Human cytomegalovirus (HCMV) end-organ disease in solid-organ transplant recipients (SOTR) may be associated with either high or low HCMV load in blood. In transplantation Centers where the preemptive therapy approach is adopted, antiviral therapy of systemic HCMV infections is initiated upon reaching pre-determined cut-off levels of viral DNA in blood, whereas no guidelines are provided for local end-organ infection/disease. In the latter case, clinicians often start antiviral treatment without defining the etiology of local symptoms. Here, we describe 14 cases of SOTR, in which a documented HCMV end-organ disease was observed. Nine patients had a systemic viral load lower than the cut-off for preemptive therapy and were treated based on viral load of local HCMV disease. The remaining five patients had a systemic viral load greater than the preemptive therapy cut-off and were efficiently treated for both the systemic and the local HCMV disease. Thus, HCMV infection in the post-transplant period must be monitored virologically both in blood and locally. End-organ disease in preemptively treated patients, seems to be associated with lack of development (primary HCMV infection) or reconstitution (reactivated infection) of HCMV-specific CD4+ and CD8+ T-cell immunity or with its functional impairment.

**KEY WORDS**: HCMV viral load, Solid organ transplant recipients, HCMV systemic infections, HCMV end-organ disease.

**INTRODUCTION**

In the post-transplant period of solid-organ transplant recipients (SOTR), human cytomegalovirus (HCMV) infection may be systemic or localized (or both). In either case, the infection may be asymptomatic, or evolve towards HCMV disease.
impedes reliable evaluation of the overall organ involvement. In addition, routine organ sampling is unfeasible, due to the invasive procedures required. This is why most clinicians are often prone to treat transplanted patients with local symptoms with HCMV-specific antiviral treatment in the absence of a documented viral diagnosis. These problems could apparently be overcome in lung transplant recipients, where lung HCMV infection may be monitored in bronchoalveolar lavage (BAL) samples. This approach is used to guide preemptive therapy of lung infection/disease (Gerna et al., 2009). However, also in this case lung biopsy is the only approach defining or resolving the diagnostic issue.

This paper documents the high variability of the relationship between systemic HCMV load in peripheral blood and organ (local) viral load (in tissue biopsies or organ fluids), whenever end-organ disease is diagnosed. Treating organ localization is not problematic when the patient reaches the established viral load cut-off for initiating preemptive therapy during systemic infection. However, when viral load in blood is below the established cut-off, anti-HCMV therapy for organ localization should be initiated, following the appearance of clinical symptoms, only based on documented viral diagnosis.

PATIENTS AND METHODS

Patients
In a group of more than 500 patients receiving heart, lung, heart-lung, or kidney transplantation at the Transplantation Center of the Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, Pavia, Italy, in the period 2003-2009, 14 patients had a virologically diagnosed end-organ disease at different times after transplantation. Along with HCMV organ localization, all these patients also presented with a systemic HCMV infection associated with different levels of HCMV viral load in blood. Immunosuppressive regimens were administered as previously reported (Grossi et al., 1995). At our center prevention of HCMV disease was based on the preemptive therapy approach, i.e. antiviral treatment of HCMV infection was initiated upon reaching predetermined cut-off levels of pp65-antigenemia until 2007 (Grossi et al., 1995) or HCMV DNA copies/ml blood thereafter (Gerna et al., 2007). On this basis, we retrospectively divided patients in this study into two groups: one had viral load in blood lower than the established cut-off for preemptive therapy, and the other showed a systemic viral load greater than the established cut-off. As a result, in the first group, antiviral treatment for organ localization was initiated independently of the systemic viral load cut-off, whereas in the second group, treatment of local organ involvement was concomitant with treatment for systemic infection.

