Early cytomegalovirus-specific T-cell response and estimated glomerular filtration rate identify patients at high risk of infection after renal transplantation


Transpl Inherit Dis 2016. All rights reserved

Abstract: Background. Assessing the risk of cytomegalovirus (CMV) viremia in kidney transplant recipients (KTR) may be helpful to indicate in which patient it is worth starting antiviral treatment during preemptive strategy.

Methods. In 40 CMV-seropositive KTR preemptively treated with ganciclovir, we used interferon (IFN)-γ ELISpot test to evaluate whether monitoring T cells directed against phosphoprotein (pp) 65 and immediate early (IE)-1 antigens could predict the onset of viremia.

Results. CMV viremia occurred in 24 patients (60%) within 120 days after transplantation. Non-viremic patients had higher anti-pp65, anti-IE-1 T cells, and estimated glomerular filtration rate (eGFR) in the first 90 days after transplantation. At logistic regression, anti-pp65, anti-IE-1 T cells, and eGFR measured at day 30 were significantly associated with CMV infection. Cutoff values of 15 spot-forming cells (SFCs)/200,000 peripheral blood mononuclear cells (PBMCs) for anti-IE, 40 SFCs/200,000 PBMCs for anti-pp65, and 46.6 mL/min/1.73 m² for eGFR, respectively, predicted the risk of CMV infection with high sensitivity and specificity (area under the receiver operating characteristic curve >0.75). Using a classification tree model, we identified as high-risk patients those showing anti-pp65 <42 SFCs/200,000 PBMCs and eGFR <62 mL/min/1.73 m², as well as anti-pp65 ≥42 and anti-IE-1 <6.5 SFCs/200,000 PBMCs.

Conclusion. Monitoring CMV-specific T-cell responses and eGFR in the first month post transplant can identify patients at high risk of CMV infection, for whom preemptive antiviral therapy is recommended.

Cytomegalovirus (CMV) infection continues to be a frequent cause of morbidity and mortality (1–4) in kidney transplant recipients (KTR). More than 50% of these patients have laboratory evidence of infection within the first year and many of them develop a life-threatening disease (5, 6). Moreover, CMV infection and disease are independent risk factors for clinical acute rejection in kidney allograft recipients (7).

Aside from naive patients, even patients with anti-CMV antibodies are prone to CMV reactivation and shedding in the post-transplant phase. The immunosuppressive regimen and pro-inflammatory conditions predispose to viral reactivation that can lead to severe symptomatic clinical manifestations (1). For a long time, it has been believed that the outcome of CMV infection was driven by the antibody-mediated
response. However, recent studies have shown that cellular immunity, particularly memory/effector ratio of CD4+ and CD8+ T cells, must be considered crucial for protection against CMV infection (7–10). Indeed, in humans, both T-cell lymphopenia and impaired lymphoproliferative responses to CMV have been demonstrated as risk factors for CMV disease (8, 9), and moreover, adoptive transfer of CMV-specific T-cell clones after solid organ transplantation has provided evidence of the importance of CMV-specific T-cell responses for protection against viral replication (10, 11). For this reason, routine immunological monitoring of transplant patients has been proposed (12, 13). Interferon-gamma (IFN-\(\gamma\)) enzyme-linked immunosorbent assay (ELISpot) might be a reliable means for evaluating the risk of infection in KTR (14, 15).

Preemptive and prophylactic antiviral strategies provide similar results, in terms of first-year CMV infection, and patient and graft survival (16). Preemptive therapy, allowing a limited degree of viral replication and antigen exposure, is thought to be beneficial for antiviral T-cell immune reconstitution (17), while prophylactic treatment is associated with a delayed priming of T-cell immune reconstitution and a higher incidence of late-onset CMV disease (2, 18, 19). However, consensus is not universal about the cutoff of CMV viremia indicating when preemptive antiviral therapy should be started, so immunological monitoring of anti-CMV T-cell reconstitution could be an attractive strategy to overcome this issue (20).

