Soluble Fas in Serum of Patients with HIV/AIDS

To the Editor:

In HIV infection, the mechanism of destruction of the CD4+ T cells is unknown. Apoptosis may play an important role in the pathogenesis of HIV. One apoptotic pathway is mediated through the Fas-Fas ligand pathway. Soluble Fas (sFas) in serum is thought to act as an inhibitor of Fas ligand binding and to block Fas-mediated apoptosis (1). We studied the role of sFas as a marker for CD4 cell destruction.

Sera were obtained from 60 HIV/AIDS patients in Chiang Mai, Thailand and stored at −20 °C until use. Serum sFas was measured by ELISA (Medical & Biological Laboratories) according to the manufacturer’s instructions (2). Briefly, serum or calibrator was incubated in wells coated with anti-Fas monoclonal antibody. After washing, a peroxidase-conjugated anti-Fas monoclonal antibody was added in each microwell and incubated. After another washing, the peroxidase substrate was added. After incubation, acid was added to each well to stop the enzymatic reaction and to stabilize the developed color. The assay is linear between 0.5 and 2.0 μg/L, and the detection limit is 0.5 μg/L.

Absolute CD4+ T-lymphocyte counts were obtained on EDTA blood (Coulter Manual CD4 Count Kit). Briefly, blood was combined with MY4 Cyto-Spheres Monocyte Blocking Reagent, and then CD4 Cyto-Sphere reagent was added. An aliquot of the mixture was added to a lysing reagent to lyse the erythrocytes, and crystal violet was used to stain the nuclear material of the leukocytes. The lymphocytes coated with CD4-coated latex spheres were counted in a hemocytometer chamber. Complete blood counts were obtained with an automated cell counter (Hemacell, DATA Cell 16; Hycel).

The sFas concentration (mean ± 2 SD) in HIV/AIDS patients was not statistically different from reference values (1.22 ± 0.58 vs 0.93 ± 0.6 μg/L; P >0.05) and did not correlate with CD4 and absolute lymphocyte number (Table 1).

sFas could block apoptosis induced by the Fas ligand in vitro. An increased serum concentration of sFas may be associated with autoimmune-like conditions, (e.g., angioimmunoblastic T-cell lymphoma and systemic lupus erythematosus), adult T-cell leukemia, B-non-Hodgkin lymphoma, bladder cancer, hepatocellular cancer, graft-vs-host disease, multiple sclerosis, Graves disease, and AIDS (3–13). The increase in serum sFas does not directly cause autoimmune disease because some healthy elderly individuals had high concentrations of sFas. sFas was correlated with soluble interleukin-2 receptor as well as with cells expressing membrane Fas.

Healthy cells undergo apoptosis as part of the normal process of development and maintenance of complex tissues. In HIV/AIDS patients, Casella and Finkel (1) in 1997 proposed one major pathway of apoptosis that is mediated through the tumor necrosis family receptor Fas. The Tat protein of HIV-1-infected cells increases Fas ligand expression and may up-regulate Fas ligand on uninfected cells. By contrast, Katsikis et al. (4) reported that apoptosis of peripheral blood T cells was Fas-independent in HIV-infected individuals. We conclude that sFas in AIDS patients is not statistically different from reference values and does not correlate with CD4 and absolute lymphocyte counts. sFas detection cannot serve as a marker for CD4 cell destruction.

Table 1. sFas concentrations (mean ± SD) and percentages of HIV/AIDS patients (n = 60) with different sFas concentrations.

<table>
<thead>
<tr>
<th>Cells/μL</th>
<th>sFas, mean ± SD, μg/L</th>
<th>% of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1</td>
<td>1-1.5</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>&lt;1000</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>1000–2000</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>&gt;2000</td>
<td>20</td>
</tr>
<tr>
<td>CD4 cells</td>
<td>&lt;200</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>200–400</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>&gt;400</td>
<td>19</td>
</tr>
</tbody>
</table>

Author for correspondence. Fax 31-433874692; e-mail C.Hackeng@klinchem.azm.nl.