Diagnosis of HCMV infection and preemptive therapy
HCMV asymptomatic and symptomatic systemic infections/disease were defined as reported (Ljungman et al., 2002). Diagnosis of HCMV infection was performed by simultaneous determination of HCMV pp65-antigenemia (Gerna et al., 1992), and DNAemia (Gerna et al., 2006). According to cut-offs established, until 2006 antigenemia-guided preemptive therapy started treatment of primary HCMV infections upon first confirmed appearance of virus in blood, and reactivated HCMV infections when level of virus in blood reached 100 pp65-positive leukocytes/2x10^5 leukocytes examined (Gerna et al., 2007). Starting from 2007, 300,000 HCMV DNA copies/ml blood were used as a cut-off for initiating antiviral treatment of both primary and reactivated systemic HCMV infections of all types of SOT (Grossi et al., 1995).

In lung transplant recipients, HCMV infection was also monitored in BAL, and preemptive therapy was initiated in the presence of 100,000 DNA copies/ml BAL (Gerna et al., 2009). Ganciclovir (GCV, 5 mg/kg b.i.d.) was used as a first line treatment, and it was replaced by foscarnet (PFA, 90 mg/kg b.i.d.) in case of GCV myelotoxicity or drug inefficacy or resistance. HCMV load was determined once a week until detection of HCMV infection, thereafter testing was performed twice a week until confirmed virus disappearance from blood.

End-organ disease diagnosis and treatment duration
End-organ disease was diagnosed as reported (Kotton et al., 2010). Immunohistochemical HCMV detection in tissue biopsies was achieved
by pre-treating paraffin-embedded tissue sections with heat-induced epitope retrieval and then staining sections with a mixture of two murine mAbs directed to p52 (Plachter et al., 1992) and the major immediate-early (IE) UL123 gene product (Wirgart, 1991). According to the different types of sample, determination of viral load in end-organ disease was performed as follows. In case of gastrointestinal (GI, esophageal, gastric, enteric) biopsy, viral load was expressed as copy number/1x10^5 cells. Cell number was determined by amplifying a human single-copy housekeeping gene (Watzinger et al., 2004). When multiple biopsy tissue samples were available, extracted DNA from different samples was combined to yield a unique DNA sample. In case of interstitial pneumonia, HCMV load was determined per ml BAL fluid (Gerna et al., 2009). In the presence of HCMV retinitis, HCMV load was determined per ml aqueous humor, as previously reported (Gerna et al., 1994). HCMV end-organ disease was treated with antiviral therapy on the basis of instrumental findings of organ pathology in association with immunohistochemical and/or virologic documentation of HCMV organ infection (at any viral load level). Discontinuation of antiviral treatment for end-organ disease was based, whenever possible, on disappearance of virus from BAL or organ biopsies in association with resolution of clinical symptoms and endoscopic (or opthalmoscopic) lesions.

**HCMV-specific T-cell immune response evaluation**

Evaluation of HCMV-specific T-cell response at time of diagnosis of end-organ disease was done by a previously established assay based on PBMC stimulation by autologous HCMV-infected dendritic cells (Gerna et al., 2011).

**RESULTS**

Fourteen cases of HCMV end-organ disease were examined. Of these, 9 initiated antiviral treatment based on diagnosis of end-organ disease, i.e. prior to reaching the cut-off in blood for preemptive therapy, whereas in 5 patients end-organ disease was diagnosed concomitantly with detection of viral load in blood above the cut-off for pre-emptive therapy. Four patients had primary infection (two per each group), while all the other patients had reactivated infections.

