C-Reactive Protein Is a Marker for Human Immunodeficiency Virus Disease Progression

Bryan Lau, PhD, MHS, ScM; A. Richey Sharrett, MD, DrPH; Larry A. Kingsley, DrPH; Wendy Post, MD, MS; Frank J. Palella, MD; Barbara Visscher, MD, DrPH; Stephen J. Gange, PhD

**Background:** Limited data on acute-phase C-reactive protein (CRP) levels in human immunodeficiency virus (HIV) infection exist.

**Methods:** We obtained a single measurement of CRP from 513 HIV-infected men in the Multicenter AIDS Cohort Study to examine the association between CRP and immune suppression and progression to AIDS. We estimated changes in CRP during the course of HIV infection in 81 of these individuals using specimens collected from October 1, 1984, to December 31, 1996.

**Results:** The cross-sectional associations between log_{10} CRP were correlated inversely with CD4 lymphocyte counts (r = −0.17; P < .001) and directly with log_{10} HIV RNA levels (r = 0.20; P < .001). Levels of CRP of more than 2.3 mg/L were associated with a decreased time to the development of AIDS (relative time to AIDS, 0.36; P < .001) compared with individuals with CRP levels of 1.2 mg/L or less, which remained significant after adjustment for CD4 lymphocyte counts and HIV RNA and hemoglobin concentrations. Levels of CRP significantly increased over time with mean slopes of 8.5% (95% confidence interval, 4.9%-12.2%) and 4.5% (95% confidence interval CI, 2.1%-6.9%) per year for individuals with and without progression to AIDS, respectively. Individuals had a geometric mean CRP level of 2.5 mg/L in the 6-month interval before progression to AIDS, which was an increase from a nadir of 1.0 mg/L at 6.3 years before progression to AIDS.

**Conclusions:** Levels of CRP were associated with HIV disease progression independent of CD4 lymphocyte counts and HIV RNA levels. In addition, regardless of progression to AIDS, HIV-infected individuals had a significant increase in CRP over time. This may have implications for cardiovascular disease among HIV-infected individuals.

Arch Intern Med. 2006;166:64-70

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**Levels of acute-phase proteins as markers of inflammation usually rise markedly during acute and chronic infections.** This rise is particularly great for C-reactive protein (CRP), an acute-phase protein recognized as an important indicator of inflammatory conditions that are often the consequence of infection. Levels of CRP change in response to the proinflammatory cytokines, interleukin 1 and 6, whose expression is induced by bacterial infections and tissue necrosis. More recently, even small elevations in CRP concentrations have been shown to indicate increased risk for cardiovascular disease and possibly colon cancer.

The 25th, 50th, and 75th percentiles of CRP levels have been estimated to be 0.6, 1.5, and 3.5 mg/L, respectively, in healthy middle-aged US individuals. Symptomatic mild inflammatory disease or viral infection is commonly thought to increase CRP concentrations from 10 to 40 mg/L, whereas concentrations of 40 to 200 mg/L are found in acute bacterial infections.

The relationship between CRP concentration and human immunodeficiency virus (HIV) is still unclear. Lower levels of CRP have been shown to predict longer survival within HIV-infected individuals. For a population with ongoing HIV infection, the level of CRP was shown to be relatively low (median <4 mg/L), which indicates that HIV infection is not a highly inflammatory disease. Furthermore, it has been suggested that measurement of CRP level may be an inexpensive method for monitoring febrile episodes and opportunistic infections in individuals with AIDS. These studies could not examine low levels of CRP, as high-sensitivity CRP assays were not used. In addition, longitudinal evaluations of plasma CRP concentrations have not been described among HIV-infected individuals, and CRP concentra-
tions have not been correlated with the degree of immunosuppression. Furthermore, it is unclear whether CRP remains prognostic for cardiovascular disease in HIV-infected individuals. Sklar et al. failed to find any predictive value of CRP for cardiovascular disease in HIV-infected individuals. However, their study was limited by a small sample size.

