To the Editor—Carpentier et al. [1] recently reported that combined determination of early-antigen (EA) IgG titers and of Epstein-Barr virus (EBV) DNA loads in peripheral-blood mononuclear cells (PBMCs) could be useful as prognosis markers to evaluate the risk of development of posttransplantation lymphoproliferative disease (PTLD). The incidence of EBV-related PTLD has been reported to vary from 1% to 33%, with a mortality rate as high as 25%–45% in pediatric recipients of organ transplants [2, 3]. The detection of a high EBV DNA load in blood usually precedes PTLD, but not all transplant recipients with a high EBV DNA load will develop PTLD. Various EBV DNA-load threshold values above which the risk for PTLD increases have been described by teams using different quantitative techniques and studying different transplant-recipient populations [4–7]. However, the specificity of these threshold values for the assessment of the risk of development of PTLD remains low. The description of an additional marker for the prediction of EBV-related PTLD is needed, and therefore Carpentier et al.’s data are interesting.

In our center, we follow children who receive kidney, liver, small-bowel, and heart transplants. For PTLD screening, an in-house semiquantitative DNA polymerase chain reaction (PCR) was performed on PBMCs between 1994 and 2002, whereas an in-house real-time PCR has been performed on whole blood since 2002. Testing for EBV serologic markers, including that used to test for EA IgG (ETI-EA-G; DiaSorin), has also regularly been performed in the follow-up of these chil-

Table 1. Prognostic value of testing for early antigen (EA) IgG, in 19 pediatric solid organ–transplant recipients with high Epstein-Barr virus (EBV) DNA loads in blood.

<table>
<thead>
<tr>
<th>Status group, patient</th>
<th>Organ(s) transplanted</th>
<th>Time between transplantation and first high EBV DNA load by PCR, weeks</th>
<th>EBV DNA load, copies/mL of whole blood</th>
<th>Time between first high EBV DNA load and appearance of EA IgG, weeks</th>
<th>PTLD treatment [result]</th>
</tr>
</thead>
<tbody>
<tr>
<td>With PTLD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBV primary infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Liver, small bowel</td>
<td>6</td>
<td>NA</td>
<td>Absent</td>
<td>Reduction in IS [dead at week 10]</td>
</tr>
<tr>
<td>2</td>
<td>Liver, kidney</td>
<td>5</td>
<td>NA</td>
<td>255</td>
<td>Reduction in IS</td>
</tr>
<tr>
<td>3</td>
<td>Liver</td>
<td>3</td>
<td>NA</td>
<td>53</td>
<td>Reduction in IS</td>
</tr>
<tr>
<td>4</td>
<td>Liver, small bowel</td>
<td>3</td>
<td>NA</td>
<td>Absent</td>
<td>Reduction in IS [dead at week 4]</td>
</tr>
<tr>
<td>5</td>
<td>Liver</td>
<td>184</td>
<td>NA</td>
<td>200</td>
<td>Anti-CD20 (4 times)</td>
</tr>
<tr>
<td>6</td>
<td>Heart</td>
<td>224</td>
<td>1,000,000</td>
<td>0</td>
<td>Chemotherapy</td>
</tr>
<tr>
<td>7</td>
<td>Lung</td>
<td>9</td>
<td>100,000</td>
<td>Absent (after week 89)</td>
<td>Anti-CD20 (5 times), chemotherapy</td>
</tr>
<tr>
<td>EBV reactivation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Liver</td>
<td>24</td>
<td>NA</td>
<td>58</td>
<td>Reduction in IS</td>
</tr>
<tr>
<td>9</td>
<td>Liver, small bowel</td>
<td>3</td>
<td>NA</td>
<td>56</td>
<td>Anti-CD20 (3 times)</td>
</tr>
<tr>
<td>10</td>
<td>Liver, small bowel</td>
<td>19</td>
<td>250,000</td>
<td>Absent (after week 66)</td>
<td>Anti-CD20 (6 times)</td>
</tr>
<tr>
<td>Without PTLD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBV primary infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Liver</td>
<td>45</td>
<td>50,000</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Liver, small bowel</td>
<td>7</td>
<td>14,000</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Small bowel</td>
<td>3</td>
<td>100,000</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Kidney</td>
<td>45</td>
<td>35,000</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Kidney</td>
<td>32</td>
<td>40,000</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>EBV reactivation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Kidney</td>
<td>90</td>
<td>10,000</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Liver, small bowel</td>
<td>3</td>
<td>100,000</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>Heart</td>
<td>2</td>
<td>210,000</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>Kidney</td>
<td>32</td>
<td>10,000</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. EA, early antigen; IS, immunosuppression; NA, quantitative results not available but high viral load observed by semiquantitative method; PTLD, posttransplantation lymphoproliferative disease.
dren. Beginning with cases from 1995, we retrospectively searched our database to identify transplant-recipient children who had high EBV DNA loads (defined as either >1000 genome copies/10⁶ PBMCs or >10,000 copies/mL of whole blood) and who regularly had been tested for EA IgG. Thirty-nine children fulfilled these criteria, and 19 were selected for this study because follow-up was complete enough to allow the results to be reliably interpreted; the results for these 19 children are presented in table 1.

