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Research Article

Keywords: cytomegalovirus, kidney transplant, RT-PCR, antigenemia

Posted Date: December 29th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1168330/v1

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Abstract

Due to the high costs, the strategy to reduce the impact of cytomegalovirus (CMV) after kidney transplant (KT) involves preemptive treatment in low and middle-income countries. Thus, this retrospective cohort study compared the performance of antigenemia transitioned to quantitative nucleic acid amplification testing, RT-PCR, in KT recipients receiving preemptive treatment as a strategy to prevent CMV infection. Between 2016 and 2018, 363 patients were enrolled and received preemptive treatment based on antigenemia (n=177) or RT-PCR (n=186). The primary outcome was CMV infection or disease. There were no differences in one-year cumulative incidence of CMV-related events (50.8% vs. 44.1%, P=0.20), neither in time to diagnosis (47.0 vs. 47.0 days) among patients conducted by antigenemia vs. RT-PCR, respectively. The length of CMV first treatment was longer with RT-PCR (20.0 vs. 27.5 days, P<0.001), while the rate of retreatment was not different (14.7% vs. 11.8%, P=0.48). In the Cox regression, the variables associated with CMV-related events were acute rejection within 30 days (HR=2.05, p=0.01) and 30-day glomerular filtration rate (HR=0.98, p<0.001). In conclusion, acute rejection and glomerular filtration rate are risk factors for CMV infection and disease, showing comparable performance in the impact of CMV-related events between antigenemia and RT-PCR for preemptive treatment.

Introduction

The cytomegalovirus (CMV) infection is one of the most common infectious events after solid organ transplantation, affecting 20 to 60% of kidney transplant recipients\(^1\)–\(^3\), increasing morbidity, costs and leading to a possible negative impact on graft survival\(^3\). The effects of the CMV infection have been traditionally characterized as direct and indirect\(^4\). Although the indirect effects have been questionable recently, the direct effects, such as symptoms and laboratory changes attributable to CMV and the invasive disease, are still a field of concern after kidney transplantation\(^4\),\(^5\). Cytomegalovirus replication occurs mainly in the first three months after the transplant, and the clinical presentation is now well defined according to international guidelines in infection, disease, and invasive disease\(^4\),\(^6\).

Considering the latent CMV infection is widely detected among candidates for kidney engraftment\(^7\), the risk of CMV active infection after transplantation should be evaluated, and a strategy to reduce the impact of direct effect has to be adopted\(^4\). Currently, there are two efficacy and safe alternatives for preventing outcomes related to CMV after transplantation: universal pharmacological prophylaxis or preemptive treatment\(^8\)–\(^10\). Although universal prophylaxis seems to be associated with lower CMV-related effects, some disadvantages have been highlighted: toxicity, late-onset CMV disease, risk of resistance, and costs\(^4\). In Brazil, for instance, prophylaxis with oral valganciclovir for three months can cost 3 to 7 times more than the preemptive treatment, depending on graft function and frequency of monitoring\(^11\). Thus, due to the high cost, the way to reduce the impact of CMV involves targeted prevention through preemptive treatment, especially in low and middle-income countries.
For preemptive treatment, patients must be strictly monitored for CMV replication throughout a laboratory method to detect viral load. For many years, many services performed a semi-quantitative test, an immunofluorescence assay based on monoclonal antibodies that detect the viral antigen, such as the pp65 antigenemia\textsuperscript{12–14}. However, in the last two decades, it has been replaced by quantitative nucleic acid testing, especially by standardization ultra-sensitivity real-time polymerase chain reaction (RT-PCR), such that it is currently the preferred method for CMV management\textsuperscript{4}. In 2017, we started implementing standardized RT-PCR for the preemptive treatment in our center, replacing the antigenemia completely one year later. This change in the clinical routine designed a natural experiment with the potential to measure CMV-related events as outcomes in two different eras. Therefore, in the present study, we aimed to compare the performance of antigenemia transitioned to quantitative nucleic acid amplification testing, RT-PCR, in kidney transplant recipients receiving preemptive treatment and to evaluate the potential clinical predictors of the CMV-related events.

