Lung CD4 Lymphocytes Predict Survival in Asymptomatic HIV Infection*

Mark D. Wewers, MD; Stanley Lemeshow, PhD; Amy Lehman, MS; Thomas L. Clanton, PhD; and Philip T. Diaz, MD

Background: Plasma viral load and blood CD4 counts are accepted indicators of severity of illness in patients with HIV-1. Lung CD4 counts have not been evaluated in asymptomatic HIV-1 patients as indicators of disease severity.

Objective: To determine if lung lymphocyte counts in asymptomatic subjects with HIV compare with plasma viral loads and blood CD4 counts in predicting survival.

Design: Retrospective, cross-sectional analysis.


Participants: HIV-seropositive subjects (n = 95) without AIDS-related pulmonary complications.

Measurements: Plasma viral load, blood hemoglobin and blood lymphocyte subtypes, lung lymphocyte subtypes from BAL, body mass index, and mortality.

Results: Eight of the 95 subjects (8.4%) had died at the 4-year follow-up. Lung CD4 counts were significantly related to mortality by univariable analysis (2.5 × 10^3/mL vs 0.9 × 10^3/mL, median values for survivors vs nonsurvivors, respectively, p = 0.010). Modeling using exact methods further showed lung CD4 counts to be a significant predictor of survival after individually adjusting for potential confounders, including plasma viral load and blood CD4 count.

Conclusions: Lung CD4 counts in patients with HIV-1 infection may provide an independent predictor of survival.

(CHEST 2005; 128:2262–2267)

Key words: BAL; HIV; load; lymphocyte; survival; viral

Abbreviations: BMI = body mass index; CI = confidence interval; DLCO = diffusing capacity of the lung for carbon monoxide; OR = odds ratio; TLC = total lung capacity

HIV infection produces a profound impairment of host immune defenses that is characterized by opportunistic infections that commonly involve the lung. HIV infection is associated with a loss of circulating CD4 lymphocytes. CD4 counts < 200 cells/μL connote severe immune compromise.¹ Measurements of plasma viral load are accurate measures of how rapidly HIV is replicating and predict how rapidly the CD4 lymphocyte counts are likely to progress over time.²³ In this context, lung macrophages are known to harbor HIV, the burden of which increases dramatically during opportunistic infections.⁴ It has been reported previously that lung lymphocyte numbers may predict survival in patients with HIV undergoing BAL for presumed lung infection.⁵ Despite this recognition, to our knowledge, there are no published data that lung lymphocyte counts in asymptomatic HIV patients may also portend outcome. The present study retrospectively evaluates 95 asymptomatic HIV patients who underwent BAL to characterize lymphocyte numbers at the same time that they were also evaluated for lung function, plasma viral load, and peripheral blood counts. Both univariable and multivariable analyses suggest that lung lymphocyte numbers may be an important independent predictor of survival.

Materials and Methods

Study Population

A study of the lung immune response to HIV infection included 95 subjects who had undergone second-generation
plasma viral load assays (ie, lower limit of detection 500 copies per milliliter) and BAL between December 1996 and August 1998. Subjects were recruited from participants of the Ohio State University AIDS Clinical Trial Group and the Columbus, Ohio community. Two subjects were excluded from the analysis after determining that their deaths were due to unnatural causes (suicide and homicide, respectively). All received informed consent by a procedure approved by the human subjects institutional review board at The Ohio State University. Subjects received payment for participation in the BAL study and were asymptomatic at the time of the study. All were weighed and completed a detailed questionnaire about drug treatment, smoking status, and medical history. Subjects also underwent blood sampling for WBC counts, blood CD4 counts, hemoglobin, and albumin. Results from BAL were also recorded, including cells counts, cell differentials, and lymphocyte subtyping for CD2, CD3, CD4, CD8, CD19, S6F1, and NK markers by flow cytometry. Subjects were also analyzed for lung function by spirometry, lung volumes, and single-breath diffusing capacity of the lung for carbon monoxide (DLCO) according to American Thoracic Society standards.

**BAL**

Bronchoscopy with BAL was performed as previously described. Briefly, after informed consent, subjects underwent standard bronchoscopy with BAL consisting of sequentially instilling and aspirating saline solution in five 20-mL aliquots into the right middle or lingual bronchus from the wedged position. Recovered lavage fluid was passed through one layer of sterile surgical gauze to remove mucus and particulate. Fluid aliquots were immediately taken for cell counting and lymphocyte phenotyping.