Although T-cell responses may target multiple CMV-specific proteins, it appears that protective cellular immunity is mainly directed against the lower matrix tegument phosphoprotein 65 (pp65) (encoded within the UL83 gene locus) and to the immune-dominant immediately early proteins (IE-1) (encoded within the UL123 gene locus) (21, 22).

Aims of this study were to evaluate if early immunological monitoring of CD4+ and CD8+ anti-CMV T-cell values in KTR could be predictive of CMV viremia onset in the first year post transplantation, and to identify cutoff levels of anti-pp65 and anti-IE-1 CMV-specific responses, indicating a decreased or increased risk of CMV infection, respectively.

**Patients and methods**

**Participants**

We performed this single-center prospective study at our Renal Transplant Unit at Annunziata Hospital in Cosenza, Italy. All de novo KTR, who received a transplant between September 2010 and December 2013, were considered for the study. Exclusion criteria included age <18 years, CMV seronegativity (CMV immunoglobulin [Ig]G negative) at transplantation, preexisting or acquired immunodeficiencies, multiorgan transplantation, severe heart failure, active neoplasia, and living-donor transplantation. Forty incident and consecutive KTR, CMV seropositive (R7) pretransplant, were enrolled in the study.

The design of the study was approved by the local Ethical Committee. All patients enrolled provided a written informed consent, according to the declaration of Helsinki and the Guidelines of the local Ethical Committee.

Patients were voluntarily recruited to donate 10 mL of peripheral blood for IFN-\(\gamma\) ELISpot at 30, 90, 180, and 360 days after transplantation, while routine surveillance for viral reactivation or infection comprised weekly determination of whole blood CMV DNAemia during the first 100 days after transplantation, and continued thereafter if clinically indicated. Demographic characteristics, as well as clinical routine measurements, were collected at the baseline visit, 30 days after transplantation. The estimated glomerular filtration rates (eGFRs) were calculated using CKD-EPI formula (23).

**Treatment**

All participants received an induction treatment based on basiliximab, steroids, and mycophenolate mofetil, followed by maintenance with calcineurin inhibitors (after the second day post transplantation), steroids, and mycophenolate mofetil as indicated by international guidelines (24). Cyclosporine and tacrolimus blood levels were measured at 30, 90, 180, and 360 days after transplantation.

Anti-CMV preemptive treatment included intravenous administration of ganciclovir (5 mg/kg daily) corrected according to renal function. Antiviral therapy was considered successful when 2 sequential negative CMV DNAemia tests were obtained. Cases of CMV-resistant strains were not detected among the transplant patients. Acute graft rejection was treated with intravenous high pulse-dose of steroids or anti-thymocyte globulin, as clinically and histologically indicated. No anti-CMV prophylaxis therapy was administered after acute rejections.

**Definitions**

CMV infection was defined as detection of viremia >650 copies/mL of whole blood. CMV disease was defined
by the presence of symptomatic clinical manifestations with fever and malaise associated with detectable CMV viremia and not ascribable to any other infection or condition. All transplant patients were treated according to a preemptive strategy, consisting of the initiation of antiviral treatment upon the detection of a viral load (CMV DNAemia) >5000 copies/mL. Acute graft rejection was scored and graded by means of the Banff schema (25). Delayed graft function (DGF) was defined as the necessity for dialysis in the first week after surgery (26).

**CMV DNAemia and CMV sero**test

CMV DNAemia was evaluated in all patients using 100 μL of whole blood extracted by EASYMAG (Biomérieux, Marcy l’Étoile, France), by adding 900 μL of lysis buffer. In all cases shown, CMV DNA was evaluated using real-time polymerase chain reaction (PCR) with an ABI Prism 7300 (Applied Biosystems, Foster City, California, USA). The DNA extract underwent quantitative reverse-transcription PCR (Elitech-Nanogen, Milan, Italy). The lowest detection limit corresponded to 650 viral copies/μL of whole blood. Anti-CMV IgG and IgM were assessed using chemiluminescence immunoassay (LIAISON, Diasorin, Saluggia, Italy).