**Results**

Between March 2016 and August 2018, a total of 2,294 kidney transplants were performed in our center. Initially, 905 patients were excluded because they were transplanted in a transition period (from March 2017 to February 2018). In the antigenemia era (March 2016 to March 2017), 932 patients had been transplanted; however, 488 recipients did not present inclusion criteria, and 267 had exclusion criteria, as depicted in figure 1. On the other hand, in the RT-PCR era (February 2018 to August 2018), 457 patients had been transplanted; however, 130 recipients did not present inclusion criteria, and 141 had exclusion criteria (figure 1). Among patients excluded due to death or graft loss (n= 65), 38 had died or had graft loss within 60 days of transplantation. All 17 deaths were verified, and no one was attributed to the CMV event. Therefore, 177 patients were enrolled for the antigenemia era, whereas 186 were for the RT-PCR era.

**Demography data according to testing era: antigenemia and RT-PCR**

Demographic data are shown in Table 1. Patients were 49.0 years old, 54.8% males and 54.8% whites. The etiology for chronic kidney disease was unknown for 44.6%, and 93.7% had undergone hemodialysis as a renal replacement treatment before transplantation; only 12.7% of patients had been submitted to a retransplantation. Donors were 52.0 years old, 52.9% male, and 52.1% white. Most transplants were performed with a deceased donor (96.1%), whose median KDPI value was 80.0. The cold ischemia time was 23.1 hours, and the delayed graft function (DGF) occurred in 187 patients (51.5%).
### Table 1
Demographic data according to the era: antigenemia and PCR

<table>
<thead>
<tr>
<th>Results</th>
<th>Total (N=363)</th>
<th>Antigenemia Era (N=177)</th>
<th>PCR Era (N=186)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient age, years</td>
<td>49.0 (37.0; 57.0)</td>
<td>50.0 (39.2; 57.0)</td>
<td>50.0 (41.0; 60.0)</td>
<td>0.38</td>
</tr>
<tr>
<td>Recipient sex, male, N (%)</td>
<td>199 (54.8)</td>
<td>92 (52.0)</td>
<td>107 (57.5)</td>
<td>0.29</td>
</tr>
<tr>
<td>Recipient ethnicity, N (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.007</td>
</tr>
<tr>
<td>White</td>
<td>199 (54.8)</td>
<td>83 (46.9)</td>
<td>116 (62.4)</td>
<td></td>
</tr>
<tr>
<td>Pardo</td>
<td>177 (32.3)</td>
<td>71 (40.1)</td>
<td>46 (24.7)</td>
<td></td>
</tr>
<tr>
<td>Afro Brazilian</td>
<td>40 (11.0)</td>
<td>21 (11.9)</td>
<td>19 (10.2)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>7 (1.9)</td>
<td>2 (1.1)</td>
<td>5 (2.7)</td>
<td></td>
</tr>
<tr>
<td>Cause of CKD, N (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>Unknown</td>
<td>162 (44.6)</td>
<td>80 (45.2)</td>
<td>82 (44.1)</td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>52 (14.3)</td>
<td>22 (12.4)</td>
<td>30 (16.1)</td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>47 (12.9)</td>
<td>18 (10.2)</td>
<td>29 (15.6)</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>29 (8.0)</td>
<td>18 (10.2)</td>
<td>11 (5.9)</td>
<td></td>
</tr>
<tr>
<td>ADPKD</td>
<td>23 (6.3)</td>
<td>10 (5.6)</td>
<td>13 (7.0)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>50 (13.8)</td>
<td>29 (16.4)</td>
<td>21 (11.3)</td>
<td></td>
</tr>
<tr>
<td>Time on dialysis, months</td>
<td>42.0 (20.0; 79.0)</td>
<td>56.0 (31.2; 88.0)</td>
<td>42.0 (22.0; 88.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>Type of dialysis, Hemodialysis N (%)</td>
<td>340 (93.7)</td>
<td>162 (91.5)</td>
<td>178 (95.7)</td>
<td>0.10</td>
</tr>
<tr>
<td>Retransplant, N (%)</td>
<td>46 (12.7)</td>
<td>24 (13.6)</td>
<td>22 (11.8)</td>
<td>0.62</td>
</tr>
<tr>
<td>PRA Class I, N (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>0 - 29%</td>
<td>273 (75.2)</td>
<td>123 (69.5)</td>
<td>150 (80.6)</td>
<td></td>
</tr>
<tr>
<td>30 - 80%</td>
<td>56 (15.4)</td>
<td>30 (16.9)</td>
<td>26 (14.0)</td>
<td></td>
</tr>
<tr>
<td>&gt; 80%</td>
<td>34 (9.4)</td>
<td>24 (13.6)</td>
<td>10 (5.4)</td>
<td></td>
</tr>
</tbody>
</table>

