Increased Peripheral Proinflammatory Cytokines in HIV-1–Infected Patients With Prolonged Viral Suppression Suffering From High Psychological Stress

To the Editor:

Despite the clinical benefits achieved with the use of antiretroviral agents, HIV-infected patients still suffer from high levels of psychological stress related to multiple inherent disease factors such as HIV nondisclosure, the presence of adverse events, or the fear of physical impairment. Many animal and human studies have demonstrated that stress accelerates HIV-1 disease pathogenesis and impairs the biological impact of antiretroviral treatment. However, we still know relatively little of the cell-mediated immune regulatory mechanisms that account for the association between psychosocial risk factors and health-related outcomes. Furthermore, the prevalence of comorbid conditions such as neurocognitive dysfunction, bone abnormalities, cardiovascular disease, lymphomas and other neoplasms have increased considerably among HIV-infected patients in recent years. The role of proinflammatory cytokines in the development of such comorbidity is demonstrated. Therefore, it seems reasonable to hypothesize that stress-derived immune deregulation could potentially modulate the development of comorbidity in HIV-infected subjects.

Our aim was to evaluate the proinflammatory cytokine profile of HIV-1–infected patients with prolonged viral suppression and different levels of reported psychological stress. For this purpose, we designed a cross-sectional observational study that included patients with documented HIV infection, aged >18 years, with nadir CD4 counts of >200 cells per milliliters, and viral load of <50 copies per milliliters during the last year and on antiretroviral therapy. Exclusion criteria were to have an AIDS-defining event and physical or mental disability that prevented participating in or understanding the study. The objective was to explain to all eligible subjects during routine visits to the HIV clinic between the months of April and June 2008. The ethics committee of Germans Trias i Pujol University Hospital, Badalona, Spain approved the study, and all subjects gave their voluntary written consent before enrollment.

Information was extracted from medical records about route of infection, years since HIV infection diagnosis, years on antiretroviral treatment, and number of antiretroviral regimens and HCV co-infection.

Blood samples were obtained from the participants to determine plasma levels of interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α), CD4 and CD8 cell counts and the CD4/CD8 ratio. Plasma levels of proinflammatory cytokines were determined by cytomeric bead array using the human T helper 1/T helper 2 cytokine kit II (BD Biosciences, San Jose, CA) according to the manufacturer’s instructions.

Patients then responded to questions on the Perceived Stress Scale (PSS) in a single visit. The PSS is the most widely used psychological instrument for measuring the perception of degree of stress in life situations. The reduced version contains 10 items with responses given on 5-point Likert scales (0–4). The scale has been translated and validated in a Spanish population.

The Kolmogorov–Smirnov test was used to explore the distribution of continuous variables. Variables were described by medians and interquartile ranges (IQR) and compared using a Mann–Whitney nonparametric test or by means and standard deviation and compared using a t test, as appropriate. The PSS score was median split, providing 2 groups of patients with high or low stress, respectively. Cytokine levels were log-transformed to normalize the distribution before analysis. The association between stress and cytokine levels was evaluated with the Pearson correlation test. P values shown represent single-variable comparisons, with statistical significance set at 0.05. All analyses were performed with SPSS 15 (SPSS Inc., Chicago, IL).

Twenty-nine patients who met the inclusion criteria were approached. Eight declined to participate and 21 (72%) gave their written consent to enrollment. The mean age of the subjects was 39.9 ± 3.5 years. Seventeen (80.9%) were men and 14 (66.7%) had acquired the infection through same-sex intercourse. Participants had been diagnosed a mean of 12.0 ± 6.2 years earlier and they had been on antiretroviral therapy for a mean of 8.6 ± 5.0 years. Six subjects (28.6%) were taking first-line antiretroviral therapy and 17 (80.9%) were HCV co-infected. The median CD4 cell count was 564.0 cells per cubic millimeter (IQR, 481.0–882.0 cells/mm³), the median CD8 cell count was 960.0 per cubic millimeter (IQR, 607.2–1260.2 cells/mm³), and the median CD4/CD8 ratio was 0.81 (IQR, 0.49–1.02).