**Cell Processing**

Total cell count and differential cell count were performed on the recovered fluid by direct cell hemocytometer counting and Giemsa-type stains (Diff-Quik; Baxter Diagnostics; McGaw Park, IL) of centrifuge (Cytospin) preparations as we have previously described. This is the standard technique that has been adopted by the American Thoracic Society and accredited by clinical pathology for the hospital laboratory analysis of BAL cells. In addition to cell counting and cell differential determinations, a sample of fluid containing approximately 1 million cells was sent to the Ohio State University Hospital Cellular Immunology Laboratory for T-lymphocyte counts and subtyping (see below). Subtyping was not possible in two individuals who died because their lymphocyte counts were too low for flow cytometry.

**FACS Quantification of BAL Cells**

Fresh BAL cells were analyzed by a fluorescence-activated cell sorter (Epics KL-MCL; Beckman Coulter; Fullerton, CA) in The Ohio State University Hospital Cellular Immunology Laboratory. Cells were analyzed using dual-staining procedures (fluorescein isothiocyanate or phycoerythrin-conjugated monoclonal antibodies) for the relative frequencies of the following phenotypes: CD3/CD4 (T lymphocytes of the helper phenotype), CD3/CD8 (T lymphocytes of the suppressor/cytotoxic phenotype), and S6F1+/CD8 (activated CD8 lymphocytes of the cytotoxic phenotype).9,10

**Definition of Smoking Status**

Subjects self-reported their smoking status category as current smoker, ex-smoker, or never-smoker. Ex-smokers were also asked to identify the length of time since quitting. Subjects who reported never smoking in their lifetime or had quit for at least 2 years were categorized as nonsmokers. None of the subjects fell into the category of having quit smoking within 2 years of evaluation. Those subjects who reported current smoking were classified as smokers.

**Viral Load Measurements**

Plasma samples were frozen and mailed to Quest Diagnostics (Pittsburgh, PA) for assay by HIV-1 RNA, quantitative bound DNA assay, second generation, in accordance with the recommendations of the manufacturer. In brief, blood is collected in ethylenediamine tetra-acetic acid and plasma separated. Viral RNA is extracted and captured by solid-phase bound synthetic oligonucleotides and hybridized to target probes. Bound DNA is detected by alkaline phosphatase-labeled probes. This generation assay had a lower limit of detection of 500 RNA copies per milliliter.

**Results**

Summary statistics are presented for all measurements. Univariable logistic regression models were applied to the data, and the significance of each potential factor was determined using the likelihood ratio test. The crude (unadjusted) relationship between lung CD4 cells per milliliter of BAL fluid and mortality was assessed via a univariable logistic regression model. Next, the presence of confounders affecting this crude relationship was explored via multivariable modeling. Potential confounders were identified by assessing the change in parameter estimates for lung CD4 cell counts between the crude and adjusted models. For this study, a change of 15 to 20% in the estimated coefficient for lung CD4 cell counts is considered indicative of confounding. Due to computational difficulties as well as concerns about the appropriateness of using large sample methods with only eight deaths in the data set, exact inference methods were used to calculate confidence intervals (CIs) and assess significance (LogXact 4 for Windows, 2000; Cytel Software; Cambridge, MA).

**Subjects**

Baseline characteristics of the 95 study subjects at the first visit are presented in Table 1. Median age of

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<tr>
<th>Table 1—Characteristics of the Study Population*</th>
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<td>Characteristics</td>
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<tr>
<td>-----------------</td>
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<tr>
<td>Patients, No.</td>
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<tr>
<td>Age, yr</td>
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<tr>
<td>Sex</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Male</td>
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<tr>
<td>Ethnicity</td>
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<tr>
<td>Nonwhite</td>
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<tr>
<td>White</td>
</tr>
<tr>
<td>Smoking status</td>
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<tr>
<td>Nonsmoker</td>
</tr>
<tr>
<td>Smoker</td>
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<td>Peripheral WBCs</td>
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*Data are presented as median (minimum–maximum) or No. (%) unless otherwise indicated.

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CHEST / 128 / 4 / OCTOBER, 2005 2263

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these subjects was 36 years (range, 25 to 63 years). The subjects were predominantly male (87%), white (74%), and smokers (71%). At the 4-year analysis, eight subjects had died.