*KDPI is applicable only for deceased donors. ADPKD, autosomal dominant polycystic kidney disease; CIT, cold ischemia time; CKD, chronic kidney disease; DGF, delayed graft function; HLA, human leukocyte antigen; KPDI, kidney profile donor index; PCR, polymerase chain reaction; PRA, panel reactive antibodies*
<table>
<thead>
<tr>
<th>Results</th>
<th>Total (N=363)</th>
<th>Antigenemia Era (N=177)</th>
<th>PCR Era (N=186)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRA Class II, N (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>0 - 29%</td>
<td>315 (86.8)</td>
<td>148 (83.6)</td>
<td>167 (89.8)</td>
<td></td>
</tr>
<tr>
<td>30 - 80%</td>
<td>29 (8.0)</td>
<td>21 (11.9)</td>
<td>8 (4.3)</td>
<td></td>
</tr>
<tr>
<td>&gt; 80%</td>
<td>19 (5.2)</td>
<td>8 (4.5)</td>
<td>11 (5.9)</td>
<td></td>
</tr>
<tr>
<td>Donor type, deceased, N (%)</td>
<td>349 (96.1)</td>
<td>170 (96.0)</td>
<td>179 (96.2)</td>
<td>0.92</td>
</tr>
<tr>
<td>Donor age, years</td>
<td>52.0 (42.0; 60.0)</td>
<td>53.0 (42.0; 62.0)</td>
<td>52.0 (42.0; 58.0)</td>
<td>0.003</td>
</tr>
<tr>
<td>Donor sex, male, N (%)</td>
<td>192 (52.9)</td>
<td>85 (48.0)</td>
<td>107 (57.5)</td>
<td>0.07</td>
</tr>
<tr>
<td>Donor ethnicity, N (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.28</td>
</tr>
<tr>
<td>White</td>
<td>189 (52.1)</td>
<td>93 (52.5)</td>
<td>96 (51.6)</td>
<td></td>
</tr>
<tr>
<td>Pardo</td>
<td>139 (38.3)</td>
<td>64 (36.2)</td>
<td>75 (40.3)</td>
<td></td>
</tr>
<tr>
<td>Afro Brazilian</td>
<td>32 (8.8)</td>
<td>17 (9.6)</td>
<td>15 (8.1)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>3 (0.8)</td>
<td>3 (1.7)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>KDPI (median of %)*</td>
<td>80.0 (49.5; 91.0)</td>
<td>82.5 (61.7; 91.7)</td>
<td>79.0 (54.0; 89.0)</td>
<td>0.003</td>
</tr>
<tr>
<td>Mismatches HLA ABDR</td>
<td>2.0 (2.0; 3.0)</td>
<td>2.0 (2.0; 3.0)</td>
<td>2.0 (1.0; 3.0)</td>
<td>0.30</td>
</tr>
<tr>
<td>CIT, hours</td>
<td>23.1 (19.4; 28.1)</td>
<td>24.7 (21.5; 31.2)</td>
<td>22.0 (18.5; 27.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>DGF, N (%)</td>
<td>187 (51.5)</td>
<td>108 (61.0)</td>
<td>79 (42.5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

[*KDPI is applicable only for deceased donors. ADPKD, autosomal dominant polycystic kidney disease; CIT, cold ischemia time; CKD, chronic kidney disease; DGF, delayed graft function; HLA, human leukocyte antigen; KDPI, kidney profile donor index; PCR, polymerase chain reaction; PRA, panel reactive antibodies]

The demographic data were compared between the era (Table 1). There is no difference in the recipients age, however in the antigenemia era, they were less frequently white (46.9 vs. 62.4%, P = 0.007), with longer length in dialysis before transplantation (56.0 vs. 42.0 months, P = 0.03), and with higher frequency of class I cPRA > 80% (13.6 vs. 5.4% P = 0.01). In this group, donors were older (53.0 vs. 52.0 years, P = 0.003), with higher KDPI (82.5 vs. 79.0 medians of %, P=0.003), and the cold ischemia time was longer (24.7 vs. 22.0 hours, P = 0.001). Consequently, the frequency of DGF was higher in the first era (61.0 vs. 42.5%, P < 0.001).

