High Human Cytomegalovirus DNAemia Early Post-Transplantation Associates With Irreversible and Progressive Loss of Renal Function

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ABSTRACT

Background
Transplant recipients are prone to viral infections, which could affect renal transplantation outcome. Our aim was to assess the effects of early human cytomegalovirus (CMV) DNAemia on transplant renal function.

Methods
264 (age 50.9±13.5; male 55%) Renal transplantation recipients undergoing preemptive anti-CMV therapy were retrospectively categorized based on early (<3 months post-Tx) CMV peak viral load (PVL); PVL≤536, PVL536-6310 or PVL>6310 International Units/ml (IU/ml). Estimated Glomerular Filtration Rate (eGFR) was analyzed between 1 and 36 months post-transplantation with Kruskal-Wallis test, linear regression and a Linear Mixed-Effects Model.

Results
CMV infection was detectable in 113 (43%) recipients within 49 [38-67] days. Subjects with PVL>6310 had statistically significant ~5-13 ml/min lower eGFR between 3 and 36 months compared to PVL≤536 and PVL536-6310. eGFR declined from 46.1 to 40.7 ml/min/1.73m2 (-12%) over three years and the annual decrease for pronounced infection with high PVL was 2.0 mL/min/1.73m2 faster than for non- or mildly infected subjects.

Conclusions
In conclusion, high CMV DNAemia early after renal transplantation was associated with significant loss of renal function, from which subjects did not recover. The severity of infection (high PVL early post-transplantation), more than the infection per se, was related to irreversible and progressive loss of renal function.
INTRODUCTION

Human cytomegalovirus (CMV) belongs to the family of β-herpesviruses, which are characterized by large infected cells, a limited host range and species- and strain-specific tropism [1]. In the general immunocompetent population, CMV establishes a lifelong latent infective state without significant clinical manifestation following primary infection [2]. The reservoir for latent CMV is probably within hematopoietic stem cells within the bone marrow, particularly in undifferentiated cells of the myeloid lineage and monocytes [3, 4]. However, immunosuppression of recipients frequently allows CMV reactivation in the post-transplantation period. The required reduction of immunosuppression permits clearance of the virus but simultaneously induces a risk for acute rejection resulting in decreased graft function and transplantation outcome [5].

Death-censored graft failure has improved over the last decades, but is still at 26% from deceased and 14% from living donors at five years post-transplantation [6]. Despite antiviral medication, CMV remains a prominent factor involved in this substantial loss of transplants [7]. CMV is the most common viral infection after renal transplantation and is associated with decreased late graft and patient survival [8, 9], CMV disease [10] and overall mortality [11]. However, the efficacy of preemptive therapy in the prevention of CMV disease remains to be clarified [12]. End-stage renal allograft dysfunction and reduced graft survival are associated with CMV protein expression in the graft, with CMV DNAemia increasing as graft function deteriorated [13].

Real-Time Quantitative Polymerase Chain Reaction (RT-PCR) enables high-throughput and accurate CMV DNA measurements [5] during monitoring of the recipient's infective state and response to CMV treatment. Previous studies have focused on the risk of CMV infection during and after (renal) transplantation along with its effect on transplant survival and rejection. However, the effect of the severity of a CMV infection on the course of renal function post-transplantation has not been addressed previously. CMV infection may affect renal function previous to actual transplant loss, and could substantially affect recipient transplant health. Therefore, the aim of this study was to determine the effect of CMV infection, characterized by early CMV peak viral load (PVL), on renal function (as determined by eGFR). Renal function decline has been demonstrated to be predictive for graft failure after transplantation [14], and as such may provide additional etiological insight. These studies demonstrate that high CMV DNAemia early post-transplantation, opposed to a mild infection, was associated with progressively lower renal function. This suggests that the severity of CMV infection is more important than the occurrence of the infection itself.
MATERIALS AND METHODS

Subject selection

From 266 consecutive renal transplant recipients undergoing a first, second or third renal transplantation in 2010 or 2011 at the University Medical Center Groningen (UMCG), 264 were selected for undergoing preemptive anti-CMV treatment. The single-center study design was evaluated by the local Ethics Committee at the UMCG and deemed exempt from formal ethical approval because it falls outside the scope of Medical-Scientific Research (decision METc 2015/448, 2015/10/05, Groningen). The authors have adhered to the Declaration of Helsinki. The clinical and research activities reported are consistent with the Principles of the Declaration of Istanbul as outlined in the ‘Declaration of Istanbul on Organ Trafficking and Transplant Tourism’.