In this study, we sought to elucidate the relationship between HIV disease and the concentration of CRP. We used a highly sensitive assay to measure the level of CRP from stored plasma specimens collected from men enrolled in the Multicenter AIDS Cohort Study (MACS). These specimens had been obtained prior to the widespread use of highly active antiretroviral therapy (HAART). We examined the relationship of CRP concentrations with markers of HIV status and time to clinical AIDS.

**METHODS**

**STUDY POPULATION AND LABORATORY ANALYSIS**

The MACS was initiated in 1983 to study the natural history of HIV infection among homosexual and bisexual men in the United States. The design of the MACS has been previously described, and only aspects pertinent to this study are presented herein. From April 2, 1984, to April 8, 1985, 4954 men, who were either seropositive or seronegative for HIV, were enrolled in the Baltimore, Md/Washington, DC, area, Chicago, Ill, Los Angeles, Calif, and Pittsburgh, Pa, with an additional 625 men enrolled from April 1, 1987, to February 25, 1991. Men with clinical AIDS or who were younger than 18 years were ineligible. At semiannual visits, the men returned to the clinics to provide specimens for laboratory analyses, undergo a physical examination, and complete self-administered data forms and an interviewer-administered questionnaire. At each visit, T-cell subset levels were measured in peripheral blood samples stained with monoclonal antibodies by means of a whole-blood lysing method and analyzed by means of 2-color flow cytometry and monoclonal antibodies specific for CD3, CD4, and CD8 lymphocytes. Absolute numbers of cells per microliter of blood were calculated using the complete blood cell count and CD4 lymphocytes. Absolute numbers of cells per microliter of blood were calculated using the complete blood cell count with an automated 10 000-cell differential. Because the MACS began before the advent of HIV RNA assays, HIV RNA data are limited. The HIV RNA data were used in this study for the baseline analyses and included only when available within 1 year of the visit.

Individuals included in this study consisted of a random sample of 513 MACS participants who were HIV seropositive on MACS enrollment and had specimens available in the national specimen repository dating from October 1, 1984, to May 31, 1987. For this study, CRP was measured using stored specimens that had been collected from each MACS participant as part of standard visits. The earliest visit from which specimens were available is referred to as the baseline (first ever) study visit. Levels of CRP were measured by means of a highly sensitive nephelometric assay using a monoclonal antibody to CRP coated on polystyrene beads with a lower limit of detection of 0.2 mg/L (Dade Behring, Marburg, Germany). To further investigate the temporal patterns of CRP, an additional subsample of individuals was randomly selected from the men with baseline measurements who had at least 6 visits through December 31, 1996 (when HAART use in the MACS became prevalent) and who were free of clinical AIDS for a minimum of 4 visits. Furthermore, men in whom AIDS developed from October 15, 1994, to December 31, 1996, were excluded (n=8) to enable evaluation of CRP patterns before and after an AIDS diagnosis was made but prior to the widespread use of HAART. This study was approved by the institutional review board, The Johns Hopkins Bloomberg School of Public Health, Baltimore.

**RESULTS**

The 513 individuals who were randomly selected reflected the overall MACS population. The sample consisted primarily of non-Hispanic white individuals (n=453 [88.3%]), with 26 (5.1%) men who reported being Hispanic white; 21 (4.1%), non-Hispanic black; and 13 (2.5%), other race/ethnicity. The median age of the population was 33.8 years (interquartile range [IQR], 29.9-37.8 years). The median body mass index (calculated as weight in kilograms divided by the square of height in meters) was 23.1 (IQR, 21.6-24.8). One hundred seventy-nine individuals (34.9%) reported smoking during the past 6 months. The median CD4 lymphocyte count was 532 cells/µL (IQR, 342-721 cells/µL); baseline CD4 lymphocyte counts were missing for 4 individuals. The HIV RNA data were available for 484 individuals (94.3%). The median HIV RNA level was 18 450 copies/mL (IQR, 5359-63 741 copies/mL). The median CRP concentration was 1.2 mg/L (IQR, 0.6-2.3 mg/L). Of these individuals, AIDS developed in 318 (62.0%). The median CRP concentration was 1.3 mg/L (IQR, 0.6-2.7 mg/L) for those in whom AIDS later developed and 1.0 mg/L (IQR, 0.5-1.8 mg/L) for those who remained free of AIDS (Wilcoxon rank sum test; P=.002). These individuals contributed a total of 2709 person-years of follow-up.