**Immunosuppression during 1-year follow-up**
Over the first year, there was a significant difference in the tacrolimus levels between eras in the mean values in three time-points (antigenemia and PCR, respectively; values expressed in ng/dL and 95% CI in the brackets): 9.8 [9.2 – 10.4] vs. 8.6 [8.1 – 9.0] in the day 42; 9.7 [9.1 – 10.2] vs. 8.4 [7.9 – 8.9] in the day 49; and 9.2 [8.7 – 9.8] vs. 8.1 [7.7 – 8.5]. The overall mean difference in the levels was 0.88 ng/dL higher in the first era (P<0.001, adjusted by Bonferroni test), and the plot summarizing the evolution over the first year after transplantation is shown in the supplementary figure 1. The doses of mycophenolate were also compared at different time points in both eras, and no differences were observed (Supplementary Table 1).

**Outcomes**

One hundred and seventy-two patients (47.4%) required treatment for CMV due to infection or disease, 47.0 days after transplantation; 79 presented symptoms or laboratory changes attributable to CMV disease (21.8% of the whole population and 45.9% of CMV diagnosed patients). The most common symptom was diarrhea (n=40), whereas the most common laboratory change was leukopenia (n=34). Symptoms and laboratory changes at the moment of CMV disease are detailed in supplementary table 2. Only one patient had an invasive disease. The length of treatment was 22.0 days.

One-year cumulative incidence of the first CMV-related event was not different according to the era: 50.8% in the antigenemia era vs. 44.1% in the PCR, P=0.20 (Figure 2.A). The time between transplantation and the first event was not different too: 47.0 (37.5; 60.2) vs. 47.0 (36.7; 64.5) days, respectively, P=0.93. There also was no difference regarding the cumulative incidence of CMV disease (23.7% vs. 19.1%, respectively, P=0.41, Figure 2.B). In the first era, the median antigenemia when the treatment was started was 18 cells, and 20 patients (21.5%) had to be treated with less than 10 cells. In the second era, the viral load when the treatment was started was 7,093 UI/mL (5,247; 12,327), while 19 patients (22.4%) had to be treated with less than 5,000 UI/mL. Furthermore, the length of treatment was longer in the RT-PCR (Table 2): 20.0 vs. 27.5 days, P<0.001. Last, there was no difference in the requirement for retreatments: 14.7% vs. 11.8%, respectively, P=0.48.
Table 2
Multivariable analyses for the first CMV-associated event and for disease

<table>
<thead>
<tr>
<th>First event (infection or disease): model 1</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>HR</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>AR within 30 days (yes vs. no)</td>
<td>2.05</td>
<td>1.18–3.56</td>
<td>0.01</td>
</tr>
<tr>
<td>30-day eGFR (each 1 mL/min/1.73m²)</td>
<td>0.98</td>
<td>0.97–0.99</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Disease: model 2</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>HR</td>
<td>95% CI</td>
<td>P</td>
</tr>
<tr>
<td>DGF (yes vs. no)</td>
<td>1.54</td>
<td>0.94–2.54</td>
<td>0.09</td>
</tr>
<tr>
<td>AR within 30 days (yes vs. no)</td>
<td>2.24</td>
<td>1.07–4.68</td>
<td>0.03</td>
</tr>
<tr>
<td>30-day eGFR (each 1 mL/min/1.73m²)</td>
<td>0.98</td>
<td>0.97–0.99</td>
<td>0.01</td>
</tr>
</tbody>
</table>

[Variables included in the model 1: era (antigenemia or RT-PCR), recipient age, hemodialysis as replacement renal therapy before the transplantation, retransplant, donor age, DGF, 30-day eGFR and AR within 30 days after transplantation. Cold ischemia time was excluded due to collinearity with DGF.

The accuracy of the multivariable modeling for predicting CMV-related event was assessed by an AUC-ROC, which achieved a result of 0.792 (95% CI 0.745 – 0.839). Variables included in the model 2: era (antigenemia or RT-PCR), diabetes as CKD etiology, donor age, DGF, 30-day eGFR and AR within 30 days after transplantation. KDPI was excluded due to collinearity with donor age. The model's AUC-ROC to predict CMV disease: 0.743 (95% IC 0.681 – 0.806). AR, acute rejection; CKD, chronic kidney disease; DGF, delayed graft function; eGFR, estimated glomerular filtration rate.]

