts (Coulter T540 system), HIV infection was documented by enzyme-linked immunoabsorbent assay (ELISA; Recombigen HIV-1 Env and gag ELISA, Cambridge BioScience, Worcester, MA). CD4 measurements were determined by flow cytometry. At screening, all subjects underwent Mantoux skin testing using 5 tuberculin units of PPD (Tubersol, Connaught Laboratories, Swiftwater, Pasadena, CA) and 0.1 mL Candida antigen (Candida albicans allergic extract, 1:50 concentration, Berkeley Biologics, Berkeley, CA). Between 48 and 72 hours after application, experienced observers recorded the results of each skin test in millimeters. Anergy was defined as absence of reactivity (0 mm of induration) to both PPD and Candida antigens. Interobserver and intraobserver reliability coefficients for these measurements were high. Subjects had positive or negative skin test results, or no reaction, and were categorized as anergic or nonanergic.

Outcome and study definitions. The primary outcome for this analysis was all-cause mortality. Subjects were censored at the last clinic visit when they were known to be alive or at the end of the study observation period. Cause of death was not determined. When a subject missed a scheduled visit, a team of trained home visitors would visit the home to encourage compliance and to ascertain their vital status. If the patient had died, the home health visitors used a structured questionnaire with surviving family members to determine the date of death.

Overall survival distributions were estimated for several key variables associated with survival (Karnofsky status, history of HIV-illnesses and CD4 lymphocyte count) using the Kaplan-Meier method and compared using the log-rank test. Variables associated with survival in Kaplan-Meier univariate analyses (P < 0.15) were evaluated in a series of Cox proportional hazards regression models which were compared using likelihood ratio tests. The relative hazard for death for each variable was estimated with 95% confidence intervals. Tuberculosis status was included in the model as a time-dependent covariate to reduce lead-time bias for TB. Once the final models were built we assessed confounding and tested for precision.

RESULTS

Of the 779 subjects included in the analysis, 316 (41%) were anergic at baseline and 463 (59%) were nonanergic, as shown in Figure 1. The distribution of baseline characteristics was...
different between the two groups and reflected more advanced disease in the anergic patients (Table 1). The median age was 30 years and two thirds of the subjects had a visible BCG scar. Anergic individuals had a lower BMI, lower Karnofsky status and were less likely to drink alcohol (32% versus 42%; *P* = 0.004). Anergic subjects were more likely than nonanergics to have a past history of HIV-associated illnesses (73% versus 59%, respectively), leukopenia, and lymphopenia. The majority of patients (56%) were symptomatic (any symptom from Table 1) at the time of enrollment, but the proportion with symptoms was similar in both groups. The most frequent symptoms were cough (42%), weight loss (38%) and malaise (25%). At baseline, the 771 available chest x-rays were normal. Anergic subjects had lower CD4+ lymphocyte counts when compared with nonanergics (278 versus 432 cells/μL, respectively). Skin-test reactivity, as measured by tuberculin skin test (TST), was weakly correlated with CD4+ lymphocyte counts (Spearman *r* = 0.299; *P* < 0.0001) but the proportion of subjects with higher CD4+ cell counts was higher in nonanergic patients (Figure 2). In an analysis stratified by anergy, the CD4+ lymphocyte cell count was lower among subjects who complained of constitutional symptoms (fever, night sweats, weight or appetite loss and malaise) compared with those without such symptoms in both the anergic subjects (261 versus 404 cells/μL, respectively; *P* = 0.0009; *t* test) and in nonanergic subjects (450 versus 541 cells/μL, *P* < 0.0001).

During a median follow-up time of 777 days (range 2–1,594) for the entire cohort, 193 (25%) subjects died. The median time of follow-up for anergic subjects was 575 days (range 2–1,329) and 884 days (range 4–1,594) for nonanergic individuals (*P* < 0.0001; Wilcoxon). In a Kaplan-Meier analysis, the overall unadjusted survival in anergic subjects was lower than in nonanergics (*P* < 0.0001, log-rank; Figure 3). Anergic individuals were more than twice as likely to die compared with nonanergics (RR 2.16; 95% CI 2.07–2.25; univariate Cox proportional hazards regression analysis). The proportion of patients dead at 1 and 2 years after enrollment was 29% and 34% for anergic and 10% and 15% for nonanergic patients, respectively (*P* < 0.001, both comparisons). In a stratified analysis, the CD4 cell level at enrollment was strongly associated to death, regardless of anergy status (Figure 4). In Kaplan-Meier univariate analyses, factors associated with survival differed between anergy-defined groups.