**STATISTICAL ANALYSIS**

Pearson product moment correlations were calculated for CRP concentrations with CD4 lymphocyte counts and HIV RNA levels at the baseline visit. Concentrations of CRP and HIV RNA were logarithmically transformed such that the data was more normally distributed.

We used Kaplan-Meier product-limit estimates to measure progression to AIDS for men with differing levels of CRP. The time at risk was calculated from the first visit from which samples underwent testing for CRP and ended when the individual was lost to follow-up, clinical AIDS had developed, or the individual was administratively censored at October 15, 1994. Lognormal survival models were used to assess the relative time for progression to AIDS after adjusting for other markers of disease progression. The distribution of events was properly distributed for a log-normal model.

To describe the pattern of CRP over time, a smooth graphical depiction (locally weighted scatterplot) was computed, stratified by whether or not progression to AIDS occurred. In addition, random-effects models were used to investigate the change in CRP level over time. In this model, the intercepts were allowed to vary among individuals. For individuals with progression to AIDS, the geometric mean of the CRP level was determined for the 6-month intervals around the AIDS diagnosis. The geometric mean was used because it is less sensitive to extreme values that may occur in measuring CRP. The mean value was determined for an individual with multiple observations within the 6-month intervals.
Figure 1 shows a scatterplot of baseline CRP concentrations with baseline CD4 lymphocyte counts and log_{10} HIV RNA levels. The CRP concentrations were inversely correlated with CD4 lymphocyte counts ($r = -0.17; P < .001$) and directly correlated with HIV RNA levels ($r = 0.20; P < .001$).

Kaplan-Meier curves of individuals categorized by CRP categories (0.0-0.6, 0.7-1.2, 1.3-2.3, and $>2.3$ mg/L) are shown in Figure 2. Individuals with higher concentrations of CRP had shorter times to AIDS (overall log-rank test, $P < .001$). Individuals with a CRP concentration of 0.6 mg/L or less had a median time to AIDS of 7.70 years (95% CI, 6.74 to 9.31 years). Similarly, those with a CRP level of 0.7-1.2 mg/L had a median time to AIDS of 6.87 years (95% CI, 6.22 to 9.33 years). However, those with higher levels had progression to clinical AIDS more quickly. Median times to AIDS were 5.07 years (95% CI, 4.15-7.26 years) for those with CRP levels of 1.3 to 2.3 mg/L and 4.48 years (95% CI, 3.17-5.61 years) for those with CRP levels of more than 2.3 mg/L. These results suggest a threshold effect, with accelerated progression only at CRP levels of more than 1.2 mg/L.

Based on these results, CRP levels were categorized into 3 groups ($\leq 1.2$, 1.3-2.3, and $>2.3$ mg/L) for the log-normal time-to-AIDS analyses. In unadjusted models, individuals with CRP levels in the 2 higher categories had significantly lower relative times to AIDS (Table 2). These relative times correspond to reductions of 43% and 64% in their time to AIDS for the CRP categories of 1.3 to 2.3 and more than 2.3 mg/L, respectively, compared with individuals who had CRP levels of 1.2 mg/L or less. This trend remained after adjusting for CD4 lymphocyte counts and other factors.
HIV RNA and hemoglobin levels (Table 2). After controlling for these factors, individuals with CRP levels of more than 2.3 mg/L showed a relative time of 0.63 (P < .001), equivalent to a 47% reduction in time to AIDS, compared with individuals with CRP levels of 1.2 mg/L or less. Models with total lymphocyte count, body mass index, and age showed that these variables did not alter these estimates.