One-year cumulative incidence of acute rejection (Figure 2.C) was 12.4% in the antigenemia era and 16.1% in the RT-PCR (P=0.35). Figure 2.D shows the eGFR over the follow-up time according to both eras. Owing to the difference in the DGF incidence, the eGFR was lower in the first era from the baseline (day 21) to day 42 (antigenemia and RT-PCR, respectively; values expressed in mL/min/1.73m²; 95% CI in the brackets): baseline 33.8 [30.7 – 36.8] vs. 39.9 [36.6 – 43.1]; day 42 40.9 [38.0 – 43.9] vs. 48.1 [45.1 – 51.2]. The overall mean difference in the graft function was 5.24 mL/min/1.73m² lower in the first era (P<0.001, adjusted by Bonferroni test).

Variables associated with CMV first event and CMV disease

In the Table 2 is shown the multivariable models for first CMV-related event (infection or disease, model 1) and for CMV disease (model 2). The variables were selected in bivariate analysis comparison between patients who had CMV-related events with those who had not (Supplementary Table 3). The same analysis was performed to CMV-disease (Supplementary Table 4). Acute rejection within 30 days after transplantation (HR yes vs. no = 2.05; 95% CI = 1.18–3.56; P = 0.01) and 30-day eGFR (HR for each 1 mL/min/1.73m² = 0.98; 95% CI 0.97–0.99; P < 0.001) were related with the probability of first CMV-related event. The accuracy of the model 1 was assessed by an AUC-ROC, which achieved a result of 0.792 (95% CI 0.745 – 0.839). Similarly, the same variable were related with the probability of CMV
disease: AR within 30 days (HR yes vs. no = 2.24; 95% CI = 1.07–4.68; P = 0.03) and 30-day eGFR (HR for each 1 mL/min/1.73m² = 0.98; 95% CI 0.97–0.99; P = 0.01). The AUC-ROC for the model 2 was 0.743 (95% IC 0.681–0.806).

**Discussion**

Despite improved kidney transplantation clinical management, the CMV infection is still a concern. According to the best clinical guidelines, both strategies available for preventing the consequences of CMV infection, universal prophylaxis or preemptive treatment, present advantages and some disadvantages, and centers should opt for one or another, considering their characteristics. For example, in Brazil, more than 90% of kidney transplantation is supported by the public health system, and the costs of universal prophylaxis are not disbursed, which can occur in other low and mid-income countries. In the present study, we compared the main CMV-related outcomes when we transitioned from pp65 to RT-PCR in kidney transplant recipients receiving preemptive treatment as a strategy to prevent CMV infection and identified potential clinical predictors of the CMV-related events.

In our primary hypothesis, we were expecting to detect a reduction in the rate of symptomatic patients, considering that the RT-PCR has high sensitivity to detect low viral load. Moreover, antigenemia presents several limitations in CMV treatment, highlighted in the updated international consensus in 2013 as the lack of standardization, the dependence of the subjective interpretation, and its performance when the count of neutrophils is low. Consequently, since that, quantitative nucleic acid amplification testing has been established as the "cornerstone for diagnosis and monitoring for CMV infection and disease." Indeed, using a threshold of 5,000 UI/mL to start the preemptive treatment in the RT-PCR era, the frequency of treatment in our cohort was not different from those observed with pp65 antigenemia, and the time to treatment onset was precisely the same. Additionally, we did not find the expected reduction in the rate of patients who had CMV disease.

When the treatment is started, the viral load seems to be associated with the clinical resolution. In an exploratory analysis from the VICTOR study, where plasma samples of 267 participants were retested, and the viral load was calibrated based on the CMV World Health Organization, the faster resolution of CMV disease after treatment with valganciclovir was 57% more likely when the initial viral load was lower than 18,200 UI/mL. Different from the VICTOR study, in our cohort, all patients were conducted under the preemptive treatment with closer viral load screening; therefore, the main target of the clinical management was to avoid the symptomatic infection. After that, the median of viral load was 7,093 UI/mL, and 75% of patients had a viral load lower than 12,327 UI/mL. Last, despite the low frequency of invasive disease, we consider that the rate of symptomatic infection was higher than we expected when we transitioned from antigenemia to RT-PCR.