### Table 1

Baseline characteristics of study subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Nonanergic (N = 463)</th>
<th>Anergic (N = 316)</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)—median [SD]</td>
<td>30 [7]</td>
<td>30 [7]</td>
<td>0.59*</td>
</tr>
<tr>
<td>Male sex—n(%)</td>
<td>141 (30)</td>
<td>106 (34)</td>
<td>0.36†</td>
</tr>
<tr>
<td>BMI—median [SD]</td>
<td>22.3 [3.6]</td>
<td>21.7 [3.3]</td>
<td>0.001*</td>
</tr>
<tr>
<td>Karnofsky—n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (70 and 80)</td>
<td>80 (17)</td>
<td>70 (22)</td>
<td>0.09†</td>
</tr>
<tr>
<td>High (90 and 100)</td>
<td>380 (83)</td>
<td>245 (78)</td>
<td></td>
</tr>
<tr>
<td>BCG scar present—n (%)</td>
<td>303 (66)</td>
<td>198 (63)</td>
<td>0.40†</td>
</tr>
<tr>
<td>PPD (mm)—median [SD]</td>
<td>14 [6]</td>
<td>0 [0]</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Symptoms present at baseline—n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any constitutional**</td>
<td>262 (57)</td>
<td>180 (57)</td>
<td>0.92†</td>
</tr>
<tr>
<td>Any non-constitutional††</td>
<td>253 (55)</td>
<td>176 (56)</td>
<td>0.77†</td>
</tr>
<tr>
<td>Past HIV-associated illnesses—n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herpes zoster</td>
<td>73 (16)</td>
<td>76 (24)</td>
<td>0.004†</td>
</tr>
<tr>
<td>Oral thrush</td>
<td>60 (13)</td>
<td>48 (15)</td>
<td>0.46†</td>
</tr>
<tr>
<td>Papules</td>
<td>128 (28)</td>
<td>139 (45)</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Genital sores</td>
<td>164 (36)</td>
<td>141 (45)</td>
<td>0.009†</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>32 (7)</td>
<td>34 (11)</td>
<td>0.06†</td>
</tr>
<tr>
<td>Any illness</td>
<td>275 (59)</td>
<td>232 (73)</td>
<td>&lt;0.0001†</td>
</tr>
<tr>
<td>Laboratory values—median [SD]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White blood cells (×10³/μL)</td>
<td>5.2 [1.8]</td>
<td>4.9 [1.6]</td>
<td>0.02*</td>
</tr>
<tr>
<td>Total lymphocytes (cells/μL)</td>
<td>2,142 [867]</td>
<td>1,773 [861]</td>
<td>0.0005*</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.6 [1.9]</td>
<td>12.5 [1.8]</td>
<td>0.29*</td>
</tr>
<tr>
<td>CD4 lymphocyte (cells/μL)</td>
<td>432 [360]</td>
<td>278 [281]</td>
<td>&lt;0.0001*</td>
</tr>
</tbody>
</table>

* Wilcoxon *t* test.
† Chi-square.
‡ n = 254.
§ n = 151.
¶ n = 281.
‖ n = 169.
** Constitutional symptoms included fever, sweats, appetite, or weight loss, and malaise.
†† Nonconstitutional symptoms included cough, dyspnea, purulent sputum, diarrhea, and bone pain.
Low Karnofsky, a body mass index (BMI) < 22, a history of HIV-illnesses, anemia and CD4 ≤ 200 cells/µL at baseline were all strongly associated with reduced survival and common to both groups, whereas the development of active tuberculosis was not (Table 2). In anergic individuals older age, the presence of symptoms and leucopenia at enrollment were all associated with reduced survival, whereas in non-anergic subjects males were more likely to die then women.