**Evaluation of immune response**

During the first year post transplant, CD4+ and CD8+ anti-CMV T-cell levels were evaluated in all patients using an ELISpot assay (Oxford Immunotech, Abingdon, UK). Peripheral blood mononuclear cells (PBMCs) were immediately extracted after blood collection and purified by Ficoll (GE Healthcare, Little Chalfont, UK). PBMCs were resuspended in RPMI-1640 medium supplemented with 10% fetal bovine serum (Sigma Aldrich, St. Louis, Missouri, USA) and were seeded at a concentration of 2 × 10^5 cells/mL per well in an IFN-γ-coated ELISpot plate (Elitech, Milan, Italy). For each patient, duplicate wells were incubated with 100 μL of positive control (pokeweed mitogen equal to 10 μg/mL; AID Diagnostika GmbH, Strassberg, Germany); 100 μL CMV pp65 peptide mix (10 μg/mL; AID); and 100 μL CMV IE-1 peptide mix (10 μg/mL; AID). For each patient, the negative control with medium alone was performed. The plate was incubated for 20–24 h in a CO2 incubator (5–7% CO2) at 37°C. The wells were washed with washing buffer and then 100 μL of secondary antibody per well was dispensed. The plate was incubated 2 h at room temperature. Afterward, the wells were emptied and washed with washing buffer, and 100 μL of substrate solution was added to each well. IFN-γ ELISpot results are expressed as spot-forming cells (SFCs)/200,000 PBMCs. The enumeration of spots and calculation of the spots’ area were performed with an ELISpot Reader (AID Diagnostika GmbH). The results were expressed as percentage of cells secreting IFN-γ, after subtracting the number of spots resulting from spontaneous IFN-γ release (measured in the control wells) from the number of spots obtained in the well incubated with CMV-specific peptides.

**Statistical analysis**

All data are presented as mean ± standard deviation or median and interquartile range (IQR) as appropriate. Groups were compared using the one-way analysis of variance or t-test for normally distributed data, and the nonparametric Kruskal–Wallis or Mann–Whitney U-test for non-normally distributed variables. Bivariate correlation analyses were done using Pearson or Spearman test for nonparametric variables. Non-normally distributed variables were log10 transformed before analysis. Nonparametric, factorial analysis of longitudinal data was performed using the corresponding R package according to Brunner et al. (27, 28). Univariate and multivariate logistic regression models were performed to test the association with CMV infection. A sensitivity/specificity receiver operating characteristic (ROC) curve test was done to investigate the value of the ELISpot test for predicting the advent of post-transplant CMV infection. Area under the ROC curve was analyzed and 95% confidence interval (CI) obtained by a bootstrap procedure, using 1000 bootstrap samples (29). The statistical significance level was defined as a 2-tailed P < 0.05.

Recursive partitioning has been used to identify a classification tree model (30). Recursive partitioning is a nonparametric classification and regression approach; its main characteristic is that the tree is built by repeatedly splitting the patients into 2 subgroups based on all predictor variables. The parameters used for splitting are selected by the tree-generating algorithm and are not specified by the analyst. The algorithm also determines the cutoff levels for continuous parameters by trying to split the patient sample at all possible values. The choice of parameters and cutoff values is made such that observations (patients here) with similar response values (CMV infection in this study) are grouped together, as determined by an
impurity measure statistic. After the partitioning is completed, a constant value of the response variable is predicted within each area (31).

All the analyses were performed using R (version 3.0.1, The R Foundation for Statistical Computing) (32).

**Results**

**Patients characteristics**

Main demographic and baseline characteristics of participants are shown in Table 1.