Similar findings were obtained using Cox proportional hazards models, despite the fact that the proportionality assumption was not always met. In the unadjusted Cox model, individuals with CRP levels of 1.3 to 2.3 mg/L and more than 2.3 mg/L had relative hazards of 1.47 (P = .008) and 1.98 (P < .001), respectively, for progression to AIDS relative to those with CRP levels of 1.2 mg/L or less. The increased hazard for the development of AIDS among higher levels of CRP remained after adjusting for CD4 lymphocyte counts and log10 HIV RNA and hemoglobin levels. However, the category of individuals with CRP concentrations from 1.3 to 2.3 mg/L was only marginally significant (relative hazard, 1.32; P = .08), whereas the highest category of CRP remained statistically significant (relative hazard, 1.57; P < .001).

**LONGITUDINAL PATTERNS OF CRP**

Four hundred of the 513 individuals met selection criteria for the longitudinal analyses. Of these, 81 were randomly selected and contributed 1065 visits with a median of 12 visits. These individuals were mainly non-Hispanic white (n=69, 85%), and all had finished high school. During follow-up, AIDS developed in 44 (54%). At baseline, the median age of these individuals was 34.1 years (IQR, 30.0-38.6 years). In addition, the baseline median CD4 lymphocyte count was 642 cells/µL (IQR, 511-874 cells/µL), and of the 76 individuals with baseline HIV RNA data, the median was 13 341 copies/mL (IQR, 4348-30 180 copies/mL). The median CRP concentration was 1.0 mg/L (IQR, 0.5-1.8 mg/L) overall, 1.25 mg/L (IQR, 0.80-2.65 mg/L) for those who progressed to AIDS, and 0.5 mg/L (IQR, 0.3-1.1 mg/L) for those who remained free of AIDS. During follow-up, 55 individuals (68%) initiated some antiretroviral therapy (ART) from 1987 to 1996.

**Figure 2.** Time to AIDS development from the baseline visit by C-reactive protein (CRP) concentration. The numbers of individuals remaining in each group is given for 0, 2, 4, 6, and 8 years and the end of follow-up.

**Table 2. Association of CRP Concentrations With Progression to Clinical AIDS: Log-normal Survival Model†**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relative Time (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Univariate model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1.2</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>1.3-2.3</td>
<td>0.57 (0.40-0.80)</td>
<td>.001</td>
</tr>
<tr>
<td>&gt;2.3</td>
<td>0.36 (0.26-0.49)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>Multivariate model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1.2</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>1.3-2.3</td>
<td>0.86 (0.68-1.09)</td>
<td>.21</td>
</tr>
<tr>
<td>&gt;2.3</td>
<td>0.63 (0.51-0.79)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CD4 lymphocyte count†</td>
<td>1.12 (1.08-1.16)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Log10 HIV RNA</td>
<td>0.34 (0.29-0.39)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>1.14 (1.06-1.23)</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; CRP, C-reactive protein; HIV, human immunodeficiency virus.

*Analysis was restricted to 474 individuals with data. Seven individuals had AIDS before the visit at which CRP was measured; 3 lacked CD4 data; 28 lacked HIV RNA data; and 1 lacked CD4 and HIV RNA data.
†Indicates per 100 cells/µL.
Levels of CRP increased over time for individuals with and without progression to AIDS (Figure 3). However, individuals with progression to AIDS had higher CRP levels than those without. In addition, the logarithmic scale of Figure 3 indicates that individuals with progression to AIDS demonstrated more rapid increases in CRP concentrations than those without progression to AIDS (this would be visually apparent if curves were plotted on an arithmetic scale). These trends were further supported by the results from the random-effects model (Table 3), which accounts for the correlation of repeated marker measurements and provides the trajectories of CRP concentrations for individuals with and without progression to AIDS.