In a Cox multivariable regression analysis the pattern of group-specific predictors of survival persisted (Table 3). Common predictors of death in both anergic and non-anergic individuals were a CD4+ lymphocyte count < 200 cells/µL at the time of enrollment, Karnofsky performance status, and the development of incident tuberculosis. We performed the same analysis using total lymphocyte count (TLC)—above or below 1,200 cells/µL—as a substitute to CD4+ cells, obtaining significant but much weaker estimates (data not shown). The onset of active TB was associated with a sixfold increase risk of death in both anergic ($P = 0.0006$) and nonanergic ($P = 0.004$) subjects, when compared with TB-free survival. When considered together, patients with TB died a median of 32 days (range 11–302) after diagnosis. Anergic patients were more likely to die if they had suffered from HIV-illnesses in the past. In nonanergic subjects, a low BMI (< 22) was associated with reduced survival.

**DISCUSSION**

In this cohort of subjects in Uganda, the health burden imposed by HIV disease was high as reflected by the number of symptomatic individuals without clinically apparent opportunistic infections or active tuberculosis. Moreover, the mortality rate was 25% despite a relatively high initial median CD4+ lymphocyte count of 373 cells/µL. The risk for death was highest among subjects with cutaneous anergy to PPD and candida antigens, especially if they had constitutional symptoms.

In the face of global initiatives to enhance access to antiretroviral medications, practical guidelines are needed for when to start antiretroviral medications in resource limited settings. The underlying principle of therapy is to start treatment when it will have the greatest effect on survival and quality of life, while minimizing the risk of complications from therapy. Recent recommendations from the WHO propose different scenarios for starting therapy, most based on WHO AIDS staging criteria and CD4+ T cell.4 The guidelines recommend initiating therapy when WHO stage III or IV disease develops and when CD4+ T cell count drops below 200 cells/µL (or stage II disease and TLC ≤ 1,200 cells/µL). The results from this study show the risk for death may be high, even before WHO stage III or IV HIV disease develops. We infer from these observations that other clinical criteria may be relevant for starting therapy, especially before overt immunosuppression develops as suggested by others.11

Early in the HIV epidemic, cutaneous anergy was used in the Walter Reed Staging system for AIDS.26 The clinical niche of anergy testing in industrialized countries has all but disappeared following the revised 1997 CDC recommendations,27 and European guidelines where it is not routinely recommended.28–30 The findings from this study suggest this practice should be reconsidered in low-income countries. Anergy skin testing provides clinical information not only about immune function but also about survival.19–23 Our results corroborate the association between anergy and reduced survival in an African setting. Anergy was not only found to predict mortality, it was also associated with past HIV-associated illnesses and, lower absolute lymphocyte counts and CD4+ T cell counts. In short, it was a good surrogate for advanced stages of HIV infection. Furthermore, when constitutional symptoms are present, the CD4+ T cell counts tend to be lower, especially in anergic subjects. Cutaneous anergy and the presence of constitutional symptoms are inexpensive markers of advanced disease that are correlated with CD4+ T cell count and risk for death. We propose that these clinical markers may be useful in deciding when to initiate antiretroviral therapy, especially in individuals who have not yet developed WHO stage IV disease.

It has been hypothesized that the deterioration in delayed-type hypersensitivity (DTH) responses may signal a shift from type 1 to a type 2 CD4 lymphocyte response to HIV.31 In the case of anergy to tuberculin skin testing, it may also be due to an innate inability to mount an antigen-specific DTH response to PPD that is genetically determined.32 One limitation is the instability of anergy over time,16–18 which may
misclassify at any point in time an individual’s true DTH function. Despite this variability, our study and others show a single measurement of anergy carries substantial information about risk of death. Moreover, we found a close association between anergy and immunosuppression level (different risks across groups), particularly among symptomatic subjects. The results of our study suggest anergic patients who develop constitutional symptoms, possibly as a result of high TNF-α levels, are at an increased risk of early death and thus would be candidates to start immediate ARV treatment. This study highlights the special burden tuberculosis poses in settings with high rates of HIV and M. tuberculosis coinfection. The overall risk of incident tuberculosis in this cohort was high (8%). Moreover, when tuberculosis developed, it was associated with an increased risk of death despite early diagnosis and treatment as reported by others, regardless of anergy group. Infection with HIV is widely recognized as the strongest known risk factor for developing active TB both postprimary as well as reactivation disease and thus significantly altering the natural history of tuberculosis.