CMV viremia was detected in 24 KTR (60%, 15 in D+ and 9 in D− patients; P = 0.652) and 17 (42.5%) with a CMV DNAemia >5000 copies/mL, required antiviral treatment. Three (7.5%) KTR experienced symptomatic CMV infection. Figure 1 shows CMV-specific anti-pp65 and anti-IE-1 ELISpot levels in all 40 KTR at 30, 90, 180, and 360 days after transplantation.

Anti-pp65 T-cell responses increased during the first year post transplantation. ELISpot levels for anti-pp65 at 30 days (36, 12.5–71) were significantly lower compared to 90 days (median value 90, IQR 20.5–137.5, P = 0.003), 180 days (122.5, 63–169.8, P = 0.006), and 360 days after transplantation. In contrast, T-cell responses to anti-IE-1 remained stable during the first year post transplantation, without significant differences among time points of ELISpot dosages.

CMV viremia was detected during the 120 days post transplantation in 23 KTR, while only 1 patient experienced viremia later. Therefore, the most interesting time points for predicting the risk of CMV infection would be 30 and 90 days.

**Viremic and non-viremic patients**

When we stratified our patients according to the development of CMV viremia (Fig. 2), we observed that, at 30 and 90 days after transplantation, non-viremic patients had higher specific T-cell responses, both to pp65 and IE-1 antigens. In non-viremic KTR at 30 days post transplantation, the median levels of anti-pp65 IFN-γ spots were 95 (IQR 47–158.5) vs. 30 (6–37) SFCs/200,000 PBMCs in viremic patients (P = 0.004); at 90 days the median levels were 130 (113–156) vs. 75 (20–111, P = 0.003). The median levels of anti-IE-1 IFN-γ spots in non-viremic KTR at 30 day were 43 SFCs/200,000 PBMCs (IQR 11–126) vs. 4 (0–20) in viremic patients (P = 0.03); they were, respectively, 40 (14–145) vs. 10 (3–25, P = 0.035) at 90 days.

Among viremic patients, only 3 experienced CMV disease: in these KTR, CMV-specific anti-pp65 and anti-IE-1 immune response was extremely low (almost undetectable) as compared with that of other patients with positive CMV DNAemia (anti-pp65 and anti-IE-1 IFN-γ spots levels ≤3 SFCs/200,000 PBMCs).

---

**Table 1**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (male/female)</td>
<td>23/17</td>
</tr>
<tr>
<td>Age (years)</td>
<td>49 ± 12.2</td>
</tr>
<tr>
<td>Donor age (years)</td>
<td>46.9 ± 12.9</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>53.4 ± 17</td>
</tr>
<tr>
<td>DGF (%)</td>
<td>19/40 (47.5)</td>
</tr>
<tr>
<td>Mismatches ≥4 (%)</td>
<td>25/40 (62.5)</td>
</tr>
<tr>
<td>BPAR (%)</td>
<td>10/40 (25)</td>
</tr>
<tr>
<td>Pre-transplant CMV donor (D)/recipient (R) serostatus</td>
<td></td>
</tr>
<tr>
<td>D+/R+ (%)</td>
<td>27 (67.5)</td>
</tr>
<tr>
<td>D−/R+ (%)</td>
<td>13 (32.5)</td>
</tr>
<tr>
<td>Pre-transplant anti-CMV IgG titers (UA/mL)</td>
<td>190.7 ± 81</td>
</tr>
<tr>
<td>CIT (hours)</td>
<td>13.1 ± 4.6</td>
</tr>
<tr>
<td>Induction immunosuppression</td>
<td></td>
</tr>
<tr>
<td>Anti-CD25</td>
<td>40/40 (100)</td>
</tr>
<tr>
<td>Maintenance immunosuppression</td>
<td></td>
</tr>
<tr>
<td>FK (%)</td>
<td>34/40 (85)</td>
</tr>
<tr>
<td>FK blood levels (ng/mL)*</td>
<td>9.8 ± 2.1</td>
</tr>
<tr>
<td>CsA (%)</td>
<td>6/40 (15)</td>
</tr>
<tr>
<td>CsA blood levels C0 (ng/mL)*</td>
<td>189 ± 22.3</td>
</tr>
<tr>
<td>CsA blood levels C2 (ng/mL)*</td>
<td>1167.7 ± 123.4</td>
</tr>
<tr>
<td>MMF (%)</td>
<td>40/40 (100)</td>
</tr>
<tr>
<td>Steroids (%)</td>
<td>40/40 (100)</td>
</tr>
<tr>
<td>Patients who experienced post-transplant CMV DNAemia (%)</td>
<td>24/40 (60)</td>
</tr>
<tr>
<td>Patients requiring antiviral treatment (%)</td>
<td>17/40 (42.5)</td>
</tr>
<tr>
<td>CMV disease (%)</td>
<td>3/40 (7.5)</td>
</tr>
</tbody>
</table>