Univariable Analysis

As demonstrated in Figure 1 and Table 2, plasma viral load and, to a lesser extent, blood CD4 counts were marginally related to survival ($p = 0.035$ and $p = 0.15$, respectively). In contrast, lung lymphocytes, more specifically lung CD4 lymphocytes, and blood hemoglobin levels were inversely associated with survival ($p = 0.01$ and $p = 0.004$, respectively). Since we have previously documented that cigarette smoking is associated with a suppression of lung lymphocyte numbers,$^{11}$ we compared smoking status with survival but no relationship was identified ($\chi^2 = 0.046$, $p = 0.83$). Furthermore, as outlined in Table 2, there was also no relationship between body mass index (BMI), lung function parameters of FEV$_1$, total lung capacity (TLC), and DLCO with survival.

Treatment Status and Survival: To characterize the role of treatment status in our population, subjects were classified into those who received at least one nonprotease inhibitor antiretroviral and those who received at least one nonprotease inhibitor antiretroviral plus one protease inhibitor (which we have defined as combination therapy). When subjects were classified by survival and treatment status, neither the use of any antiretroviral nor the use of any antiretroviral agent in combination with a protease inhibitor at the time of the initial evaluation was associated with survival ($\chi^2 = 2.054$, $p = 0.36$).

Identification of Confounders

Based on the crude (unadjusted) model, the number of lung lymphocyte CD4 cells per milliliter of

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*Figure 1. Comparison of nonsurvivors to survivors for blood and lung lymphocyte counts. Blood and lung lymphocyte subtyping was performed on 95 asymptomatic HIV-infected subjects upon study entry. Shown are the (top left, A) blood CD4 counts, (top right, B) blood CD8 counts, (bottom left, C) BAL CD4 counts, and (bottom right, D) BAL CD8 counts at entry for survivors and nonsurvivors. The difference between BAL CD4 counts in survivors vs nonsurvivors was statistically significant ($p < 0.01$).*
BAL fluid was a significant predictor of mortality (p = 0.010). The univariable odds ratio (OR) for a 1 × 10^3 cells/mL decrease is 1.87 (95% CI, 1.03 to 5.48).

Consistent with the univariable results, the number of lung CD4 lymphocytes per milliliter of BAL fluid was a significant predictor of survival, even after controlling separately for other potential confounders (Table 3). Of all the factors considered, plasma viral load elicited the largest change from the crude model; controlling for viral load increased the OR for lung CD4 cell counts from 1.87 to 2.87, a 53% increase in the unadjusted OR. Note that even after controlling for blood CD4 cell counts and hemoglobin separately, the adjusted model coefficients and OR for lung CD4 counts did not drastically change (OR, 1.99; 95% CI, 1.03 to 6.52; and OR, 1.98; 95% CI, 1.03 to 6.83, respectively). Modeling for more than two factors using exact methods was not possible due to the limited size of the data set.

**Table 3—OR for Mortality Based on Lung CD4 Levels Unadjusted and Controlling for Potential Confounders**

<table>
<thead>
<tr>
<th>Potential Confounder in Model</th>
<th>Estimated OR*</th>
<th>95% CI for OR</th>
</tr>
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<tbody>
<tr>
<td>None (unadjusted)</td>
<td>1.87</td>
<td>1.03–5.48</td>
</tr>
<tr>
<td>Smoking</td>
<td>2.00</td>
<td>1.06–6.02</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>1.82</td>
<td>1.01–5.55</td>
</tr>
<tr>
<td>Blood CD4, μL</td>
<td>1.99</td>
<td>1.03–6.52</td>
</tr>
<tr>
<td>Lung CD4, × 10^3/μL</td>
<td>1.73</td>
<td>1.02–4.23</td>
</tr>
<tr>
<td>DLCO, %</td>
<td>1.88</td>
<td>1.01–6.33</td>
</tr>
<tr>
<td>FEV₁, %</td>
<td>1.86</td>
<td>1.04–5.52</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>1.98</td>
<td>1.03–6.83</td>
</tr>
<tr>
<td>TLC, %</td>
<td>1.83</td>
<td>1.02–5.32</td>
</tr>
<tr>
<td>Viral load, 10^7 copies/mL</td>
<td>2.87</td>
<td>1.14–22.65</td>
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</table>

*OR for a 1 × 10^3 cells/mL decrease in BAL fluid for lung CD4.

