Measurements of HIV Viral Loads From Different Levels of the Respiratory Tract*

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**Background:** The lung is a common site of disease in HIV infection. Virus has been detected in BAL fluid (BALF) and saliva. However, the relationship between viral loads detected at different levels of the respiratory tract is unknown.

**Method:** We measured simultaneous HIV viral loads in parotid saliva (PS), bronchial fluid (BF), BALF, and plasma by reverse transcription polymerase chain reaction in 20 HIV-infected individuals.

**Results:** HIV was detected in 53% of BALF samples, 15% of BF samples, 5% of PS samples, and 88% of plasma samples. Viral loads in plasma and BALF samples were positively correlated. There were significantly higher levels of HIV viral load in both plasma and BALF in subjects with CD4 counts of < 200 cells/μL compared to those with higher counts. Antiretroviral therapy (ART) was associated with lower BALF and plasma viral loads, and the effect in BALF was independent of the plasma viral load. Interestingly, smoking also was associated with lower levels of both BAL and BF viral loads, independent of the plasma viral load.

**Conclusion:** These data demonstrate that while HIV can be detected in the respiratory tract, the viral load is influenced by both local factors (ie, level of the respiratory tree and cigarette smoking) and systemic factors (ie, ART and peripheral CD4 count).

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**Key words:** BAL fluid; bronchial fluid; HIV; polymerase chain reaction; saliva; viral load

**Abbreviations:** ART = antiretroviral therapy; BALF = BAL fluid; BF = bronchial fluid; PS = parotid saliva; RML = right middle lobe; RT-PCR = reverse transcription polymerase chain reaction

Infectious and noninfectious complications are common in the lung during HIV infection and increase in frequency with HIV disease progression. In order to monitor HIV progression, plasma viral load measurement by reverse transcription polymerase chain reaction (RT-PCR) is now one of the most widely used measures of disease activity in the blood and is used, side-by-side, with CD4 cell count. While much is known about plasma viral load, less is known about the viral burden in the lungs and other portions of the respiratory tract, and how this affects pulmonary disease.

Previous studies measuring viral loads in separate anatomic sites have suggested that productive HIV infection is compartmentalized. In studies of semen, virus has been detectable, but the levels were poorly correlated with plasma viral loads. Others have measured viral loads by RT-PCR in the saliva of subjects infected with HIV. While oral secretions rarely transmit HIV infection, detectable virus has been found in salivary specimens. Saliva HIV concentrations are lower than those in plasma, and HIV viral inhibitors have been found in saliva.

Although the lung is a separate immune compartment, it represents only one level of the respiratory system. Virtually all studies that have examined the lung compartment usually did so by BAL, which samples the distal respiratory tree. We have previously shown that HIV is easily detectable in the...
alveolar space and that the viral burden increases with HIV disease progression. However, pulmonary disease, especially infection, frequently begins with abnormalities in the upper airway, including the bronchi and oral pharynx. Detectable virus at these sites could impact local immunity. To date, the detection of HIV has not been attempted in bronchial fluid (BF). Furthermore, while some studies have looked at the viral load in BAL fluid (BALF) or saliva, none have examined various compartments simultaneously to determine whether a relationship exists between these distinct anatomic sites. In this study, we have examined the HIV viral load at several levels of the respiratory tract (ie, BALF, BF, and parotid saliva [PS]) and in the plasma of HIV-infected subjects. All samples were collected simultaneously in each patient, allowing accurate comparisons between different sites.

**Materials and Methods**

Twenty confirmed HIV-seropositive subjects underwent bronchoscopy with BAL between October 19, 1999, and January 23, 2001. Subjects had BF secretions, PS, and plasma collected in addition to BALF for viral load measurement. All subjects underwent bronchoscopy solely for research purposes and had no active pulmonary disease, as determined by symptoms or radiograph. Written informed consent was obtained from all subjects. All subjects were participating in a human study protocol that was approved by the institutional review board at Indiana University-Purdue University in Indianapolis.