Although the local clinical approach in the first era had preconized one-week extension in the treatment after the last negative antigenemia, the duration of treatment to reach a viral load suppression was longer with RT-PCR. There was an initial concern that highly sensitive assays to manage the CMV infection
resulted in prolonged treatments and unnecessary exposure to antiviral therapy\textsuperscript{14}. However, a shorter time of treatment using standardized quantitative nucleic acid testing has been demonstrated in a previous study\textsuperscript{17,18}. Additionally, as a direct consequence of a more prolonged treatment time with RT-PCR observed in our study, we expected that the rate of the retreating requirement was reduced, considering that reaching a virological suppression seems to be predictive of clinical response\textsuperscript{17}. Indeed, the need for retreatment was slightly lower in the PCR era (11.8\% vs. 14.7\%); however, this difference was not significant. Taking these results together and the indirect evidence figured in the present study, it is possible to speculate that a cut-off lower than 5,000 UI/mL could reach lower rates of symptomatic patients than we observed. On the other hand, it seems to be that a cut-off of 10 positive cells associated with a seven-day extended treatment would be equivalent to the viral load suppression achieved by treatment guided for PCR, and this find can be helpful for centers that have only antigenemia as the option to conduct the preemptive treatment.

In a secondary analysis, we sought predictors of CMV-related events in patients receiving preemptive treatment. More recently, the quantification of the T-cell-specific response against CMV antigens has been considered a promisor tool for predicting the CMV-related events\textsuperscript{19}, and it would be helpful for preemptive treatment optimization\textsuperscript{20}. However, its use is not standardized for wieldy clinical use. Here, two clinical predictors were associated with the probability of the first CMV-related event: early acute rejection and 30-day graft function.

The association between acute rejection and CMV replication is mainly supported by immunosuppression intensification to treat the immunological event\textsuperscript{21}. Therefore, we included the acute rejection within 30 days as an independent variable in the multivariable model. Of note, early acute rejection was also an independent predictor for CMV disease, which supports the correlation between the intensity of immunodepression and the spectrum of infection. Furthermore, the association of early graft function and CMV-related events has been previously reported\textsuperscript{22}, which was confirmed by our group in an independent cohort of 938 patients transplanted between 2014 and 2015, where the 30-day eGFR was a strong predictor of CMV infection or disease: OR for each mL/min/1.73m\textsuperscript{2} = 0.98; 95\% CI, 0.97-0.99\textsuperscript{23}.

How could these three variables converge to the likelihood of the CMV-related events? It is known that some cytokines expressed in tissue damage, especially in the ischemia and reperfusion injury, and acute rejection (TNF\textsubscript{α}, IL-6, and IL-1β), promote CMV replication\textsuperscript{24}. Nevertheless, the initial high exposure to tacrolimus and the levels of mycophenolate influenced by the immunological compatibility could increase the risk of CMV replication.

Our study has several limitations. First, a historical study, carried out in a single-center, with groups followed in two different eras, is associated with some biases. Second, in both periods, the thresholds for starting the preemptive treatment were defined by the clinical routines due to the lack of robust evidence to support a prespecified cut-off. Third, some differences in the baseline characteristics were observed when both eras were compared, mainly in the donor’s demography, which could be associated with worse
graft function 30 days after transplantation, although the model to evaluate predictors of CMV-related events has been adjusted for eras. Last, the adherence to the local approach was not directly measured.

In conclusion, in the present study, we did not observe a reduction in the frequency and in the time for CMV-related events, as well as in the requirement for retreatments when the antigenemia was transitioned to quantitative nucleic acid amplification testing, using a threshold of 5,000 UI/mL in the standardization RT-PCR for starting the preemptive treatment in kidney transplant recipients. Finally, we defined 30-day graft function and early acute rejection as clinical predictors of CMV replication after transplantation.

Methods

Study Design and population

This was a retrospective sequential single-center cohort study carried out at Hospital do Rim – São Paulo, Brazil. Considering that the study was aimed to evaluate a transition in methods chosen to assess CMV viremia in the preemptive treatment (antigenemia or PCR), patients were grouped in two different eras: the use of antigenemia in the first era and the use of RT-PCR in the second one. The study was conducted following the Declaration of Helsinki and was approved by the Ethics Committee at Federal University of São Paulo (identification number CAEE 05677618.6.0000.5505, and approval number 3.164.538). Being a retrospective study, the informed consent form was waived by the Ethics Committee at Federal University of São Paulo.