### Table 2
Kaplan-Meier univariate analysis of variables associated with mortality

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonanergic (N = 463)</th>
<th>Anergic (N = 316)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number at risk</td>
<td>Deaths n (%)</td>
</tr>
<tr>
<td>BMI*&lt;22</td>
<td>213</td>
<td>61 (29)</td>
</tr>
<tr>
<td>≥22</td>
<td>242</td>
<td>27 (11)</td>
</tr>
<tr>
<td>Karnofsky Low</td>
<td>80</td>
<td>28 (35)</td>
</tr>
<tr>
<td>Karnofsky High</td>
<td>380</td>
<td>62 (16)</td>
</tr>
<tr>
<td>Const. symptoms† Yes</td>
<td>262</td>
<td>59 (23)</td>
</tr>
<tr>
<td>Const. symptoms† No</td>
<td>201</td>
<td>32 (16)</td>
</tr>
<tr>
<td>HIV illnesses‡ Any</td>
<td>275</td>
<td>69 (25)</td>
</tr>
<tr>
<td>HIV illnesses‡ None</td>
<td>188</td>
<td>22 (12)</td>
</tr>
<tr>
<td>Active TB Yes</td>
<td>41</td>
<td>3 (7)</td>
</tr>
<tr>
<td>Active TB No</td>
<td>422</td>
<td>88 (21)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)&lt;12.5</td>
<td>211</td>
<td>56 (27)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)≥12.5</td>
<td>250</td>
<td>34 (14)</td>
</tr>
<tr>
<td>Lymphocytes (cells/μL)&lt;1,200</td>
<td>21</td>
<td>12 (57)</td>
</tr>
<tr>
<td>Lymphocytes (cells/μL)≥1,200</td>
<td>233</td>
<td>40 (17)</td>
</tr>
<tr>
<td>CD4 (cells/μL)&lt;200</td>
<td>65</td>
<td>26 (40)</td>
</tr>
<tr>
<td>CD4 (cells/μL)≥200</td>
<td>216</td>
<td>27 (13)</td>
</tr>
</tbody>
</table>

<sup>§ Median values for BMI were 22.3 and 21.7 for nonanergic and anergic group, respectively.</sup><br><sup>† Constitutional symptoms included fever, weight loss, appetite loss, and malaise.</sup><br><sup>‡ Previous HIV-illnesses included thrush, cutaneous papules, genital sores, and history of diarrhea.</sup><br>All P values (log-rank) ≥ 0.0001 except for: a 0.07, b 0.0002, c 0.10, d 0.35.

### Table 3
Factors associated with mortality in Cox multivariate analysis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Nonanergic</th>
<th>Anergic</th>
<th>Nonanergic</th>
<th>Anergic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relative risk</td>
<td>95% CI (P value)&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Relative risk</td>
<td>95% CI (P value)&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>History of HIV illness† Yes</td>
<td>NS</td>
<td>–</td>
<td>2.5</td>
<td>1.02–5.9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>(0.05)</td>
<td>2</td>
<td>1–2</td>
</tr>
<tr>
<td>Body mass index</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;22</td>
<td>2.6</td>
<td>1.4–4.8</td>
<td>NS</td>
<td>–</td>
</tr>
<tr>
<td>≥22</td>
<td>1</td>
<td>(0.002)</td>
<td>1</td>
<td>(0.05)</td>
</tr>
<tr>
<td>Karnofsky status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1.9</td>
<td>1.1–3.5</td>
<td>2.2</td>
<td>1.3–4.0</td>
</tr>
<tr>
<td>High</td>
<td>1</td>
<td>(0.03)</td>
<td>1</td>
<td>(0.006)</td>
</tr>
<tr>
<td>CD4 at enrollment (cells/μL)&lt;200</td>
<td>4.5</td>
<td>2.6–7.8</td>
<td>7.1</td>
<td>3.6–13.9</td>
</tr>
<tr>
<td>≥200</td>
<td>1</td>
<td>(&lt; 0.0001)</td>
<td>1</td>
<td>(&lt; 0.0001)</td>
</tr>
<tr>
<td>Incident tuberculosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>6.0</td>
<td>1.8–20.5</td>
<td>6.6</td>
<td>2.3–19.3</td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>(0.004)</td>
<td>1</td>
<td>(0.0006)</td>
</tr>
</tbody>
</table>