**Sample Collection and Processing**

After anesthetizing the upper airway with 4% topical lidocaine, a flexible bronchoscope was introduced through the nares or mouth. Immediately on entering the right middle lobe (RML), 20 mL balanced salt solution (Plasmalyte; Baxter; Deerfield, IL) was introduced and immediately suctioned back for the collection of BF, with a usual return of 5 mL. The bronchoscope then was wedged into a subsegment of the RML, and BALF was obtained. One hundred fifty milliliters of plasmalyte was instilled in 50-mL aliquots into two separate subsegments of the RML. Typically, 300 mL fluid was instilled for a usual return of 50%, or 150 mL. After collection, samples were kept on ice until processing. BALF was filtered through 70-nm nylon filters and then was centrifuged for 10 min at 400g to collect the acellular supernatant. After recovery from sedation, approximately 1 to 3 mL PS was collected using a previously established method.26 Sterile intraoral cups were placed directly over both parotid ducts (ie, Stensen ducts) by applying pressure and a vacuum to the buccal mucosa. PS flow was stimulated by the application of lime juice to the dorsal surface of the tongue at 3-min intervals for 30 to 45 min. The samples were centrifuged at 2,000g for 10 min. At the time of bronchoscopy, peripheral blood was collected from each subject in a heparinized syringe. Plasma was isolated and saved along with BALF, BF, and PS supernatants at −70°C until viral load measurements were conducted.

**Measurement of HIV Viral Loads**

HIV-1 viral loads were measured (Amplicor HIV-1 Monitor Test; Roche Diagnostic Systems, Inc; Branchburg, NJ), as previously described in our laboratory.19 The ultrasensitive specimen-processing procedure was used and has a detection range of 50 to 75,000 copies of HIV-1 RNA per milliliter of sample. For each sample, the presence of inhibitors was evaluated by adding an internal standard to each specimen. If the recovery of the internal standard was less than expected, then an inhibitor of the assay was present in the sample.

**Statistical Analysis**

A comparison of the number of samples with detectable viral loads between anatomic compartments was performed using the Fisher exact test. The Mann-Whitney rank sum test was used to compare the number of viral copies between two groups. Linear regression analysis was used to examine the relationship between viral loads in different compartments. Logistic regression analysis was used to compare the effects of smoking and antiretroviral therapy (ART) on viral loads using a statistical software package (SAS, version 8.01; SAS Institute Inc; Cary, NC). Plasma, BALF, BF, and PS viral load data were log10-transformed prior to regression analysis. The data are expressed as the mean ± SEM.

**Results**

Twenty HIV-infected subjects were studied. The mean age was 37.5 ± 1.34 years. There were 13 active smokers and 7 nonsmokers. Twelve of the 20 subjects were receiving ART. Subjects were divided based on CD4 count, with 10 subjects having CD4 counts of >200 cells/μL and 10 subjects having CD4 counts of <200 cells/μL (Table 1). Nineteen of the 20 subjects had BALF samples available, and 17 of the 20 subjects had plasma samples available for viral load analysis. All subjects had BF and saliva samples available.

HIV was detected significantly more often in plasma than in all respiratory compartments (Fig 1, top, A). A detectable viral load was present in 15 of 17 plasma samples, 10 of 19 BALF samples, 3 of 20 BF samples, and 1 of 20 saliva samples. Six of the saliva specimens had inhibitors detected by the RT-PCR assay. Virus was detected significantly more often in BALF compared to BF or PS. As a group, there were not significant differences in the number

<table>
<thead>
<tr>
<th>Table 1—Patient Characteristics*</th>
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<tr>
<td>Variables</td>
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<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>Ethnicity</td>
</tr>
<tr>
<td>Black</td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>CD4 count</td>
</tr>
<tr>
<td>Tobacco use</td>
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<tr>
<td>ART</td>
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*Values given as mean ± SEM or No. of patients.
of copies of the virus detected per milliliter of fluid in HIV-positive samples among the different levels of the respiratory tract (Fig 1, bottom, B). However, several BALF samples contained very high viral loads.