Ten subjects (47.6%) had high psychological stress (PSS score >14). The sociodemographic and clinical variables of patients with and without stress were comparable. Subjects with high stress had significantly higher IL-6 levels (mean, 13.0 ± 23.0 pg/mL) than those with low stress (mean, 1.1 ± 2.4 pg/mL; P = 0.005). Nine subjects (42.9%) had IL-6 levels >2.5 pg/mL. Likewise, subjects with high stress had higher levels of TNF-α (mean, 5.1 ± 2.3 pg/mL) than those with low stress (mean, 2.4 ± 3.4 pg/mL; P = 0.025). A positive correlation was observed between degree of psychological stress and IL-6 concentration (r = 0.48, P = 0.03). Figure 1 illustrates IL-6 and TNF-α levels in patients with high and low psychological stress.

No differences in CD4 and CD8 cell counts or the ratio were observed between subjects with high and low stress (data not shown).

In summary, patients who reported high levels of stress had significantly higher levels of peripheral proinflammatory cytokines, especially IL-6. The levels in our patients who reported stress averaged more than 6 times the standard cut-off of 2.5 pg/mL, which has been determined to be predictive of physical decline. TNF-α levels were also significantly higher in patients with stress.
Despite the limited sample size in this pilot study, the findings suggest that psychological stress could be a cofactor in the comorbidity of HIV-infected patients. This hypothesis requires further investigation.

**REFERENCES**

In this study, we assessed the impact of ribavirin plasma levels on SVR in patients previously treated for their hepatitis C without HCV clearance and receiving peginterferon alfa plus ribavirin as retreatment.

HIV–1–infected patients aged 18–65 years with chronic hepatitis C previously treated without virological response were eligible for the study. They had previously received a first anti-HCV treatment as follows without virological response: (1) interferon alfa alone at least 3 MU 3 times per week for at least 12 weeks; (2) interferon alfa at least 3 MU 3 times per week and ribavirin at least 600 mg per day during a minimal duration of 24 weeks. They had a CD4 cell count >200 cells per microliter and a plasma HIV RNA level <10,000 copies per milliliter (Versant HIV–1 RNA 3.0 Assay; Bayer Corporation, Berkeley, CA; cutoff 50 copies/mL), with or without antiretroviral therapy. Patients received pegylated interferon alfa-2a (Pegasys, Produit Roche, 180 μg subcutaneously once a week; Neuilly-sur-Seine, France) and ribavirin (Copegus, Produit Roche, 800, 1000, or 1200 mg twice per day for a bodyweight <65 kg, between 65 kg and 85 kg, and ≥85 kg, respectively) for 48 weeks. HCV genotype was determined at the beginning of the study (TruGene 5’ NC HCV Genotyping Assay; Bayer Diagnostics, Tarrytown, NY). The plasma HCV load (Cobas Amplicor HCV Monitor 2.0; Roche Diagnostics, Meylan, France, cutoff 600 IU/mL) was measured before and at weeks 4 and 12 of the treatment. SVR was defined as plasma HCV RNA undetectability 24 weeks after the end of the treatment, nonresponse if HCV RNA was detectable at this time.

The ribavirin concentration was measured on stored frozen plasma from blood samples taken 2 and 12 weeks after treatment initiation and collected before the next dose of ribavirin. Samples were processed in the 2 hours after blood sampling and were kept frozen at −80°C. Ribavirin concentrations were measured using an automated solid phase extraction process and a validated high-performance liquid chromatography–ultraviolet assay adapted from previously published methods at weeks 2 and 12 after treatment initiation. Association between trough plasma concentration of ribavirin (RBV C₀) at these times and SVR was determined according to the best cutoff.

Analysis of HCV treatment efficiency was conducted on an intention-to-treat basis. The value of efficacy cutoff was evaluated by considering the median, the first, and third quartiles of the concentration of ribavirin, respectively. To compare the proportions of patients who achieved a SVR, a χ² or a Fisher exact test was used. For each comparison, patients were divided in 2 groups after the observed plasma RBV C₀ (≤ or >cutoff). For each series of experiments, the variability of ribavirin concentration was estimated by determination of the relative standard deviation.