*Measurements at 30 days after transplantation.

eGFR, estimated glomerular filtration rate; DGF, delayed graft function; BPAR, biopsy-proven acute rejection; CMV, cytomegalovirus; R+, CMV-seropositive transplant recipient; D+, CMV-seropositive transplant donor; D−, CMV-seronegative transplant donor; CIT, cold ischemia time; FK, tacrolimus, CsA, cyclosporine; MMF, mycophenolate mofetil.
Considering CMV immune response, in this cohort a significant linear inverse correlation was noted between anti-IE-1 IFN-γ spots levels and CMV DNAemia (30 days: \( r = -0.332, P = 0.036 \); 90 days: \( r = -0.334, P = 0.037 \)) as well as between anti-pp65 IFN-γ spots levels and CMV DNAemia (30 days: \( r = 0.332, P = 0.036 \); 90 days: \( r = 0.334, P = 0.037 \)).

Interestingly, anti-pp65 IFN-γ spots levels, unlike anti-IE-1, were directly correlated with eGFR at 30 days after transplantation (\( r = 0.487, P < 0.0001 \); Fig. 3) and
Viremic patients showed significantly lower eGFR levels (median 31.9 mL/min/1.73 m², IQR 25.5–40.6) than non-viremic patients (56.7, 50.5–81.3, P < 0.001).

**Prediction of CMV reactivation**

To determine the impact of anti-pp65 and anti-IE-1 T cells on CMV viremia onset, we performed univariate and multivariate logistic regression analysis. At univariate analysis, anti-pp65 and anti-IE-1 spots at 30 days, eGFR, DGF, and biopsy-proven acute rejection were all associated with CMV viremia. At multivariate analysis, only anti-pp65, anti-IE-1 IFN-γ spots levels, and eGFR at 30 days remained independently associated with CMV infection (Table 2).

We also performed a ROC analysis, to identify cutoff values of anti-pp65, anti-IE-1, and eGFR at day 30, indicating a decreased or increased risk of CMV infection (Fig. 4). Using 15 SFCs/200,000 PBMCs as anti-IE-1 IFN-γ spots cutoff levels, we obtained a sensitivity and specificity of 70% and 73%, respectively.

![Fig. 3. Correlation between estimated glomerular filtration rate (eGFR) and ELISpot assay values at 30 days after transplantation. (A) Correlation between log10 eGFR and log10 anti-pp65 interferon (IFN)-γ spots. (B) Correlation between log10 eGFR and log10 anti-IE-1 IFN-γ spots. IE, immediate early; pp, phosphoprotein.](image-url)
with a positive predictive value (PPV) and a negative predictive value (NPV) of 80% and 78%, respectively. Using 40 SFCs/200,000 PBMCs as anti-pp65 IFN-γ spots cutoff levels, we obtained a sensitivity and specificity of 83% and 82%, respectively, with correspondent PPV and NPV of 88% and 75%. Using 46.6 mL/min/1.73 m² as the eGFR cutoff level, we obtained a sensitivity of 82% and specificity of 88%, respectively, with a PPV and NPV of 91% and 76%, respectively.