We next examined the following three variables that could impact the ability to detect virus in each compartment: disease state; smoking status; and ART (Table 2). In agreement with previous work,19 individuals with late-stage disease (ie, CD4 count, < 200 cells/μL) had a greater amount of detectable virus found in BALF. There was no difference in the amount of virus detected in BF secretions and saliva based on CD4 count. As expected, there was a significantly higher mean plasma viral load in those patients with a CD4 count of < 200 cells/μL than in those with a CD4 count of > 200 cells/μL.

The ability to detect HIV in BALF was also significantly decreased in patients receiving ART. There were no differences in BF or saliva viral load based on whether ART had been initiated. As expected, ART influenced plasma viral loads, with the mean plasma viral load for subjects receiving ART being lower than for those not receiving ART.

Finally, we examined the effect of smoking on HIV viral loads in the respiratory tract. Forty-two percent of subjects who were smokers (5 of 12 subjects) had detectable viral loads found in BALF samples, whereas 71% of nonsmokers (5 of 7 subjects) had detectable viral loads found in BALF samples. This did not meet statistical significance in univariate analysis. In the BF samples, none of the smokers had detectable viral loads, whereas significantly more of the nonsmokers had detectable viral loads. There was no difference, based on smoking status, in the number of copies of HIV detected per milliliter of BALF or BF samples.

Because we found higher viral loads in both the plasma and BALF of subjects with late-stage disease, we next examined the relationship between the BALF viral load and plasma viral load (using log-transformed data). There was a significant positive correlation between the BALF viral load and the plasma viral load ($R^2 = 0.54; p < 0.01$) [Fig 2].

Because of the effect of plasma viral load on the respiratory tract (ie, BALF) viral load, we wondered whether the effects of ART and smoking on BALF and BF viral loads might be accounted for by differences in plasma viral load. Therefore, a logistic regression model was used to adjust for plasma viral load when measuring the effects of the other variables (ie, ART and smoking) on BALF viral load. We first examined a model with BALF viral load as the response (dependent) variable, ART as the independent variable, and plasma viral load as the covariate. Adjusting for plasma viral load, subjects receiving ART had lower viral load in BALF compared to subjects not receiving ART. For a given plasma viral load, those persons receiving ART had a 37% lower log10 BALF viral load ($p < 0.01$). The use of ART was not associated with differences in BF viral load.

A similar model was used to test the effect of smoking on BALF, adjusting for plasma viral loads. Nonsmokers had higher levels of viral load in BALF compared to smokers. For a given plasma viral load, smokers had a 21% lower log10 BALF viral load ($p < 0.03$). Figure 3, top, A, shows the correlation of BALF viral load and plasma viral load in both smokers and nonsmokers. Smoking had lower levels of BALF viral load for a given plasma viral load compared to nonsmokers.

Finally, the effect of smoking on BF viral load was examined, adjusting for plasma viral load. Nonsmokers had higher BF viral loads than smokers. For a given plasma viral load, smokers had a 16% lower log10 BF viral load ($p = 0.095$). Figure 3, bottom, B,
shows the correlation of BF viral loads and plasma viral loads in both smokers and nonsmokers.

**Discussion**

In this study, we have examined HIV viral loads in plasma and various compartments of the respiratory tract concurrently in individual subjects. Our data demonstrate that the viral load was higher in plasma compared with BALF, BF, or saliva. In the respiratory tract, detectable viral RNA was found most often in the BALF compared with BF or PS. It was uncommon to detect HIV RNA in BF or saliva. The viral load was higher in the BALF of subjects with later stage disease compared to those with earlier stage disease, as determined by CD4 counts. There was a strong correlation between BALF and plasma viral loads. In subjects who smoked, there were lower levels of HIV RNA found in BALF and less detectable levels of HIV RNA found in BF, when adjusted for plasma viral load. Receiving ART also was associated with lower viral loads in the BALF when adjusted for plasma viral loads. These data demonstrate that the concentration of HIV RNA varies at different levels of the respiratory tract and is influenced by smoking status and ART.

