Investigation of the Prevalence of Cardiovascular-Associated Cytomegalovirus among Patients with Coronary Artery Disease at Louisiana State University Medical Center at Shreveport

To the Editor—Adler et al. [1] reported that infection with human cytomegalovirus (CMV) is not a major risk factor for angiographically demonstrated coronary artery atherosclerosis. In that report, they found that CMV seropositivity was not associated with coronary artery disease (CAD). We have data that support their studies.

We examined arterial plugs and plaque from patients undergoing cardiovascular surgery at Louisiana State University Medical Center at Shreveport. CMV DNA was assessed by polymerase chain reaction (PCR) using the primers and probes (Genosys Biotechnologies, Houston, TX) described previously [2]. Specimens were collected in sterile tubes containing EDTA (Vacutainer; Becton Dickinson, San Jose, CA) and taken immediately to the PCR laboratory.

All tissues were processed using the Isoquick DNA Extraction Kit (Orca Research, Bothell, WA), following the manufacturer's instructions except that the DNA pellet was rehydrated with 100 μL of RNase/DNase-free water. Unlabeled and biotin-labeled CMV major transforming region (mtrII) primers and probe, with sequences as previously described [2], were synthesized by Genosys Biotechnologies. The CMV master mix contained water, 10× PCR buffer II (Perkin-Elmer, Applied Biosystems, Foster City, CA), 15 nmol of each dNTP (Applied Biosystems), 50 pmol of each primer, and 250 nmol of MgCl2. Tissue DNA (15 μL) was used as the target. The thermocycler was set using a hold cycle of 94°C for 10 min and cooling to 80°C. While the tubes were sitting at 80°C, 5 U of AmpliTaq (Applied Biosystems) was added to each PCR tube. Fifteen cycles were set for 1 min at 94°C, 2 min at 60°C, and 1 min at 72°C, followed by 15 cycles of 1 min at 94°C, 1 min at 60°C, and 2 min at 72°C. The last setting was for 25 cycles of 1 min at 94°C, 2 min at 60°C, and 3 min at 72°C.

Amplicon detection was by gel electrophoresis plus either spectrophotometry or Southern blot. For spectrophotometric detection of CMV amplicons, a custom-made detection kit was used (Synthetic Genetics, San Diego), and the manufacturer's instructions were followed. For Southern blots, a chemiluminescent detection system (Aurora Southern Blot; ICN, Costa Mesa, CA) was used with biotin-labeled probes (Genosys Biotechnologies).

β-globin amplification was performed on all specimens to ensure a sufficient quantity of DNA and the absence of PCR inhibitors. Unlabeled β-globin primers were synthesized by Synthetic Genetics. These primer sequences are as follows: CO4 5′-CAACCTTCATCCACGTTACC-3′; GH5′, 5′-AATAAACCGTACCTACGTGACC-3′. The β-globin master mix was as described for CMV PCR except that it contained 20 nmol of each dNTP and 3.5 U of AmpliTaq (Applied Biosystems). For β-globin amplification, the thermocycler was set using a hold cycle of 94°C for 10 min, followed by 40 cycles of 1 min at 94°C, 1 min at 65°C, and 1 min at 72°C. The last extension step was continued for 10 min. β-globin amplicons were detected by gel electrophoresis.

To date, we have examined specimens from 57 patients. Sixteen were excluded from consideration in our study because they tested PCR-negative for β-globin DNA, indicating either an inadequate amount of DNA or the presence of PCR inhibitors. We detected mtrII DNA of CMV in 6 (14.6%) of 41 patients. Three patients were positive by both gel electrophoresis and spectrophotometric analysis, whereas 3 were positive by spectrophotometry only. No additional positive specimens were detected using gel electrophoresis plus a chemiluminescent Southern blot. Four patients known not to have CAD all tested negative for CMV. Of the 37 patients for whom a diagnosis of CAD was confirmed, 6 (16%) tested positive for CMV. The average age of the CMV-positive group was 61 years and of the CMV-negative group with known CAD, 69 years. Half (3/6) of the CMV-positive group and 58% of the CMV-negative group were white; all of the CMV-positive patients were male.

Our incidence of 16% CMV positivity for patients undergoing surgery for cardiovascular disease falls far below the incidence of 90% reported on a cohort of surgical specimens from patients with atherosclerotic disease by researchers at Baylor College of Medicine. Using the same primer sequences to detect the mtrII region by PCR, Melnick et al. [2] found CMV in 90% of atherosclerotic plaque tissues and uninvolved aortic biopsies collected from 60 patients undergoing vascular surgery. In that study, amplification of the late gene region resulted in only 76% positivity, and an immediate-early primer pair amplified none of the mtrII-positive specimens.