Next, we used a binary classification tree analysis to define subgroups of KTR at high risk of CMV infection identified by a combination of cutoff values of the 3 factors selected. The tree model identified as being at high risk, patients showing an anti-pp65 <42 IFN-γ spots and eGFR <62 mL/min/1.73 m², as well as KTR with anti-pp65 ≥42 and anti-IE-1 <6.5 IFN-γ spots (Fig. 5). Analyzing the area under the ROC curve (7), the classification tree reached the best predictive value for CMV onset respect to the 3 factors alone (Fig. 4, solid black line).

Discussion

Our study demonstrates that, in CMV-seropositive recipients 30 days after renal transplantation, CMV-specific immune response together with eGFR are
helpful for early identification of patients at high risk to develop CMV infection.

In our prospective cohort, patients who experienced CMV infection within 12 months after transplantation showed lower pp65 and IE-1 specific T-cell responses compared to those who did not have infection. The first-month levels of CD4⁺ and CD8⁺ anti-pp65 and anti-IE-1 T cells were predictive for CMV infection with high sensitivity and specificity. Most importantly, with this approach, it was possible to discriminate patients at high risk for CMV viremia onset during the first year after transplantation, only 1 month after transplantation. Thus, the use of anti-IE-1 and anti-pp65 IFN-γ spots cutoff levels, associated with CMV DNA monitoring, could be important for the decision to start antiviral therapy during preemptive strategy.

Previous meta-analyses suggested that prophylaxis is the most effective strategy to reduce the CMV disease incidence and to improve graft survival (33–35). Nonetheless, it is also the most expensive strategy and the optimal use of this approach has generated substantial debate (36). The use of CMV viral load monitoring to guide preemptive antiviral treatment in patients at moderate risk of CMV disease has been shown to be effective (24) and has several potential advantages compared to the use of universal chemoprophylaxis. Actually, this strategy prevents antiviral drug toxicity, poor patient compliance, development of drug-resistant strains, and the risk of late-onset CMV disease (2, 37), and can limit the exposure to antiviral agents only to patients who have demonstrated evidence of subclinical CMV infection. Although no CMV DNA cutoff to start treatment is universally accepted (38, 39), the addition of information about anti-CMV-specific T-cell values in the first month after transplantation to CMV DNAemia, could be effective to reduce exposure to antiviral drug and ameliorate the efficacy of preemptive antiviral strategy.

Previous studies have demonstrated that monitoring IE-1-specific CD4⁺ and CD8⁺ T-cell responses before transplantation may be useful for predicting post-transplant risk of CMV infection and in guiding decision-making regarding CMV preventive or prophylactic treatment (40–42). Bestard et al. (40) found, retrospectively, that pre-transplantation IE-1-specific CD4⁺ and CD8⁺ T-cell cutoff levels predict the risk for post-transplantation CMV infection. It is well known that, early after transplantation, induction and aggressive maintenance immunosuppressive therapy severely bring down levels of CD4⁺ and CD8⁺ anti-pp65 and IE-1. However, after transplantation, several factors influence the immune reconstitution for CMV, independently from pre-transplant immune status, such as acute rejection, DGF, susceptibility to immunosuppressive drugs, sufficient exposure to virus antigen, and other factors not yet elucidated (43). Moreover, it seems that CMV-specific T cells against pp65 antigens would also have a strong role in controlling CMV replication (44, 45), as reported by Egli et al. (46) showing the pp65 overlapping peptide-induced IFN-γ response in seropositive KTR. Therefore, cutoff values of anti-pp65 and anti-IE-1 after transplantation could be more effective in predicting CMV infection and useful for guiding CMV antiviral treatment.