Detectable levels of HIV were found in only 1 of 20 saliva samples. This is lower than the incidence found in most studies that have measured HIV RNA levels in saliva.\(^7^–^1^2\) However, most previous studies have examined whole saliva. In contrast, we have collected PS in this study in order to examine the pure glandular component of saliva without the mixture of gingival crevicular fluid, which is primarily an exudation of serum components. Several studies\(^7^,^1^4\) have suggested that the presence of HIV in the saliva correlates with the number of lymphocytes and that in active inflammatory conditions both the number of lymphocytes and the HIV viral load increase. Furthermore, inhibitors both to the RT-PCR assay and to HIV may decrease the detection of virus in saliva. Inhibitors of the PCR assay can be found in many nonplasma body fluids, including semen, breast milk, and saliva.\(^2^1\) We detected inhibitors to the RT-PCR assay in 30% of our saliva samples. Other important inhibitors that are present

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**Table 2—Factors Influencing Virus Detection and Viral Load Levels in Different Compartment***

<table>
<thead>
<tr>
<th>Site</th>
<th>CD4 Count</th>
<th>ART</th>
<th>Smoking Status</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 200, cells/μL</td>
<td>&gt; 200, cells/μL</td>
<td>+</td>
</tr>
<tr>
<td>Plasma</td>
<td>% positive</td>
<td>89</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Copies/mL</td>
<td>65,857 ± 9,142</td>
<td>8,298 ± 5,660</td>
</tr>
<tr>
<td>BALF</td>
<td>% positive</td>
<td>70</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Copies/mL</td>
<td>3,692 ± 1,665</td>
<td>145 ± 52</td>
</tr>
<tr>
<td>BF</td>
<td>% positive</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Copies/mL</td>
<td>203 ± 19</td>
<td>211 ± 211</td>
</tr>
<tr>
<td>PS</td>
<td>% positive</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Copies/mL</td>
<td>0 ± 270</td>
<td>270 ± 270</td>
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</tbody>
</table>

*Values given as mean ± SEM, unless otherwise indicated.
†Copies per milliliter of fluid in samples with detectable virus.
\(p = 0.002\) compared to plasma samples with CD4 > 200 cells/μL.
\(p = 0.001\) compared to BAL samples of subjects not receiving ART.
\(p = 0.02\) compared to BAL samples with CD4 > 200 cells/μL.
\(p = 0.03\) compared to BF samples of nonsmoking subjects.

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**Figure 2.** Correlation between log\(_{10}\) BALF viral load and log\(_{10}\) plasma viral load. There is a strong correlation between the two compartments in the 16 subjects who had both plasma and BALF samples available.
in saliva are direct anti-HIV factors. These inhibitors are found in salivary gland secretions, including PS secretions. We suspect that one reason for the lower level of virus detection in pure PS in this study is the lower contribution of serum and oral inflammatory cells combined with inhibitors that are present in the saliva.

There are several potential explanations for why HIV is found more frequently in BALF than in the upper respiratory tract. First, BALF contains epithelial lining fluid that has been diluted in balanced salt solution. Epithelial lining fluid represents transudation from plasma. Thus, higher levels of detection of HIV in BALF may be a direct reflection of the plasma viral load. In support of this, we found that BALF and plasma viral loads were strongly correlated. In contrast, no correlation was found between plasma viral load and HIV in the other respiratory tract compartments.

A second possibility to explain why HIV is detected more frequently in BALF than in BF and saliva may be found in the cellularity of the three compartments. BALF has a significant cellular component that is composed of alveolar macrophages and lymphocytes. Alveolar macrophages have been shown to harbor HIV, and we have shown previously that the lymphocytic alveolitis that is present in HIV-infected patients is predictive of elevated BALF viral loads. BF has a higher percentage of neutrophils and epithelial cells, and a lower percentage of macrophages and lymphocytes. As stated previously, HIV is most readily detected in saliva when lymphocytes are present. Thus, the difference in HIV RNA concentrations may be a reflection of the difference in the cellular component of BF and PS.