References
5. Dobmeyer TS, Findhammer S, Dobmeyer JM, Klein SA, Raffel B, Hoejler D, et al. Ex vivo induction of apoptosis in lymphocytes is medi-
Comparison of Cardiac Troponin I Measurements on Whole Blood and Plasma on the Stratus CS Analyzer and Comparison with AxSYM

To the Editor:

Assays for cardiac troponin T and troponin I (cTnI) are powerful tools for early identification of patients with acute myocardial infarction (AMI) and for pointing out subjects at highest risk among patients with unstable angina pectoris (1–3). These applications require increasingly rapid and more sensitive tests. To this end, one of the most commonly used automated assays for cTnI (Stratus® II; Dade) has undergone extensive revision (4, 5). A second-generation quantitative radial partition immunoassay is now used in combination with the Stratus CS fluorometric analyzer, a system designed to meet the needs of STAT and point-of-care testing. The Stratus CS yields cTnI results that are clinically consistent with those obtained with the Stratus II (6).

The Stratus CS uses closed routine sample tubes containing anticoagulated whole blood (lithium heparin). The system takes ≤14 min to centrifuge the tube and perform the analysis. The Stratus CS can also manage precentrifuged plasma samples. In the present study, the Stratus CS was used to determine cTnI on whole blood and on preprocessed plasma samples drawn from the same patients. Our purpose was to check the similarity of the results obtained for both samples in the entire measuring range. We also aimed at comparing results from the Stratus CS with cTnI results yielded by the Abbott AxSYM analyzer, with special attention to concentrations below the threshold for AMI.

The Stratus CS is a fluorometric enzyme immunoassay analyzer for quantitative determination of the cardiac markers creatine kinase-MB mass, myoglobin, and cTnI (Dade Behring). The test system uses radial partition immunoassay technology, which has been enhanced through the use of a monoclonal capture antibody coupled to Starburst® dendrimers (7). According to the insert for the cTnI method, coronary risk increases at cTnI concentrations >0.4 μg/L, and this value was considered as the cutoff for AMI in the present study. Reference values are <0.08 μg/L (6). The two monoclonal antibodies used in the method recognize both free and complexed cTnI. The results were compared with those obtained by the AxSYM cTnI microparticle enzyme immunoassay (Abbott Laboratories). This system also allows for automated quantification of cTnI in serum or plasma (lithium heparin) (8, 9). According to the manufacturer’s package insert, cTnI values >0.4 μg/L are increased above the reference values established in blood donors, whereas values >2.0 μg/L are indicative of AMI.

Blood samples were obtained from 85 patients hospitalized at the University Hospital of Liège, Belgium. Twenty-three patients were admitted for suspected AMI; diagnosis was confirmed in 16 cases (14 Q-wave AMI and 2 non-Q-wave AMI), and a myocardial lesion was excluded in 7 cases. Twelve patients had unstable angina pectoris, 9 had stable angina, and 19 had undergone cardiac surgery. In the remaining patients were 5 patients with polytrauma, 1 non-cardiac surgery patient, and 16 chronically hemodialyzed patients.

In this study, we used two different types of samples: one tube (tube I; Hemogard lithium heparin, Vacutainer; Becton Dickinson) to be analyzed without further treatment on the Stratus CS, and one (tube II; Venoject-II lithium heparin + separator; Terumo Europe) to provide the plasma to be analyzed on the two analyzers. A sample was collected into tube II from each of the 85 above-mentioned patients. In a subgroup of 53 patients, tube I was taken in addition to tube II. Both tubes were taken within periods of time not exceeding 2 min. The order in which the tubes were sampled (tube I first or tube II first) was randomized in each category of patients. Tube I was kept at room temperature and analyzed within 2 h. No manual treatment of the tube was required prior to the assay.

cTnI results obtained for whole blood were compared with the corresponding results obtained for plasma aliquots from tube II, also analyzed by the Stratus CS using the special design for plasma samples (comparison A1). For 15 patients, selected at random among the patients with cTnI results <0.15 μg/L on the Stratus CS, tube I was removed from...