To the Editor—Boivin et al. [1] compared the diagnostic value of quantitative polymerase chain reaction (PCR) using polymorphonuclear leukocytes (PMNL) with qualitative plasma PCR to monitor human immunodeficiency virus–infected individuals at risk for cytomegalovirus (CMV) disease. Symptomatic patients had significantly higher CMV DNA copies in both leukocytes and plasma than asymptomatic individuals, but plasma PCR was far less sensitive than leukocyte PCR due to significantly higher CMV DNA load in leukocytes. Although the specificity and positive predictive value were much lower for a positive leukocyte PCR versus plasma PCR result, this problem was overcome by defining a cutoff value for the CMV DNA load in leukocytes. The authors concluded from their data that quantitative leukocyte PCR was superior to plasma PCR.

We would like to comment on three of the issues raised in the discussion. First, Boivin et al. [1] see a discrepancy between their own observations and those reported by others, who were unable to define a reliable cutoff value for leukocyte PCR. However, we previously showed for renal allograft recipients [2] that CMV DNA load in total peripheral blood leukocytes (PBL) of ≥10,000 copies of CMV/10^7 copies of β-globin DNA (the theoretical average DNA yield from ∼1 mL of whole blood) is highly indicative of clinically manifest CMV infection. This is almost the same as the cutoff of 16,000/mL described by Boivin et al. [1]. Furthermore, we observed similar sensitivity, specificity, and positive and negative predictive values (100%, 94%, 83%, and 100%, respectively). These similarities are all the more remarkable because we investigated a different patient clientele and also used a completely different quantification method based on competitive PCR [3].

Second, Boivin et al. [1] hint that reliable performance of quantitative PCR can be hazardous. In our experience with leukocyte PCR, the DNA extraction procedure is the most crucial step. We regard the parallel quantitation of cellular DNA sequences and CMV genomes as indispensable to assess the efficiency of DNA extraction, since we have observed high interassay variability of total DNA yield from leukocyte samples [3].

Finally, due to the technical demands of quantitative PCR procedures, we find it desirable to improve the diagnostic value of qualitative CMV PCR. Since it is generally accepted that leukocyte PCR is more sensitive and usually becomes positive earlier than plasma PCR, the challenge is to improve the inferior specificity of leukocyte PCR by selective detection of CMV DNA originating from active infection. CMV DNA is found predominantly in PMNL during active infection [4], whereas latently CMV-infected cells belong mostly to the peripheral blood mononuclear cell (PBMC) population [5]. PCR analysis of purified PMNL is therefore the most promising strategy. However, the dextran sedimentation method as used by Boivin et al. [1] and many others for isolating “PMNL” in reality yields leukocyte mixtures containing up to 30% PBMC. This might be a reason for the low specificity of leukocyte PCR. In contrast, using ficoll-metrizoate density centrifugation, which yields ~95%-pure PMNL and PBMC fractions, we recently found [6] that with leukocyte samples of 35 CMV IgG-positive kidney transplant patients with no laboratory evidence for active CMV infection, only 6% of PMNL but 66% of PBMC fractions were PCR-positive.

To assess the diagnostic feasibility of our approach, we performed qualitative PCR from total PBL, ficoll-purified PMNL, and PBMC, and plasma samples from 96 renal allograft recipients to diagnose (1) active CMV infections (n = 37) as defined by pp65 antigenemia [7] or (2) symptomatic CMV infections (n = 19) as defined by previously described criteria [8] (table 1). Active as well as symptomatic CMV infections were detected in the PMNL fraction with maximal sensitivity and similar specificity compared with plasma. In contrast, the vast majority of unwanted positive results in leukocytes was attributable to the PBMC. These results suggest that qualitative CMV PCR using ficoll-purified PMNL is a true alternative to plasma PCR and to quantitative leukocyte PCR for monitoring patients at risk for CMV infections.