**SOT recipients with end-organ disease and viral load below the cut-off for pre-emptive therapy**

The clinical and virologic findings relevant to the nine SOT recipients with HCMV end-organ disease diagnosed when viral load in blood was below the established cut-off for pre-emptive therapy are reported in Table 1. In this group of patients, end-organ disease was diagnosed at a median time of 62 (range 32-1385) days after transplantation. Five patients (three heart and two kidney transplant recipients) had HCMV gastrointestinal (GI) disease (4 with gastritis and one with colitis, as documented by both endoscopy and immunohistochemistry), three had HCMV interstitial pneumonia (Figure 1A, pt #7), and one HCMV retinitis. Organ viral load reached a median value of 4.0x10^4 (range 6x10^1-3.0x10^7) DNA copies/1x10^5 cells in the 5 patients with GID. In all these patients, diagnosis was established by endoscopy, and in 2/5 pts. (# 2 and 4) it was confirmed by immune histochemistry. Ganciclovir treatment, initiated concomitantly with first viral DNA detection in GI biopsies, had a median duration of 14 (range 14-35) days, and, by definition, was associated with low viral load in blood (median 2.2x10^4, range 1.2x10^3-3.9x10^4 DNA copies/ml blood). Treatment was successful in all patients, as shown by the disappearance of clinical symptoms and/or endoscopic resolution of mucosal lesions, in 4 patients in association with virologic documentation of viral DNA disappearance in the biopsy tissue (pt. #3). In the three patients (one lung, one heart-lung, and one kidney transplant recipient) with radiological diagnosis of interstitial pneumonia, HCMV load in BAL samples ranged from 5.3x10^5 to 2.7x10^6/ml BAL, and in blood from 5.4x10^3 to 6.4x10^4/ml. Diagnosis was supported by immunohistochemistry of lung biopsies in two of these patients (pts.# 6 and 7), while in the third patient (# 8) only virus in BAL was detected at an high level concomitantly with CT signs of interstitial pneumonia. However, antiviral treatment of different duration (12-67 days) was able to resolve lung infection in all three patients, as shown by clinical, radiological and virological findings. Finally, patient #9 had a severe hemorrhagic
HCMV retinitis 4 years after heart transplantation with a HCMV load in aqueous humor (4.2 x 10^5 DNA copies/ml) about one log_{10} higher than in blood. A prolonged antiviral treatment with VGCV in association with bilateral vitrectomy and immune suppression reduction failed to prevent a severe vision impairment.

**SOT recipients with end-organ disease and viral load above the cut-off for pre-emptive therapy**

Table 2 summarizes the clinical and virologic findings relevant to the five patients in whom organ localization was diagnosed concomitantly with the presence of viral load in blood above the

### Table 1 - Clinical and virologic findings relevant to HCMV organ localization in SOT recipients with viral load in blood lower than the established cut-off for pre-emptive therapy (3x10^5 DNA copies/ml blood).