The eligible participants were kidney transplant recipients who underwent kidney transplants between March 2016 and August 2018, under preemptive treatment for the risk reduction of CMV disease, and who completed one year of follow-up. Other inclusion criteria were: age at the transplantation time older than 18 years, immunological induction with thymoglobulin, and the immunosuppression regime of maintenance based on tacrolimus and mycophenolate, owing to it was the main indication for the preemptive treatment according to the local approach. In addition, recipients of kidney transplants combined with another solid organ or negative CMV serology were excluded. The time for transition from antigenemia to RT-OCR was from March 2017 to February 2018, when patients started treatment based on antigenemia, but the antiviral was usually interrupted based on the result of PCR. Therefore, patients transplanted in this period were excluded too.

Immunosuppression and prophylaxis

All patients received a single dose of 3.0 mg/kg of Thymoglobulin as an induction strategy, following the local practice, which was previously published\(^{25}\). The maintenance immunosuppression regime consisted of a combination of tacrolimus, prednisone, and acid mycophenolate. The dose of tacrolimus was adjusted to maintain $C_0$ levels between 5-15 ng/mL. In addition, all patients underwent prophylaxis with albendazole for parasitic infections and sulfamethoxazole-trimethoprim for *Pneumocystis jirovecii*. Monitoring and treatment of CMV infection
For the preemptive treatment, viremia was collected every two weeks from the 21st after transplantation. The CMV tests results were available one day after sample collection. When patients presented the preemptive treatment criteria, the antiviral was started two or three days after the sample collection. For pp65 antigenemia, after peripheral blood extraction, leukocytes were incubated with C10/C11 antibodies and other reagents from the CMV Brite Turbo kit (IQ® Products, Groningen, Netherlands). The presence of pp65 antigen was detected by a homogeneous yellow-green nuclear pattern in a fluorescence microscope, and the final result was expressed by the number of positive cells per 200,000 leukocytes26. The RT-PCR was performed with a commercial Abbott RealTime CMV kit. The DNA extraction, amplification, and detection were performed in the automated Real-Time m2000 system (Abbott Molecular Inc), having the DNA sequences of the UL34 and UL80.5 CMV genes as targets15. The procedure consisted of a real-time amplification reaction on a microplate, with programmable temperature control and variation, and simultaneously an optical fluorescence detection system with the reaction in a thermocycler16,27. The reported limits of detection and quantification were 31.2 IU/mL.

In the antigenemia era, the preemptive treatment was indicated in the presence of 10 or more positive cells in asymptomatic patients or in patients who presented symptoms attributable to CMV infection, independent of the number of positive cells. In the RT-PCR era, the preemptive treatment was indicated in the presence of 5,000 UI/mL or more in asymptomatic patients or in patients who presented symptoms attributable to CMV infection, independent of viral load in the RT-PCR. The treatment consisted of intravenous ganciclovir 5 mg/kg twice a day, adjusted for renal function. During treatment, monitoring was carried out weekly. For the antigenemia era, the treatment was extended for seven following days from the first negative result. On the other hand, in the RT-PCR era, the treatment was interrupted when the result was undetectable (>31 UI/mL)28. Monitoring after the treatment interruption was maintained over the following three months.

Definitions

Cytomegalovirus infection was classified according to the Third International Cytomegalovirus Consensus as CMV infection, CMV disease, and invasive disease4: infection was defined by the evidence of viral replication in the absence of symptoms attributable to the viral activity, whereas disease was determined by evidence of CMV replication, associated with attributable symptoms or laboratory abnormalities, and the invasive disease was defined by the presence of the virus in the histological analysis of any tissue regardless of the result of the viremia or by retinitis, meningitis, or encephalitis. Recurrences of CMV infection or disease were defined by the need for a new treatment after the complete remission of the previous episode. Delayed graft function was defined by the need for dialysis during the first week, and acute rejection (AR) as treated rejections, proven by biopsy or not, according to Banff’s classification29. The estimated glomerular filtration rate (eGFR) was calculated by the CKD-EPI equation30.