**Discussion**

Although blood CD4 counts and plasma viral loads are generally accepted as the best predictors of severity of involvement in patients with HIV infection, to our knowledge, only one prior report has addressed the question as to whether lung lymphocyte counts are related to severity of illness as determined by survival. We therefore chose to analyze our findings in 95 HIV-positive individuals who were evaluated by BAL at The Ohio State University between August 1996 and November 1998. At the 4-year follow-up, eight individuals had died. We analyzed the relationship between plasma viral load, lymphocyte counts in the blood and lung, lung function parameters, BMI, smoking status, and treatment status on survival. Lung lymphocyte counts were statistically linked with mortality, a relationship that was not diminished by controlling for potential confounders. In fact, when controlling for plasma viral load, the association of lung CD4 cells with mortality (OR, 1.87; 95% CI, 1.03 to 5.48) actually increased to an OR of 2.87 (95% CI, 1.14 to 22.65), lending further support to the hypothesis that lung CD4 counts are independent predictors of survival. As expected, baseline viral load measures on their own were negatively associated with survival (p = 0.035); however, blood CD4 counts were not (p = 0.15). In addition, antiviral treatment status, smoking status, lung function measurements, and BMI were not associated with survival. Thus, our findings expand on the prior work in symptomatic individuals to demonstrate for the first time that lung lymphocyte counts may be predictive in asymptomatic individuals as well.

A shortcoming of the present study is the relatively small sample size. However, despite this, the study reproduced prior observations that document a rela-
relationship among blood hemoglobin level, plasma viral load, and survival.\textsuperscript{2,3,12,14} Importantly, despite the sample size, there is a highly significant association between lung CD4 count and survival. This supports the concept that HIV-related alterations in lung immune cell populations have broad biological and clinical implications.

We did not find a significant association between survival and current cigarette smoking. This is noteworthy since we have previously identified a link between smoking and low lung CD4 lymphocyte counts.\textsuperscript{11} Smoking induces a suppression of proinflammatory cytokine release, as we and others have shown.\textsuperscript{11,14,15} This immunosuppressive effect of smoking could suppress viral replication since HIV replication is linked to nuclear factor-κB activation, a pivotal signaling event in proinflammatory conditions.\textsuperscript{16–18} Although there are published data to suggest that smokers’ lungs have higher viral burdens, prior work\textsuperscript{19} only analyzed smokers with active symptoms. It has been readily demonstrated that smokers are at a higher risk for respiratory tract infections,\textsuperscript{20–26} and it has been shown that lung viral burdens expand dramatically during the course of Pneumocystis pneumonia.\textsuperscript{4} Nevertheless, our data failed to identify a connection between cigarette smoking and survival in this asymptomatic population.

The negative association of survival with hemoglobin levels confirms several prior reports\textsuperscript{12,13,27} that have shown this connection. Hemoglobin levels may be markers for underlying disease in the marrow or may reflect changes in iron regulation that are clearly important in antibacterial defenses.\textsuperscript{12} Interestingly, there is some suggestion that correction of hemoglobin levels improves survival but a cause and effect relationship is not proven.\textsuperscript{27} In the context of the present study, our confirmation of an association between survival and hemoglobin level lends further credence to our modeling approach and to the additional association we show between lung CD4 counts and survival.

Although it is tempting to speculate about a reason for a relationship between lung lymphocytes and survival, we do not have uniform access to the specific cause of death in our cohort. Nevertheless, it is interesting to speculate. Persistent viral burden in dendritic cells and lung macrophages has been described.\textsuperscript{28–31} Thus, the lung may be a source of viral replication that seeds the peripheral blood. In this context, it has been shown that viral burden in lung lavage predicts survival and does not necessarily correlate with blood lymphocyte values.\textsuperscript{31} Thus, one interpretation of our findings is that regulation of organ-specific viral burden is critical to host survival. It is possible that the lung, at the front line of many opportunistic infections, may depend on local lung CD4 lymphocytes more than on the peripheral CD4 population to drive efficient host defenses against pathogens. Low lung CD4 levels may not only allow excessive viral replication during opportunistic infections, but also predispose to an inadequate host response to infections and hence increased mortality. Although we do not propose that this association be used as a clinical tool to predict survival, we believe that this finding places renewed emphasis on the functional significance of lung immunity in HIV-infected individuals. Further studies are warranted to investigate the significance of lung immune cell populations on survival since this relationship may have implications for a number of immunocompromised states.

ACKNOWLEDGMENT: We thank Janice Drake, Tina Bees, and Patricia Farmer for help with subject recruitment; Judith Hart for technical work; Drs. Thomas Yunger, Nitin Bhatt, and Anuj Goyal for help with bronchoscopy; and Dr. M.E. Wewers for helpful comments.

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