Nucleic acid extraction and CMV detection

CMV DNAemia was monitored post-transplantation using an in-house CMV PCR (detection threshold: 536 IU/ml) on DNA extracted from whole blood. All samples were extracted according to the manufacturer instructions, using 190 µl whole blood samples with the addition of 10 µl seal herpes virus (PhHV) as an internal control [13]. Samples collected before January 2012 were extracted using the MagNAPure LC with the MagNA Pure LC Total Nucleic Acid Isolation kit (Roche Diagnostics, Mannheim Germany). After January 2012, DNA was extracted with the MagNAPure 96 system using the MagNAPure 96 DNA and viral NA small volume kit (Roche Applied Bioscience, Mannheim, Germany).

Primers for CMV were targeted against the DNA polymerase; Forward primer; 5’-GCCGATCGTAAAGAGATGAAGAC; Reverse primer; 5’-CTCGTGCGTGTGCTACGAGA; Probe 1, 5’-VIC-AGTGCAGCCCCGACCATCGTTC. Probe 2 (5’-VIC-AGTGCAAGCCCCGACCATCATTC) was added to the reaction from March 2012 onwards. The PCR was performed in a 50 µl reaction consisting of 2X TaqMan Universal Mastermix (Life Technology, Carlsbad, USA), 200 nM CMV forward primer, 800 nM of CMV reverse primer, 200nM of CMV probe 1, 100nM of CMV probe 2, 50nM PhHV forward, 200nM PhHV reverse, 100nM PhHV probe, 5mg/ml Bovine serum albumin and 20 µl of isolated DNA. The ABI Prism 7500 sequence detection system (Life Technologies, USA) was used for amplification and detection with the following thermal conditions: 2 min at 50°C, 10 min at 95°C followed by 42 repeats of 15 sec at 95°C and 1 min at 60°C. Viral loads were derived from the raw Ct-values using a previously established standard curve for both copies/ml (based on electron microscopy counted CMV stock (Advanced Biotechnologies Incorporated)) and International Units (IU)/ml (based on the 1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification techniques 09/162 (National Institute for Biological Standards and Control). Validity of these external standard curves was continuously monitored by using a low and high range positive control calibrated to these standards.
Cytomegalovirus DNAemia Early Post-Transplantation

CMV monitoring was protocolled at every outpatient visit post-transplantation (weekly in the first month, biweekly in month 2 and 3 and monthly afterwards) for all recipients. Anti-CMV therapy with valganciclovir was initiated at first detection of CMV DNA irrespective of exact viral load for primary infections (CMV IgG-negative pre-transplantation and >536 IU/ml), at all symptomatic reactivations (CMV IgG-positive pre-transplantation and >536 IU/ml) irrespective of viral load and for reactivations at viral load exceeding 64,575 IU/ml. Treatment is started orally, and switched to intravenous ganciclovir during occurrence of diarrhea/colitis or severe symptoms. Therapeutic or curative dosages were adjusted for renal function and applied until CMV DNA was negative (<536 IU/ml) for two consecutive measurements. The peak viral load (PVL) is the maximum value of CMV DNA measurements in the first three months post-transplantation.

Collection of clinical data

Virological data, histopathology data, recipient and donor characteristics were obtained from subjects’ local electronic files at the University Medical Center Groningen.

Subject PVL categorization

Subjects were retrospectively categorized based on CMV PVL in whole blood within the first three months after transplantation (“early”); 1) PVL≤536: PVL below the detection limit of 536 IU/ml, 2) PVL536-6310: PVL of 536-6310 IU/ml and 3) PVL>6310: PVL above 6310 IU/ml. The cut-off value of 6310 IU/ml was selected because this represents the median peak viral load in CMV-positive recipients.