EBV serologic status before transplantation was negative in 12 of the 19 children and was positive in the other 7. PTLD occurred in 10 children—after primary infection in 7 cases and after EBV reactivation in 3 cases; in 9 of these 10 children, the results of tests for EA IgG were negative at the time of PTLD; in 4 of these 9 children, the results remained negative during the entire follow-up, and the appearance of EA IgG was delayed (53–255 weeks) in the other 5. One child with PTLD (patient 6) had a high level of EA IgG concomitantly with both the first high positive result of PCR and the occurrence of PTLD and before she received any immunoglobulins intravenously. It is worth noting that this child had an uncommon PTLD presentation, with proliferation of T cell lineages in the skin and adenoids [8, 9]. In 6 of the 9 children who did not develop PTLD, the results of tests for EA IgG were positive concomitantly with the first high EBV DNA load, and they were positive 3–53 weeks after the first high EBV DNA load in the other 3. Therefore, with regard to the development of PTLD, the results in our series of transplant-recipient patients show that a high EBV DNA load concomitant with negative results of tests for EA IgG has a positive predictive value of 75% and a negative predictive value of 86%.

Therefore, our results partly confirm those of Carpentier et al., in that testing for EA IgG, in association with quantification of EBV DNA loads in blood, may allow more-accurate screening of patients at risk for PTLD. However, difficulties will remain: first, false-positive results of tests for EA IgG could occur in transplant-recipient patients treated with immunoglobulins intravenously, and this possibility must be evaluated; second, results of tests for EA IgG might also be correctly positive in some children with uncommon PTLD with proliferation of T cell lineages; and, finally, in pretransplant seronegative children, a negative result of a test for EA IgG lacks specificity in the prediction of PTLD, particularly when the latter occurs early after transplantation. Indeed, in these cases, the first positive result of a test for EBV PCR is often detected within the first 3 weeks after transplantation of an EBV-positive organ, and the EBV DNA load in blood is often high, whereas the results of tests for EA IgG are always negative: in our study, for example, 7 children with primary EBV infection (patients 1–4, 7, 12, and 13) had high EBV DNA loads early after transplantation and concomitantly negative results of tests for EA IgG. It was impossible, at this early stage of the infection, to distinguish between the 5 children who rapidly developed PTLD and the 2 who did not.

In conclusion, testing for EA IgG will probably help in the interpretation of high EBV DNA loads in pediatric recipients of organ transplants; however, the predictive value of these 2 tests done simultaneously must be analyzed prospectively.

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References

Reply to Leruez-Ville et al.