From the random-effects model, individuals with progression to AIDS had higher initial CRP concentrations than individuals who remained free of AIDS (1.39 vs 0.91 mg/L, respectively). Furthermore, the rate at which CRP concentration increased was greater for individuals with progression to AIDS, with an 8.5% change per year compared with a 4.5% change per year for individuals who remained free of AIDS (Table 3).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean Estimate (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline geometric mean for CRP, mg/L</td>
<td></td>
</tr>
<tr>
<td>Individuals remaining free of AIDS</td>
<td>0.91 (0.69-1.19)</td>
</tr>
<tr>
<td>Individuals progressing to AIDS</td>
<td>1.39 (1.08-1.79)</td>
</tr>
<tr>
<td>Change per year, %</td>
<td></td>
</tr>
<tr>
<td>Individuals remaining free of AIDS</td>
<td>+4.5 (2.1-6.9)</td>
</tr>
<tr>
<td>Individuals progressing to AIDS</td>
<td>+8.5 (4.9-12.2)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; CRP, C-reactive protein.

Concentrations of CRP are shown for individuals with progression to AIDS in Figure 4. The overall trend of CRP concentration indicates a gradual rise in CRP before the development of AIDS.
The role of inflammation as defined by CRP levels has not been fully explored in the context of HIV disease. These results indicate that elevated CRP levels were modestly correlated with low CD4 lymphocyte counts and elevated HIV RNA levels. These correlations were determined from baseline values (before June 1, 1987), and no individual had initiated any ART by their baseline visit. The association of higher CRP with progression to AIDS was independent of any HAART effects, as the specimens were collected before any ART use, and individuals were censored before the HAART era. The baseline association indicates long-term prognostic value for CRP levels, which remains after adjusting for CD4 lymphocyte counts and HIV RNA levels. These results suggest a potential role for CRP in monitoring the clinical course of HIV-infected individuals. Our data also indicated that individuals with progression to AIDS have a faster elevation in CRP concentrations over time than individuals remaining free of AIDS. It is unclear whether the underlying CRP pattern is a continuous gradual rise over time or, alternatively, an initially relatively stable level that eventually increases prior to AIDS. The random effects model on a logarithmic scale indicates an acceleration of the increase in CRP concentrations over time. Use of ART was not significantly associated with a decrease in CRP levels when included in the random-effects model, indicating that early treatment regimens did not have an effect on CRP concentrations.

An inherent difficulty in this research is the adequate exploration of the temporal relationship between the immunocompromised state induced by HIV infection and the potential for subclinical or apparent low-level infections. Although it is possible and perhaps likely that CRP elevations may result from such infectious processes, the survival models described herein used CRP concentrations assessed at the baseline visit, when CRP levels were less than 2.7 mg/L for 75.8% of the individuals with progression to AIDS. These data are perhaps the strongest to mitigate the potential for subclinical or apparent low-level infections. Because this study was restricted to individuals with prevalent HIV infection, it was impossible to examine CRP changes around the time of seroconversion. Individuals with rapid progression to AIDS after seroconversion may not have been included. Furthermore, this study population consisted of homosexual or bisexual non-Hispanic white men. Although this group constitutes a large proportion of the HIV epidemic, the demographics of the epidemic have shifted toward other populations (eg, women and injecting-drug users). Further research in these populations is warranted.

Although these results suggest that CRP concentrations may have prognostic value, such measurements clearly cannot replace CD4 lymphocyte counts and HIV RNA levels for monitoring HIV-infected individuals. In addition, in resource-poor regions where alternative markers for disease progression would be of most value, the use of high-sensitivity CRP assays could be prohibitively expensive. However, measurement of CRP concentrations may provide additional prognostic information when used in conjunction with standard markers for HIV care. Furthermore, data on CRP concentrations in HIV infection with or without antiretroviral therapy is limited. To our knowledge, this is the only study that includes longitudinal CRP data from HIV-infected individuals prior to the widespread use of HAART. These results indicate increasing inflammation with disease progression, which may have implications for cardiovascular risk in HIV-infected populations.