<table>
<thead>
<tr>
<th>Pt. #, sex, Type of tx</th>
<th>Days post-tx</th>
<th>HCMV disease</th>
<th>Local/systemic HCMV load</th>
<th>Organ pathology</th>
<th>Antiviral therapy (day duration)</th>
<th>Criteria for antiviral drug discontinuation</th>
<th>Outcome of HCMV disease</th>
<th>Type of HCMV-specific infection</th>
<th>HCMV-specific T-cells CD4&lt;sup&gt;+&lt;/sup&gt; CD8&lt;sup&gt;+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. M, 51 Heart</td>
<td>144</td>
<td>gastritis</td>
<td>4.0x10^4 (bps)/ 2.1x10^5</td>
<td>EGDS: gastritis (IHC neg)</td>
<td>GCV (14)</td>
<td>Clinical+EGDS</td>
<td>Resolved</td>
<td>R</td>
<td>ND</td>
</tr>
<tr>
<td>2. M, 50 Heart</td>
<td>519</td>
<td>gastritis</td>
<td>3.0x10^5 (bps)/ 2.5x10^4</td>
<td>EGDS: gastritis (IHC pos)</td>
<td>GCV (14)</td>
<td>Clinical+EGDS</td>
<td>Resolved</td>
<td>P</td>
<td>+</td>
</tr>
<tr>
<td>3. M, 23 Heart</td>
<td>35</td>
<td>gastroenteritis</td>
<td>3.0x10^7 (bps)/ 3.9x10^4</td>
<td>EGDS: gastric and enteric lesions</td>
<td>GCV(28)</td>
<td>HCMV DNA negative in blood and biopsy</td>
<td>Resolved</td>
<td>P</td>
<td>-</td>
</tr>
<tr>
<td>4. M, 24 Kidney</td>
<td>33</td>
<td>gastroenteritis</td>
<td>5.8x10^4 (bps)/ 2.2x10^4</td>
<td>EGDS: multiple mucosal lesions (IHC pos)</td>
<td>GCV (35)</td>
<td>Clinical</td>
<td>Resolved</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>5. M, 60 Kidney</td>
<td>62</td>
<td>enterocolitis</td>
<td>6.0x10^4 (bps)/ 1.2x10^3</td>
<td>Colonoscopy: aphthous ileal lesions</td>
<td>GCV (14)</td>
<td>Clinical</td>
<td>Resolved</td>
<td>R</td>
<td>ND</td>
</tr>
<tr>
<td>6. M, 26 Lung</td>
<td>44</td>
<td>pneumonia</td>
<td>5.3x10^5 (BAL)/ 4.3x10^4</td>
<td>Interstitial pneumonia (x-ray, IHC pos)</td>
<td>GCV (21)</td>
<td>Clinical+x-ray (HCMV DNA negative in blood)</td>
<td>Resolved</td>
<td>R</td>
<td>ND</td>
</tr>
<tr>
<td>7. F, 29 Heart-lung</td>
<td>32</td>
<td>pneumonia</td>
<td>9.9x10^5 (BAL)/ 6.4x10^4</td>
<td>Pneumonia (IHC pos)</td>
<td>GCV (12)</td>
<td>HCMV DNA negative in blood</td>
<td>Resolved</td>
<td>R</td>
<td>ND</td>
</tr>
<tr>
<td>8. M, 50 Kidney</td>
<td>346</td>
<td>pneumonia</td>
<td>2.7x10^6 (BAL)/ 5.4x10^5</td>
<td>CT: interstitial pneumonia</td>
<td>VGCV (67)</td>
<td>HCMV DNA negative in BAL</td>
<td>Resolved</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>9. M, 53 Heart</td>
<td>1,385</td>
<td>retinitis</td>
<td>4.2x10^6 (AH)/ 3.2x10^6</td>
<td>Ophthalmoscopy: hemorrhagic retinitis</td>
<td>VGCV (76 d induction, then maintenance)</td>
<td>Clinical+fundus examination</td>
<td>Severe vision impairment</td>
<td>R</td>
<td>-</td>
</tr>
</tbody>
</table>