To the Editor—Although our study used a small study cohort, my colleagues and I had hoped that, in publishing our initial findings, we might encourage other investigators to expand available data and, hence, to support or disprove our assertion that a high peripheral-blood Epstein-Barr virus (EBV) DNA load coupled with a second marker—namely, an absent or
weak EBV early-antigen (EA) serologic re-
response—has prognostic value for post-
transplantation lymphoproliferative dis-
ease (PTLD). The data provided by Lerue-
z-Ville et al. [1] largely agree with the
results of our recently published study
[2]. After careful analysis of their results,
we note that Leruez-Ville et al. have
raised several concerns with regard to the
use of the interpretation of EA serology
as a second marker in the diagnosis of
PTLD.

Leruez-Ville et al. point out that, in
their study cohort of 19 patients, 1 was
diagnosed with a rare T cell tumor of the
skin. This patient showed strong IgG
seroreactivity to EA, concurrent with a
high EB viral load, an observation that
runs counter to our claim, indicating a
possible lack of correlation between these
2 viral markers in atypical presentations
of PTLD. It is noteworthy, however, that Le-
ruez-Ville et al.’s findings for the B cell
PTLDs in their cohort are in line with
our results.

Another concern raised by Leruez-Ville
et al. is the possibility of false-positive EA
serotiters after passive transfer of EA IgG
antibodies to patients treated with intra-
venous immune globulin (IVIG) for cy-
tomegalovirus prophylaxis in cases of
mismatched donor/recipient pairs. We
had considered this possibility and con-
sequently sought to test patient sera after
infusion of IVIG, but we found no titers
with detectable EA IgG. Moreover, we ex-
amined the timing of the appearance of
EA IgG in the 3 patients (i.e., patients 7,
9, and 10 in [2, table 2]) in our study
cohort who had been treated with IVIG.
Surprisingly, in the development of sig-
nificant titers of EA IgG, these patients
showed a mean additional delay of 24.8
weeks, compared with the 11 patients not
treated with IVIG.

Leruez-Ville et al. also observe that their
EBV-negative patients infected during the
early posttransplantation period (i.e., 0–
10 weeks) showed high EB viral loads but
no detectable EA IgG. Seven of their pa-
ients—namely, patients 1–4, 7, 12, and
13—belong to this category, and 5 of these
7 developed PTLD. After analysis based
on the viral-load and EA-erosology tests,
Leruez-Ville et al. note that the 2 patients
who did not develop PTLD could not be
distinguished from the 5 who did develop
PTLD, and they therefore conclude that
absence of EA IgG in patients with a high
viral load is not an absolutely reliable pre-
dictor of the occurrence of PTLD. On
their point that the use of the EA-eroso-
gy test as a second marker has limitations,
we agree with Leruez-Ville et al. None-
theless, the salient message of both studies
is that the positive predictive value of the
EA-erosology test used in conjunction with
the viral-load test, although not 100%, is
still considerably higher than that of the
viral-load test alone.

Last, Leruez-Ville et al. call for a pro-
spective study to further analyze the pre-
dictive value of EA serology in patients
with a high viral load. Scientifically, we
concur; however, we feel that it would be
more practical to perform retrospective
studies. Large serum banks from major
transplantation centers should allow for
ready analysis of viral load and EA sero-
logy of transplant-recipient patients with
and without PTLD. Such an undertaking
would add some much-needed statistical
power to the existing data, thereby clari-
fying the importance of EA serology as a
second marker for the prognosis of PTLD.

Caroline Alfieri
Sainte-Justine Hospital, Research Center,
Montreal, Canada

Reference
1. Leruez-Ville M, Talbotec C, Iserin F, Salomon
R, Lacaille F, Rouzioux C. Epstein-Barr virus
(EBV) early-antigen antibodies and EBV DNA
load in blood, in posttransplantation lympho-
proliferative disease. J Infect Dis 2004; 190:
1524–5 (in this issue).
2. Carpentier L, Tapiero B, Alvarez F, Viau C, Al-
fieri C. Epstein-Barr virus (EBV) early-antigen
serologic testing in conjunction with peripheral
blood EBV DNA load as a marker for risk of
posttransplantation lymphoproliferative disease.
J Infect Dis 2003; 188:1853–64.