Immunosuppression

Immunosuppressive therapy consisted of a quadruple regimen for the majority of subjects, with individual adaptions based on clinical indication: induction with Basiliximab; calcineurin-inhibitors tacrolimus or cyclosporine A; a proliferation inhibitor mycophenolate mofetil (MMF), mycophenolic sodium (MPS) or azathioprine (AZA); and prednisolone. Basiliximab and prednisolone are common to all regimens, for distribution of the other components see Table 1.

eGFR measurements

Glomerular Filtration Rate was estimated (eGFR) using the Modification of Diet in Renal Disease (MDRD) [15, 16] formula based on blood creatinine during follow-up at 1, 3, 6, 12, 24 and 36 months post-transplantation (2010-2014). Recipients were put to 0 ml/min/1.73m² from the moment of return to dialysis or nephrectomy. Values closest around the intended eGFR measurement were used within specific intervals (±2 weeks, ±3 weeks; ±1.5 months; ±3 months; ±5 months and ±6 months, respectively).
Statistical analyses

Data are reported as means with standard deviations, medians with [25 – 75] interquartile ranges, or proportions where appropriate. Statistical analyses were performed and figures created using IBM SPSS Statistics 22 and GraphPad Prism v6.07. P-values are two-tailed, were considered statistically significant when <0.05, and are represented together with 95% confidence intervals where applicable. Comparison of baseline characteristics between the different PVL groups was performed using the Kruskal-Wallis test followed by multiple comparisons for continuous data, and the Chi-squared test for discrete data. Interstitial fibrosis/tubular atrophy (IF/TA) in biopsies was compared using a Chi-squared test.

At every time point, the association between eGFR and recipient sex and age, donor sex and age, recipient cause of disease, Caucasian race, previous transplantation, immunosuppressive regimen, Tacrolimus, type of renal replacement therapy, duration of renal-replacement therapy, antibody-mediated rejection, BK nephropathy, delayed graft function, cellular rejection, Valganciclovir resistance, primary infection, donor-recipient CMV serostatus, HLA AB and DR mismatches, donor living, donation after brain death, ischemia time and PVL>6310 was analyzed using univariable linear regression. All variables with P<0.10 at at least one time point between 1 and 36 months were included in the multivariable linear regression analyses at 1, 3, 6, 12, 24 and 36 months post-transplantation.

To estimate the association between PVL>6310 and the change in eGFR over time a Linear Mixed-Effects Model analysis on all eGFR-measurements was applied with the patient as grouping variable, with a random intercept and an unstructured covariance structure, including PVL>6310, predefined time (time post-transplantation, year), and the interaction term between PVL>6310 and time post-transplantation (PVL>6310*time post-transplantation). This model was adjusted for the same variables as the multivariable regression analyses.