AH, aqueous humor; ATG, anti-thymocyte globulin; BAL, bronchoalveolar lavage fluid; bps, GI biopsy; CT, computerized tomography; EGDS, esophago-gastro-duodenoscopy; EMB, endomyocardial biopsy; GCV, ganciclovir; IHC, immunohistochemistry; IS, immunosuppression; ND, not done; P, primary infection; PFA, phosphonoformic acid; R, reactivated infection; VGCV, valganciclovir; -, negative T-cell response; +, positive T-cell response.
FIGURE 1 - Immunohistochemical detection (arrows) of HCMV-infected (A) alveolar epithelial cells in a transbronchial biopsy (pt #7), and (B) endothelial cells in a cardiac/esophageal biopsy (pt #10). (C). The lower part of the figure shows the low-power view of three sections of endomyocardial biopsy of pt #14, while the high-power view of the immunostain with anti-HCMV antibodies of the inflammatory infiltrate (squared low-power middle section) with HCMV-positive cells (arrows) is shown in the upper part of the figure. Myocytes, interstitial and endothelial cells of low-power left and right sections do not show either cytopathic effect or signs of inflammation.
cut-off established for pre-emptive therapy. Four patients had HCMV GID (one with esophagitis - pt #10- Figure 1B, one with gastritis –pt #11-, and two with gastritis in association with interstitial pneumonia –pts #12 and 13-), while one patient was apparently affected by HCMV myocarditis (pt #14, Figure 1C). At the time of diagnosis all these patients showed a systemic viral load in blood greater than \(3 \times 10^5\) HCMV DNA copies/ml (range \(6.1 \times 10^5\) to \(1.7 \times 10^6\)) and, according to protocol, they were treated with antiviral therapy. Local viral load in GI biopsies of pts. 10-13 ranged from \(6.6 \times 10^3\) (pt#13) to \(1.4 \times 10^5\) (pt#10). In patients #12 and 13 levels of viral DNA in BAL were both greater than \(1 \times 10^5\). However, for patient #14 (Figure 1), cardiomyocytes were not found to be involved by HCMV infection at any section level of the biopsy sample, while HCMV-infected cells were only found in the inflammatory infiltrate within the endomyocardial tissue. Thus, this was not a case of true myocarditis, but of pseudomyocarditis. In all of these patients, the antiviral treatment was effective in resolving both systemic and local infection, although treatment duration varied according to different patients (median 31, range 31-48, days). Discontinuation of antiviral therapy was determined in all patients by viral DNA disappearance from blood, as well as by resolution of local clinical symptoms and/or disappearance of endoscopic lesions.

<table>
<thead>
<tr>
<th>Pt. #, age (yrs)</th>
<th>Type of tx</th>
<th>Days post-tx</th>
<th>HCMV disease</th>
<th>Local/systemic HCMV load</th>
<th>Organ pathology</th>
<th>Antiviral therapy (day duration)</th>
<th>Criteria for antiviral drug discontinuation</th>
<th>Outcome of HCMV disease</th>
<th>Type of infection</th>
<th>HCMV-specific T-cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>10. F, 62</td>
<td>Heart</td>
<td>41</td>
<td>Esophagitis</td>
<td>(1.4 \times 10^7) (bps)/(6.1 \times 10^6)</td>
<td>EGDS: ulcer (IHC pos)</td>
<td>GCV (48)</td>
<td>Clinical-healing of endoscopic ulcer</td>
<td>Resolved</td>
<td>P</td>
<td>-</td>
</tr>
<tr>
<td>11. F, 40</td>
<td>Kidney</td>
<td>213</td>
<td>Gastritis</td>
<td>(3.2 \times 10^4) (bps)/(1.3 \times 10^6)</td>
<td>EGDS: gastric lesions</td>
<td>GCV (35)</td>
<td>Clinical-HCMV DNA neg. in blood</td>
<td>Resolved</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>12. M, 49</td>
<td>Kidney</td>
<td>49</td>
<td>Gastritis+ interstitial pneumonia</td>
<td>(2.4 \times 10^4) (BAL)/(2.1 \times 10^4) (bps)/(1.0 \times 10^6)</td>
<td>EGDS: gastritis; CT, x-ray; interstitial pneumonia</td>
<td>GCV (28)</td>
<td>Clinical-HCMV DNA neg. in blood</td>
<td>Resolved</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>13. M, 47</td>
<td>Right lung</td>
<td>84</td>
<td>Interstitial pneumonia+gastritis</td>
<td>(1.4 \times 10^6) (BAL)/(6.6 \times 10^5) (bps)/(6.8 \times 10^5)</td>
<td>X-ray: interstitial pneumonia; EGDS: gastric lesions</td>
<td>GCV+PFA (31)</td>
<td>Clinical-HCMV DNA neg. in blood</td>
<td>Resolved</td>
<td>R</td>
<td>+</td>
</tr>
<tr>
<td>14. M, 52</td>
<td>Heart</td>
<td>112</td>
<td>Myocarditis?</td>
<td>ND/(1.7 \times 10^6)</td>
<td>EMB: HCMV-positive inflammatory cells (IHC pos)</td>
<td>GCV (21)</td>
<td>HCMV DNA neg. in blood</td>
<td>Resolved</td>
<td>P</td>
<td>ND</td>
</tr>
</tbody>
</table>

See footnote to Table 1.