**Fig. 5.** Classification tree for the onset of cytomegalovirus (CMV) viremia in the first year after transplantation. Subgroup of patients identified by the tree model are presented in the boxes on the lower part of the figure and are defined by the predicted response class (VIREMIC or NON VIREMIC) and the number of patients correctly classified/total number of patients belonging to that subgroup. Among patients not having a number of anti-pp65 T-cells spots >42, those with eGFR <62 mL/min/1.73 m² are classified as VIREMIC, while those showing a higher eGFR are NON VIREMIC. Patients with anti-pp65 ≥42 are distinguished by a cutoff value of anti-IE-1 T cells of 6.5 spots. IE, immediate early; pp, phosphoprotein.
An unexpected but very interesting observation was that patients who experienced CMV infection showed lower 1-month eGFR and CMV immune cellular reconstitution, directly correlated with glomerular filtration. These data are unique in literature and fit well with a clinical observation: usually, patients who develop CMV infection are those with a slow recovery of renal function after kidney transplantation. This observation could be partially explained by more DGF events or acute rejection (47, 48). These events usually require stronger immunosuppressive regimens that can favor viral reactivation. Moreover, the inflammatory state, closely linked to the uremic state, blunts the immune response in humans and in experimental animals (49).

By the multivariate logistic regression model, we observed that eGFR was also associated with onset of CMV viremia and, using a ROC analysis, we identified a cutoff level for eGFR able to predict onset of CMV viremia with high sensitivity and specificity. Therefore, we used a classification tree to allow early identification of patients at high risk of infection, using our 3 predictive parameters. This tree reached the best predictive value for CMV onset in the first year, and identified a model defined by the interactions among the 3 most predictive factors that would be easy to use by clinicians. Such a tree model allows identification in the first month of those patients with slow renal function reconstitution who are at high risk for CMV infection.

In agreement with previous reports (40), our results suggest that it takes at least 6 months after transplantation to reconstitute an effective CMV-specific immune response. Abate et al. (43) demonstrated that high ELISpot counts, 100 SFCs/200,000 PBMCs for anti-pp65 T cells, may be considered to be protective against CMV infection. In our prospective cohort of transplant recipients, we have found low values of CMV-specific immune response to anti-pp65 in the first 3 months after transplantation. ELISpot counts reached this threshold of protectiveness from CMV infections only at 6 months after transplantation, and remained steadily above this threshold after 1 year. The attainment of protectiveness also is demonstrated by the observation that, in our cohort, all patients but 1 experienced onset of CMV viremia within 120 days after transplantation.

Moreover, our results showed a linear correlation between anti-pp65 T cells and CMV DNAemia, and anti-IE-1 T cells and CMV DNAemia, only in the first and third months after transplantation, but this correlation is lacking after 3 months. This might indicate that, even if CMV triggers both humoral and cellular response, only the latter seems to be crucial for immediate post-transplantation control of viral replication: in particular, CD4+ and CD8+ anti-pp65 and anti-IE-1 could play a role in short-term viral control (8, 10). This hypothesis was corroborated by the observation that our patients who developed CMV disease in the first year post transplantation, despite a pre-transplant congruous CMV IgG titer, showed extremely low specific T-cell responses.

A limitation of this study is the small number of events. Nevertheless, this technique can allow clinicians to identify those KTR who do not have early reconstitution of CMV-specific responses, and to organize an effective preemptive treatment.

In conclusion, we have shown that monitoring not only CMV-specific T cells, but also eGFR levels early after transplantation, may be useful for predicting risk of viremia onset, thus being potentially valuable for guiding decision-making regarding CMV preemptive treatment. Further studies are needed to confirm our data and to validate the CMV-specific response monitoring and eGFR cutoff, to better personalize preemptive antiviral strategy.

Acknowledgements:

The authors gratefully acknowledge F. Armentano and A. Greco for their nursing assistance in this study.

Author contributions: P.G., F.L., and M.V.M. performed the study; D.L. analyzed the data; F.G., R.T., and D.P. collected the data; T.P., A.M., A.P., D.V., A.L.R., G.T., and S.L. contributed with interpretation of results and review of the article; C.G. and R.B. designed the study, and approved the submitted and final versions.

References