Finally, the variability in HIV detection between respiratory compartments may reflect the differing antibody concentrations at each site. IgG and IgA concentrations are similar in the BALF of healthy subjects. However, in PS, IgA is the major antibody. During HIV infection, IgA secretions have been shown to decrease in the lung, whereas IgA levels in PS have been shown to be similar between HIV-infected persons and control subjects. Both in PS and BALF, IgG and IgA antibodies specific for HIV have been detected. Thus, it is possible that local concentrations of HIV-specific antibodies may impact the ability to detect HIV in various respiratory compartments.

Two previous studies have measured the level of HIV RNA in BALF in asymptomatic HIV-infected subjects and found detection rates of 10 to 16%. In contrast, we found that 52% of subjects had detectable viral RNA levels. One difference that would explain these differences is the higher sensitivity assay that was used in the present study. Our findings are not likely to be explained by differences in sample dilution between compartments. To examine this, the dilution factor of BALF and BF was calculated using the urea method. BALF was diluted an average of 30 times, whereas BF was diluted 97 times compared to plasma. Even after correcting for dilution, the relationship of detectable HIV RNA along the respiratory tract remains intact, as the average BALF viral RNA level was greater than three times that in BF. In addition, the PS sample was not diluted.

Using a logistic regression model, we found significant associations between respiratory tract viral loads and both smoking and ART. Subjects receiving ART had a lower viral load found in BALF. This is not surprising by itself, since a decreased BALF viral load may just be due to lower plasma viral loads. However, when accounting for plasma viral loads in the model, there was still an association between lower BALF viral loads and receiving ART.

A more surprising result was the negative effect of...
smoking on both BALF and BF viral loads. Smoking is known to be a respiratory tract immunosuppressant.32,33 Intuitively, one would therefore expect that smoking would increase the viral load found in the lung. We found the opposite effect, and subjects who smoked had lower viral concentrations in both BALF and BF. This can be explained when looking at the cellular differential recovered in the alveolar space. It has been shown previously that smoking decreases the number of lymphocytes recovered in the alveolar space, both in healthy control subjects and in HIV-infected subjects. We have shown previously that lymphocytic alveolitis is a predictor of poor prognosis and elevated viral burden in the lungs of HIV-infected subjects. Therefore, the lower viral burden in the lungs of smokers may just reflect lower lymphocyte concentrations. Alternatively, it is known that HIV replicates best in activated and proliferating cells.34,35 Thus, the immunosuppressive properties of smoking may actually inhibit viral replication.

The significance of detectable HIV levels in various respiratory compartments remains to be elucidated. Besides the obvious issues of transmissibility, free HIV has the potential to influence local immune responses. HIV may directly infect local immune cells and alter their ability to initiate immune responses.36 Even in the absence of direct cellular infection, HIV proteins can inhibit lymphocyte activation, and subsequent cellular and humoral responses.37,38 Clearly, immune abnormalities occur in the lungs of HIV-infected subjects.36 It has been more difficult to document abnormalities in the more proximal respiratory tract. This may be due to there being different viral loads in these anatomic sites.

In summary, while respiratory secretions rarely transmit HIV, viral RNA is detectable at several levels of the respiratory tract and is detected more frequently as one progresses toward the alveolar space. We speculate that the differences in detection at different levels can be explained by the fact that BALF has a significant cellular component and represents sampling from a tissue compartment, whereas increased mucosal immunity (ie, IgA and viral inhibitor levels) is found in the proximal respiratory tract. Factors such as ART and smoking also affect the viral burden in the respiratory system. The impact of detectable levels of HIV RNA being found at different levels of the respiratory tract on local immunity, both against HIV itself as well as against infectious agents, remains to be elucidated.

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CHEST / 124 / 2 / AUGUST, 2003 541


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