**HCMV-specific T-cell immune response in SOT recipients with end-organ disease**

HCMV-specific T-cell response was determined in 9/14 (64.3%) patients at time of end-organ disease detection. While CD4+ T-cell count showed a high variability, ranging from 22 to 649 (median 138) cells/µl blood, in 7/9 (77.8%) patients, no HCMV-specific T-cell response was detected, while in patients #2 and #13, both CD4+ and CD8+ T-cells were present. However, in patient #13 (reactivated infection) steroid treatment for...
heart rejection was administered, while in patient #2 (primary infection) HCMV disease occurred >18 months after transplantation, concomitantly with development of primary T-cell response.

**DISCUSSION**

Two entirely different clinical scenarios may occur in SOT recipients with HCMV end-organ disease. HCMV disease may take place in the presence of low HCMV viral load in blood, or in association with a high systemic viral load. In transplantation centers where the preemptive therapy approach is routinely adopted, therapeutic decisions may vary widely according to these two clinical situations.

In the presence of clinical symptoms suggesting an HCMV organ localization, even in the presence of low (or absent) viral load in blood, the etiologic diagnosis of HCMV organ involvement must be established as rapidly as possible. On the other hand, in the case of high viral load in blood, the initiation of antiviral therapy, based on systemic viral DNA cut-off, is effective also for end-organ disease. In both cases, however, therapy discontinuation should not be decided solely on the basis of virus disappearance from blood, but additional requirements need to be met, i.e. amelioration/resolution of local clinical symptoms, resolution/healing of endoscopic lesions possibly in association with disappearance of viral DNA in biopsy samples, or in local secretions. In this study, a special warning comes from the analysis of pt #14 in whom myocardial involvement by HCMV infection was only apparent, i.e. due to the inflammatory infiltrate in the absence of cardiomyocyte infection.

Among HCMV tissue-invasive diseases, GID is the most frequently occurring in SOT recipients, including esophagitis, gastritis, duodenitis and small bowel enteritis, and colitis (Lemonovich and Watkins, 2012). Other less frequently involved organ are lungs (pneumonitis), liver (hepatitis), pancreas (pancreatitis), and eye (retinitis). In general, risk factors associated significantly with the development of HCMV disease are primary infections following universal prophylaxis (often associated with late-onset HCMV disease, allograft failure and mortality), and the level of the immunosuppression or reconstitution of HCMV-specific immunity (Arthurs et al., 2008; Eid et al., 2010). In our series, using pre-emptive therapy, we had 4/14 (28.6%) patients with primary infection, thus confirming that primary infection is a risk factor for end-organ disease also in association with pre-emptive therapy. It has been reported that gastrointestinal lesions were present in 3/4 solid organ transplant recipients developing HCMV drug-resistance in the era of valganciclovir prophylaxis (Eid et al., 2008a). In addition, Halme et al. detected HCMV in biopsies of liver transplant recipients, as well as in patients suffering from chronic liver disease or even in otherwise healthy patients with dyspeptic symptoms (Halme et al., 2008). Therefore, the sole presence of cells with nuclear inclusion bodies does not seem to be enough for initiating the antiviral therapy, since same findings have also been observed in GI tract of immunocompetent individuals.

Similarly, control endoscopy at the end of treatment to verify resolution of lesions and/or viral DNA disappearance does not necessarily mean protection from HCMV relapse (Eid et al., 2010). In our opinion, tissue section examination must be performed whenever possible. However, results must be interpreted with caution, both for antiviral therapy initiation and discontinuation.