RESULTS

Study population and CMV infection specifications

In order to determine the effect of CMV infection on renal function, 264 renal transplant recipients receiving preemptive anti-CMV treatment were included (2010-2011). 11 Patients had returned to dialysis, 10 had undergone nephrectomy, 21 had deceased and 2 lacked eGFR measurements at the end of follow-up (Figure 1). For all 264 transplantations combined, recipient and donor age were 50.9±13.5 and 50.9±12.6 years with 55% male recipients and 51% male donors. 44.7% of donor organs originated from living donors. CMV infection was detectable in 113 recipients (43%) with a median PVL of 6310 [2397 - 63,834] IU/ml within 49 [38-67] days post-transplantation.
<table>
<thead>
<tr>
<th>Table 1. Subject characteristics, categorized per PVL category.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PVL ≤ 536</strong></td>
</tr>
<tr>
<td>No. of subjects</td>
</tr>
<tr>
<td>Recipient sex, males</td>
</tr>
<tr>
<td>Recipient age, years</td>
</tr>
<tr>
<td>Recipient race, Caucasian</td>
</tr>
<tr>
<td>Graft failure within 3 years</td>
</tr>
<tr>
<td>Return to dialysis</td>
</tr>
<tr>
<td>Nephrectomy</td>
</tr>
<tr>
<td>Death</td>
</tr>
<tr>
<td>Graft failure, time post-Tx, days</td>
</tr>
<tr>
<td>Underlying disease pre-transplantation</td>
</tr>
<tr>
<td>Primary glomerular disease / glomerulonephritis / vasculitis</td>
</tr>
<tr>
<td>Cystic kidney disease</td>
</tr>
<tr>
<td>Dysplasia/hypoplasia</td>
</tr>
<tr>
<td>Renovascular disease / hypertension</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
</tr>
<tr>
<td>Tubulo-interstitial nephritis</td>
</tr>
<tr>
<td>Urological complications</td>
</tr>
<tr>
<td>Other/unknown causes</td>
</tr>
<tr>
<td>Previous transplantation</td>
</tr>
<tr>
<td>Renal replacement therapy</td>
</tr>
<tr>
<td>Preemptive transplantation</td>
</tr>
<tr>
<td>Peritoneal dialysis</td>
</tr>
<tr>
<td>Hemodialysis</td>
</tr>
<tr>
<td>Duration of RRT, months</td>
</tr>
<tr>
<td>No. of HLA AB mismatches</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>No. of HLA DR mismatches</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>Delayed graft function</td>
</tr>
<tr>
<td>BK nephropathy &lt;3 months</td>
</tr>
<tr>
<td>Antibody-mediated rejection &lt;3 months</td>
</tr>
<tr>
<td>T-cell-mediated rejection &lt;3 months</td>
</tr>
<tr>
<td>Rejection, days post-Tx</td>
</tr>
</tbody>
</table>
### Protocol biopsies, n

| Protocol biopsies, n | 36 (24) | 15 (26) | 9 (16) | 0.23 |

### Primary immunosuppression

| CyA + MMF/MPS | 97 (64) | 46 (81) | 51 (91) | 0.005 |
| Tac + MMF/MPS | 48 (32) | 11 (19) | 5 (9) |
| CyA + AZA | 1 (1) | 0 (0) | 0 (0) |
| Tac + AZA | 5 (3) | 0 (0) | 0 (0) |
| Cyclosporine A (vs. Tac) | 98 (65) | 46 (81) | 51 (91) | <0.001 |

### Cyclosporine A blood concentrations, µg/L (at 3 months)

| Cyclosporine A blood concentrations, µg/L (at 3 months) | 185 [167 - 216] | 189 [156 - 220] | 171 [133 - 205] | 0.17 |

### Tac blood concentrations, µg/L (at 3 months)

| Tac blood concentrations, µg/L (at 3 months) | 10.2 [8.6 - 11.8] | 9.5 [6.7 - 11.2] | 8.5 [7.4 - 10.6] | 0.39 |

### Valganciclovir resistance

| Valganciclovir resistance | 0 (0) | 1 (2) | 1 (2) | 0.26 |

### First positive CMV RT-PCR, IU/ml

| First positive CMV RT-PCR, IU/ml | N/A | 851 [634 - 1,806] | 1832 [882 - 8,396] | <0.001 |

### First positive CMV RT-PCR, days post-Tx

| First positive CMV RT-PCR, days post-Tx | N/A | 33 [22 - 49] | 31 [23 - 37] | 0.19 |

### Peak viral load, IU/ml

| Peak viral load, IU/ml | N/A | 2,397 [1,483 - 3,754] | 64,105 [19,490 - 324,131] | <0.001 |

### Peak viral load, days post-Tx

| Peak viral load, days post-Tx | N/A | 50 [35 - 65] | 48 [39 - 74] | 0.77 |

### Primary infection, n

| Primary infection, n | N/A | 3 (6) | 23 (42) | <0.001 |

### Donor sex, males

| Donor sex, males | 77 (51) | 29 (51) | 29 (52) | 0.99 |

### Donor age, years

| Donor age, years | 52 [45 - 60] | 50 [39 - 60] | 53 [45 - 58] | 0.35 |

### Transplant source

| Transplant source | 0.14 |
| Port-mortem | 73 (48) | 34 (60) | 39 (70) |
| DBD (remainder DCD) | 46 (31) | 25 (44) | 25 (45) |
| Ischemia time, hours | 7 [3 - 14] | 12 [3 - 16] | 13 [3 - 17] | 0.01 |