We chose to use the mtrII primers in our study in order to closely adhere to the protocol of the Baylor study. As stated, our positivity rate of 16% falls far below that of the Houston study (90% positivity). However, our data are similar to the reported incidence of CMV DNA in coronary arteries collected from autopsy at the Mayo Clinic [3], in which 21 coronary artery specimens obtained from 11 (22%) of 49 cases tested positive for CMV DNA using two separate pairs of CMV primers, with detection by gel electrophoresis and confirmation by Southern blot. In that latter study [3], no obvious correlation with the presence of CMV and the degree of atherosclerosis was found. It could be argued that the chemiluminescent detection system that we used is less sensitive than the radioactive probe assay used by Melnick et al. in the Houston study [2]; however, inasmuch as we used the same primers and probe sequences, we do not believe that this can explain the large discrepancy in the data (16% vs. 90%).

In summary, we have molecular findings to support the se-
rologic data of Adler et al. [1], suggesting that infection with CMV is not associated with CAD.

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**References**


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### Pentoxifylline in Human Immunodeficiency Virus–Positive Tuberculosis: Safety at 4 Years

**To the Editor**—Two years ago, we reported in this Journal a randomized clinical trial of the tumor necrosis factor-α (TNF-α) inhibitor, pentoxifylline, as adjunctive therapy in human immunodeficiency virus type 1 (HIV-1)-infected persons with pulmonary tuberculosis (TB) [1]. The study found that pentoxifylline therapy resulted in decreased plasma HIV-1 RNA and β₂-microglobulin and, in a subset of moderately anemic subjects, improved blood hemoglobin. At the time the study was planned, concern was raised that, given the role of TNF-α in immune defenses against Mycobacterium tuberculosis, inhibition of TNF-α might lead to increased rates of treatment failure or relapse. The report, reflecting 1.5 years of follow-up, did not completely resolve this question, as it noted trends toward increased adverse outcomes in TB therapy in the pentoxifylline arm (7 vs. 2 controls) that did not reach statistical significance.

We recently completed a second analysis of outcomes in this cohort, after nearly 4 years of follow-up. Of the original study subjects, 91% could be included in this analysis; the rest were lost to follow-up. As expected, the number of adverse events has increased significantly with time. However, the trend toward increased adverse events in the pentoxifylline arm has not continued. As indicated in table 1 above, TB adverse events and total deaths do not differ in the two treatment arms. The sample size is sufficient to identify with 80% certainty a 2.8-fold increase in the rate of TB adverse events—less than that observed in the original report.

This represents the longest period of observation of HIV-1–infected subjects with active opportunistic infections treated with a TNF-α inhibitor. It indicates that such treatment does not increase the risks of relapse, other opportunistic infections, or death. However, the extent of the reduction in TNF-α observed with pentoxifylline is modest, as is that for plasma HIV RNA. It is therefore appropriate that future studies of other, more highly active TNF-α inhibitors should similarly evaluate both short- and long-term safety with respect to potential infectious complications of such therapy.


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**Table 1. Outcome measures 4 years after pentoxifylline treatment in HIV/TB patients.**

<table>
<thead>
<tr>
<th></th>
<th>Pentoxifylline (n = 50)</th>
<th>Placebo (n = 47)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment failure</td>
<td>4 (8.0)</td>
<td>3 (6.4)</td>
<td>1.00</td>
</tr>
<tr>
<td>Relapse</td>
<td>5 (10.0)</td>
<td>4 (8.5)</td>
<td>1.00</td>
</tr>
<tr>
<td>TB death</td>
<td>3 (6.0)</td>
<td>1 (2.1)</td>
<td>0.62</td>
</tr>
<tr>
<td>All TB adverse events</td>
<td>11 (22.0)</td>
<td>7 (14.9)</td>
<td>0.44</td>
</tr>
<tr>
<td>All deaths</td>
<td>5 (10.0)</td>
<td>5 (10.6)</td>
<td>1.00</td>
</tr>
</tbody>
</table>

NOTE. Treatment failures had cultures positive for *Mycobacterium tuberculosis* on multiple occasions after 4th month of therapy. Patients with relapse had positive cultures accompanied by clinical evidence of active disease after completion of therapy. All deaths during first 2 months of therapy were attributed to TB. Subsequently, deaths were attributed to TB only if they occurred in setting of worsened clinical disease without other identifiable cause. Significance was determined by 2-tailed Fisher’s exact test.

References


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