HCMV presence in lungs and BAL merits special analysis (Gerna et al., 2009). While diagnosis of HCMV pneumonitis requires the presence of HCMV-infected epithelial cells surrounded by inflammatory infiltrates in biopsy lung sections, the sole presence of nuclear inclusion body bearing cells is not sufficient to make a diagnosis of HCMV pneumonitis. In addition, the HCMV presence in BAL is not *per se* indicative of HCMV lung pathology. A recent study correlating viral load in BAL with HCMV detection in lung biopsies found that levels >1x10⁵ DNA copies /ml BAL were detected in 100% of LTR with HCMV pneumonia (lung infection + inflammatory infiltrates), in 25-30% of patients with HCMV infection in lungs and no inflammatory infiltrates, and in only 3% of LTR with no sign of lung infection. HCMV-specific T-cell immunity seems to control HCMV infection during the post-transplantation period (Gerna et al., 2009). However, preliminary findings seem to indicate that different T-cell populations are responsible for local lung and systemic immunity (Shlobin et al., 2006).
A more uncommon feature of HCMV end-organ disease is HCMV retinitis (Eid et al., 2008b), which is currently diagnosed based on funduscopic examination, but may be virologically diagnosed by disclosing viral DNA in aqueous humor, as we demonstrated in a series of AIDS patients (Gerna et al., 1994). In the only patient of our series, the amount of viral DNA detected in aqueous humor was rather high (>10^5 DNA copies/ml).

In the present study, lack of specific T-cell immune response appears to be consistently associated with HCMV end-organ disease. In fact, of the 9 patients tested, 6 were lacking both HCMV-specific CD4+ and CD8+ T-cell response, one had only specific CD8+ T-cells, while of the two patients showing a T-cell response, one (#13, reactivated infection) was under steroid treatment, and the other (#2, primary infection) had end-organ disease while developing HCMV-specific T-cell primary response.

We already observed in the past that HCMV-specific CD8+ T-cells are often unable to control HCMV infection, while steroid therapy may impair T-cell response (Gerna et al., 2011). In addition, for patient #2 it can be hypothesized that primary T-cell response developing concomitantly with HCMV end-organ localization was not fully protective against HCMV disease.

It has been a matter of debate which viral assay is more sensitive in detecting HCMV end-organ disease in SOT recipients. HCMV pp65-antigenemia and qualitative/quantitative PCR have been compared for sensitivity and specificity in a number of published reports (Fica et al., 2007; Jang et al., 2009; Grim et al., 2010). Both assays have shown levels of sensitivity between 50% and 80%, with a slightly higher level of specificity for both assays. In addition, sensitivity increased if tests were performed within 6 months after transplantation (Fica et al., 2007). In our series, we consistently found the presence of viral DNA in blood of transplanted patients at the time of organ involvement diagnosis by using real-time PCR (Gerna et al., 2006). It cannot be excluded that in some cases end-organ disease may not be associated with detection of virus in peripheral blood. However, in some studies symptomatic systemic HCMV infection/disease was not differentiated from end-organ disease (Marchetti et al., 2011). On the other hand, virus in blood may not provide specific indications on which organ may be involved in the HCMV end-organ disease.

The pre-emptive therapy approach appears generally effective in preventing HCMV end-organ disease, considering that it was diagnosed in less than 3% of patients in our series. However, HCMV disease prevention is not 100% successful, especially when HCMV organ localization occurs in the presence of low viral load in blood or early after transplantation. In these cases, local infection must be timely diagnosed endoscopically and virologically, and documentation of HCMV infection is needed before deciding on the therapeutic approach to be adopted.

ACKNOWLEDGEMENTS
We are indebted to all the technical staff of the Virology section of the Struttura Complessa of Virology and Microbiology for performing the viral assays. We acknowledge the careful and devoted secretarial support of Daniela Sartori and the English revision by Laurene Kelly. This work was partially supported by grants from the Fondazione Carlo Denegri, Torino, and by the Ministero della Salute, Ricerca Corrente Fondazione IRCCS Policlinico San Matteo, grant 80207.

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