Outcomes
The primary outcome was a CMV-related event defined as infection or CMV disease, which occurs first. Additionally, the incidence of disease, the time for detecting events, the length of treatment with ganciclovir, and the frequency of retreatment requirement were compared according to the era. The incidence of acute rejection and 1-year graft function were secondarily evaluated.

**Statistical analysis**

Continuous variables are summarized as the median and interquartile range (1st; 3rd) and compared by test U of Mann-Whitney, and categorical variables are summarized as absolute and relative frequencies and compared by the $X^2$ test or Fisher's exact test. These comparisons were fitted for the era (antigenemia vs. RT-PCR).

The cumulative incidence of CMV-related events, CMV disease, and acute rejection were calculated by Kaplan-Meier and compared by log-rank test. The frequency of retreatment requirement was compared by the $X^2$ test. Time for detecting CMV-related events and the length of treatment with ganciclovir according to era were compared by test U of Mann-Whitney. For graft function, a generalized estimated equation was performed to compare the mean of eGFR between eras (antigenemia and RT-PCR). The model was adjusted by the Bonferroni test. The same approach was performed to compare tacrolimus levels between eras.

The potential clinical predictors for the primary outcomes (CMV-related events and CMV disease) were analyzed by the proportional hazard ratios (HR) throughout the Cox regression modeling (backward stepwise). The variables for the model were selected in bivariable analyses comparisons of patients who had CMV-related events with those who did not (supplementary material). The same approach was performed to select candidates variables related to CMV disease. Variables that reached a P-value < 0.20 were considered for the final modeling. The median was imputed for the only variable with missing values, 30-day eGFR (1.38%). The accuracy of the final model was assessed by the area under a receiver operating characteristic (AUC-ROC). Statistical analyses were performed using Statistical Package for the Social Sciences (version 26; IBM, Armonk, NY, USA), and statistical significance was defined as P<0.05, with the 95% confidence interval.

**Abbreviations**

ADPKD: Autosomal dominant polycystic kidney disease

AR: Acute rejection

CI: confidence interval

CIT: Cold ischemia time

CKD: Chronic kidney disease
CMV: Cytomegalovirus

cPRA: Calculated panel reactive antibody

CTS: Collaborative transplant study

DGF: Delayed graft function

DNA: Deoxyribonucleic acid

eGFR: Estimated glomerular filtration rate

GEE: Generalized estimating equation

GLMM: Generalized linear mixed models

HLA: Human leukocyte antigen

HR: hazard ratio

KDPI: Kidney donor profile index

mTOR: Mammalian target of rapamycin

OR: odds ratio

RT-PCR: Real-time polymerase chain reaction

Declarations

Acknowledgements:
The authors thank to CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for the support received.

Author contributions:

MRN: designed and performed the study, collected and analyzed the data and wrote the paper.

LRRM: performed the study, analyzed the data and wrote the paper.

RMG: collected data and approved the manuscript.

CB: collected data and approved the manuscript.

JT: collected data and approved the manuscript.
LAV: performed the study, analyzed the data and approved the manuscript.

CRF: designed and performed the study, analyzed the data and approved the manuscript.

JMP: analyzed the data and approved the manuscript.

HTS: designed and performed the study, analyzed the data and wrote the paper.

Conflict of interest:

They authors have no conflict of interest to disclose.

References


**Figures**

**Figure 1**

Flowchart of the population.

[The transition period is from March 2017 to February 2018, when the service had adopted both methods for viremia detection. CMV, cytomegalovirus; HLA, human leukocyte antigen; mTOR, mammalian target of rapamycin; RT-PCR, real-time polymerase chain reaction]

**Figure 2**

Primary and secondary outcomes: CMV-related events, CMV disease, acute rejection and graft function.

[2.A) cumulative incidence of the first CMV-related event (infection or disease) according to the era. 2.B) cumulative incidence of CMV disease according to the era. 2.C) cumulative incidence of acute rejection according to the era. 2.D) graft function assessed by estimated glomerular filtration rate according to the era. In the figures A, B and C, the P-value was calculated by log-rank. The means of graft function over time and according to era were compared by generalized estimating equation modeling and adjusted by Bonferroni test. Squares and circles in the plot represent mean and bars the 95% confidence interval. RT-PCR, real-time polymerase chain reaction.]
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.docx