<sup>NS, not significant.</sup><br><sup>* P = Chi-square.</sup><br><sup>† HIV-associated illnesses include oral thrush, chronic diarrhea, herpes zoster genital sores, and pruritic papules.</sup>
there is a growing body of evidence suggesting that tuberculosis has a deleterious impact on HIV disease progression. Our results are insufficient to comment on the nature of the HIV/TB interaction. Our findings, and that of others, indicate preventing TB disease before it develops should remain a focus in HIV health programs.

This study has several limitations. The ascertainment of study groups was done at different time frames as a result of the design in the parent study. Although this influenced our decision to perform a separate survival analysis for anergy group, this strategy provided insight about HIV disease in sub-Saharan Africa. We were unable to assess the full effect of tuberculosis on AIDS-associated deaths because we could not determine cause-specific mortality. A potential limitation of the study is the large number of censored events, but because of the good long-term follow-up we observed in this cohort, the majority of censoring occurred at study close out. The panel used to assess anergy may be suboptimal. The poor survival among anergic individuals, however, indicates that our measure of anergy did identify individual with more advanced disease. Another limitation was that HIV viral load information was not available in study patients so we could not examine the effect of viral burden on skin anergy or survival. We believe this limitation does not influence our results because CD4+ lymphocytes are a better predictor of death than HIV viral load and the latter is not directly associated with worsening cell-mediated immunity or anergy. Finally, the results of our study may only be generalized to our study population, somewhat circumscribed by the parent study exclusion criteria. However, we believe our results are valid as our primary objective was to understand clinical factors (anergy and TB in particular) influencing long-term survival in relatively healthy HIV-infected subjects at baseline. Other studies in sub-Saharan Africa have shown the burden and diversity of active opportunistic infections is significant and associated with a high, short-term mortality. Inclusion of patients with active TB and other opportunistic infections (OI) or, more advanced AIDS, as measured by very low Karnofsky performance scores, would have obscured our results as it would have overestimated the influence of OI-specific factors (case-fatality rates). In this context, we believe our screening protocol was successful in identifying a relatively healthy population at baseline as the observed difference in survival between anergics and non-anergics begins after almost a year of follow-up, as shown in Figure 3.

In this study from sub-Saharan Africa, HIV infection causes substantial morbidity and mortality. Tuberculosis poses a special burden because of its high and increasing prevalence and, its detrimental impact on survival. Cutaneous anergy was a useful surrogate for CD4+ T cell count and a strong predictor of mortality. We are fully aware that anergy testing is not the ideal field test as it requires a repeat visit 48-72 hours after placement and handling injectable materials (syringe, cold chain, etc.) and a cadre of trained personnel. However, we believe testing for cutaneous anergy is simpler, cheaper, less technologically demanding and more readily available than current recommendations based on CD4 and/or total lymphocyte counts. In addition, it offers the added benefit of screening for M. tuberculosis infection and thus makes it an attractive test at point of entry for HIV-TB treatment programs, as currently recommended by the WHO and others. A patient with a positive tuberculin skin test not eligible for ART therapy should have treatment of latent tuberculosis infection. The effect ART therapy has on immune reconstitution is only partially understood. At this time, we are not proposing to perform follow-up anergy testing to evaluate a clinically relevant outcome to ART therapy. Future longitudinal studies would be needed to explore such a strategy. The findings from this study suggest efforts to combat HIV disease in sub-Saharan Africa should be directed on two fronts: initiating ART treatment on patients at highest risk of death and developing targeted preventive strategies against opportunistic infections, particularly to tuberculosis.

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