Seventeen patients (14 males, median age 39 [interquartile range (IQR)] 28–44) were included in the study. The median CD4 T-lymphocyte count was 609/μL (IQR 302–1140) at the initiation of anti-HCV therapy. All the patients received an antiretroviral treatment, 12 had an undetectable HIV RNA level. Eleven patients were infected with HCV genotype 1, 3 with genotype 2 or 3, and 3 with genotype 4. Eleven had previously been treated with standard interferon alfa alone, the others having received standard interferon alfa plus ribavirin. The median baseline HCV level was 6.44 (IQR: 5.77–6.73) log10 IU/mL. Concerning the fibrosis score, 6 patients were F1 or F2, the others F3 or F4 (META VIR

![Box plots of ribavirin plasma concentration in patients with SVR and in NR at W2 (A) and W12 (B). NR, nonresponders. Squares, are medians; boxes, lower and upper limits are the 25th and 75th percentiles; and T bars are minimum and maximum values.](image-source)
have been performed in patients never in whom the immune effects of in-HCV therapy in HIV-coinfected patients, optimize virological response to anti-HCV alone–infected patients, adequate retreatment in this population. As in which confirms the interest of anti-HCV a SVR was observed in 40% of cases, 1.8 m

In summary, trough plasma concentration of ribavirin must be monitored during anti-HCV therapy in previously treated patient, as described in naive patients. Adjustment of dosage of ribavirin according to a ribavirin concentration determined shortly after initiation of anti-HCV therapy could be crucial to obtain a SVR.

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REFERENCES
Tenefovir Coadministration Is Not Associated With Lower Unboosted Atazanavir Plasma Exposure in the Clinical Setting

To the Editors:

Use of unboosted atazanavir (ATV) in selected patients is an attractive option due to its convenience and favorable metabolic profile. In Induction-Maintenance With Atazanavir in HIV Naive Patients (INDUMA) study, switching from 300 mg boosted by 100 mg of ritonavir to unboosted 400 mg ATV-containing regimen was recently shown to be virologically equivalent as compared with continuation of ATV/ritonavir as a first-line regimen. However, lower plasma concentrations and higher interindividual variability associated with unboosted dosing could lead to inadequate ATV plasma exposure and increased risk of virological failure. Coadministration of tenefovir (TDF) with unboosted ATV is contraindicated because the former has been shown to further decrease ATV plasma exposure (area under the curve and minimum concentration by ATV plasma exposure (area under the curve) has been shown to further decrease ATV plasma exposure and increased risk of virological failure.

Coadministration of tenefovir (TDF) with unboosted ATV is contraindicated by current guidelines because the former has been shown to further decrease ATV plasma exposure (area under the curve and minimum concentration by 25% and 40%, respectively). However, it is not clear at which stage this interaction occurs, and there are no data on the impact of the latter in the clinical setting. Considering that this association was not allowed in any trials. Nevertheless, some observational studies pointed out a good clinical outcome, and ATV plasma levels above the minimum effective concentration (150 ng/mL) in patients switching from a fully suppressive boosted ATV-containing regimen regardless of TDF use. In such context, off-label use of such association is not infrequent among Italian prescribers due to the off-label use of TDF and ATV concomitant use could not be considered a priori contraindicated as generally reported in guidelines, but it could be feasible with TDF of ATV.

Further clinical investigation is warranted to assess individual factors predicting the feasibility of unboosted ATV and TDF coadministration.

Andrea Calcagno, MD*
Stefano Bonora, MD*
Maria Cristina Tettoni, MD*
Antonio D’Avolio, BSc, MSc*
Giovanni Di Perri, MD, PhD†
Massimiliano Lanzafame, MD†
Giovanni Penco, MD‡

*Department of Infectious Diseases, University of Torino, Turin, Italy
†Unit of Infectious Diseases, University of Verona, Verona, Italy
‡Unit of Infectious Diseases, Galliera Hospital, Genova, Italy

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