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References
To the Editor—We appreciate the interesting comments of Schäfer et al. [1] regarding our manuscript describing the utility of quantitative and qualitative polyme
erase chain reaction (PCR) tests using leukocytes or plasma for the diagnosis of cytomegalovirus (CMV) disease in human immunodeficiency virus-infected subjects [2]. First, we are pleased to see that Schäfer and colleagues previously found a similar cutoff value for their leukocyte PCR in a totally different target population, namely, renal allograft recipients [3]. This result is not totally unexpected, since we have previously reported a similar CMV DNA load in leukocyte populations of AIDS patients and a mixed group of solid organ transplant recipients with established CMV disease [4]. In contrast, bone marrow transplant recipients with CMV disease had a much lower viral DNA burden in their leukocytes.

We agree with the comments made by Schäfer et al. [1] regarding the potential high interassay variability of quantitative PCR tests and, consequently, the difficulty in comparing CMV virus load results generated from different research laboratories. This problem has become even more crucial since the adoption of preemptive protocols in many transplant centers on the basis of the measure of the circulating CMV virus load. In that regard, PCR tests should be highly reproducible but also not too complex. It is hoped that the availability of commercial quantitative PCR kits (such as the Amplicor Monitor CMV test; Roche Molecular Systems, Branchburg, NJ) should be a basis for future interpretation of the results in different clinical settings.

Finally, Schäfer et al. [1] present data suggesting higher specificity and positive predictive values associated with a qualitative CMV PCR test using ficoll-purified polymorphonuclear leukocytes (PMNL) rather than mixed peripheral blood leukocytes for the diagnosis of active or symptomatic CMV infections. In a recent paper [5], these authors have suggested that peripheral blood mononuclear cells (PBMC) were the main reason for the low specificity attributable to a qualitative PCR assay using leukocytes. In that study, PBMC harbored equally low CMV DNA levels both in patients with active infections and in those with latent infections with no decline of the CMV DNA load observed in these cells during antiviral therapy.

However, those results are different from those reported by us [4] and others [6] using quantitative-competitive PCR and blot hybridization, respectively, from ficoll-purified leukocyte populations. In the latter studies, the largest amounts of viral DNA were generally found in the PMNL fractions of patients with visceral CMV disease. However, CMV DNA levels in PBMC were significantly higher in patients with disease compared with asymptomatic viremic subjects. Furthermore, a 10-day course of intravenous ganciclovir induced a significant decrease in the number of CMV copies in PMNL and PBMC of subjects with AIDS.

Thus, we feel that additional comparative studies are needed to resolve this issue. Importantly, leukocyte populations should be purified using a fluorescence-activated cell sorter to avoid contamination of the cell fractions and aberrant PCR results.

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References

Table 1. Utility of leukocyte fractions and plasma for monitoring CMV infections in renal allograft recipients.

<table>
<thead>
<tr>
<th>Differential diagnosis, PCR assay</th>
<th>Se (%)</th>
<th>Sp (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active infection vs. no active infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total PBL</td>
<td>100</td>
<td>52.5</td>
<td>56.9</td>
<td>100</td>
</tr>
<tr>
<td>PBMC</td>
<td>100</td>
<td>55.9</td>
<td>58.7</td>
<td>100</td>
</tr>
<tr>
<td>PMNL</td>
<td>100</td>
<td>94.9</td>
<td>92.3</td>
<td>100</td>
</tr>
<tr>
<td>Plasma</td>
<td>73.0</td>
<td>96.6</td>
<td>93.1</td>
<td>85.1</td>
</tr>
<tr>
<td>Symptomatic infection vs. no symptomatic infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total PBL</td>
<td>100</td>
<td>40.3</td>
<td>29.2</td>
<td>100</td>
</tr>
<tr>
<td>PBMC</td>
<td>100</td>
<td>42.9</td>
<td>30.2</td>
<td>100</td>
</tr>
<tr>
<td>PMNL</td>
<td>100</td>
<td>72.7</td>
<td>47.5</td>
<td>100</td>
</tr>
<tr>
<td>Plasma</td>
<td>78.9</td>
<td>81.8</td>
<td>51.7</td>
<td>94.0</td>
</tr>
</tbody>
</table>

NOTE. Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; PBL, peripheral blood leukocytes; PBMC, peripheral blood mononuclear cells; PMNL, polymorphonuclear leukocytes. In each PCR reaction, equivalents of 10⁵ total PBL or 10 µL of plasma were analyzed.


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