The fact that the correlations of CRP concentrations with CD4 counts and HIV RNA levels were weak was somewhat surprising and may be related to the chronic immunosuppression consequent to HIV infection. Common viral infections that result in mild systemic inflammation are associated with much greater elevations in CRP levels (10-40 mg/L) than those seen in our subjects. However, because this is a seroprevalent cohort of HIV-infected individuals with whom the duration of infection is unclear, it is possible that these individuals experienced a more dramatic rise in CRP concentrations during acute HIV infection that eventually diminished and assumed a relatively stable lower level of inflammation seen in this study at baseline. Just before the development of AIDS, the CRP geometric mean level approached 2.5 mg/L, which may have clinical implications for these individuals. This level of CRP has been shown to confer an increased risk for cardiovascular disease relative to those with lower CRP concentrations. The American Heart Association and Centers for Disease Control and Prevention have recommended that the CRP concentration be used as a marker for cardiovascular disease in individuals with a Framingham risk score from 10% to 20%, considering CRP values between 1 and 3 mg/L as average risk and those of more than 3 mg/L as high risk for cardiovascular disease. The proportion of individuals with CRP of more than 3 mg/L ranged from 34% to 50% in the 2.5 years preceding AIDS development, whereas the proportion ranged from 9% to 24% when there were at least 15 individuals prior to this period. Whether CRP predicts cardiovascular disease equally well in the presence of a chronic infection like HIV is unknown. One study of HIV-infected individuals found that CRP did not significantly add predictive value when compared with traditional risk factors for identifying cardiovascular disease, although a small sample size and lack of matching on length of follow-up time were acknowledged as limitations.

These data support a modest association between the level of inflammation as measured by CRP concentration and the degree of immunosuppression and HIV RNA levels in HIV-infected men. The results indicating increasing levels of inflammation for individuals with progression to AIDS and those who remained free of AIDS may have important implications for potential cardiovascular disease within this population, especially as infected individuals are living longer with effective therapies.

Accepted for Publication: July 18, 2005.
Correspondence: Bryan Lau, PhD, MHS, ScM, Department of Medicine, The Johns Hopkins School of Medicine, 1830 E Monument St, Room 8070, Baltimore, MD 21287 (blau1@jhmi.edu).
Author Contributions: Dr Lau had full access to all the data in this study and takes responsibility for the integrity of these data and the accuracy of the data analysis.

Financial Disclosure: None.

Funding/Support: The Multicenter AIDS Cohort Study is supported by grants UO1-AI-35042, 5-M01-RR-00052 (GCRC), UO1-AI-35043, UO1-AI-37984, UO1-AI-35039, UO1-AI-35040, UO1-AI-37613, and UO1-AI-35041 from the National Institute of Allergy and Infectious Diseases, with additional supplemental funding from the National Cancer Institute and the National Heart, Lung, and Blood Institute, Bethesda, Md.

Acknowledgment: Data in this manuscript were collected by the Multicenter AIDS Cohort Study (MACS) with centers located at The Johns Hopkins University Bloomberg School of Public Health, Baltimore, Md (principal investigator, Joseph Margolick, MD, PhD); Howard Brown Health Center and Northwestern University Medical School, Chicago, Ill (principal investigator, John Phair, MD); University of California–Los Angeles (principal investigator, Roger Detels, MD); and University of Pittsburgh, Pittsburgh, Pa (principal investigator, Charles Rinaldo, PhD); and the Data Coordinating Center, The Johns Hopkins University Bloomberg School of Public Health (principal investigator, Lisa Jacobson, ScD).

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