### CMV serologic status

| CMV serologic status | <0.001 |
| Donor-negative, recipient-negative | 56 (37) | 0 (0) | 0 (0) |
| Donor-negative, recipient-positive | 28 (18) | 19 (33) | 11 (20) |
| Donor-positive, recipient-negative | 29 (19) | 3 (5) | 23 (41) |
| Donor-positive, recipient-positive | 34 (23) | 31 (55) | 21 (37) |
| Unknown | 4 (3) | 4 (7) | 1 (2) |

Kruskal-Wallis test followed by multiple comparisons for continuous data and Chi-squared test for discrete data. Unless otherwise noted, data represent median [Q1-Q3] for continuous variables and n (%) for discrete variables. RRT: Renal replacement therapy; HLA: Human Leukocyte Antigen; CyA: cyclosporin A; MMF: mycophenolate mofetil; MPS: Mycophenolate sodium; Tac: tacrolimus, AZA: azathioprine; CMV: human cytomegalovirus; RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; Post-Tx: post-transplantation, DBD: Donation after brain death; DCD: Donation after cardiac death; CMV: human cytomegalovirus; D-/R-: donor-seronegative, recipient-seronegative, N/A, not applicable.
PVL was determined in the first three months post-transplantation, because viral infections occur relatively soon after transplantation and the suspected most pronounced effect of infection in this period. Categorization according to PVL resulted in 151 recipients (57%) with PVL≤536, 57 recipients (22%) with PVL 536-6310 and 56 recipients (21%) with PVL>6310 (Table 1). The first detection of CMV DNA occurred at similar time for PVL536-6310 (33 [22 - 49] days) and PVL>6310 (31 [23 - 37]) as did time to PVL (50 vs. 48 days). However, PVL was higher for PVL>6310 (2,397 vs 64,105 IU/ml). Additional recipient and donor characteristics are depicted in Table 1. The three PVL categories differed in recipient age, race, type of renal replacement therapy, primary immunosuppressive therapy, Cyclosporine A, primary infection, ischemia time and CMV serostatus (Table 1). All were considered during subsequent univariable analyses, and included in multivariable analyses when P<0.10.

PVL>6310 associated with lower eGFR at 3, 6, 12, 24 and 36 months after transplantation

In order to determine the effect of PVL on renal function, eGFR was compared between PVL≤536, PVL536-6310 and PVL>6310 (Figure 2). In subjects with high CMV DNAemia (PVL>6310), eGFR was lower compared to non-infected and mildly-infected recipients at 3, 6, 12, 24 and 36 months post-transplantation (Kruskal Wallis; P=0.02, P=0.01, P=0.02, P=0.006 and P=0.01). Nevertheless, eGFR was similar at 1 month post-transplantation (P=0.20), suggesting that renal function did not differ immediately after transplantation. eGFR for PVL>6310 declined from 46.1 to 40.7 ml/min/1.73m2, a substantial and clinically relevant 12% decrease (Figure 2).
Table 2. Multivariable linear regression for eGFR at various months post-transplantation.

<table>
<thead>
<tr>
<th>Change</th>
<th>95% CI</th>
<th>P</th>
<th>Mean eGFR PVL≤6310 (ml/min/1.73 m²)</th>
<th>Mean eGFR PVL&gt;6310 (ml/min/1.73 m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 month</td>
<td>-1.81</td>
<td>0.57</td>
<td>51.95 ± 22.76</td>
<td>46.73 ± 25.32</td>
</tr>
<tr>
<td>3 months</td>
<td>-6.88</td>
<td>0.03</td>
<td>52.34 ± 21.27</td>
<td>44.25 ± 19.28</td>
</tr>
<tr>
<td>6 months</td>
<td>-7.46</td>
<td>0.01</td>
<td>51.26 ± 20.44</td>
<td>42.55 ± 16.32</td>
</tr>
<tr>
<td>12 months</td>
<td>-10.88</td>
<td>0.01</td>
<td>51.09 ± 22.60</td>
<td>43.00 ± 18.98</td>
</tr>
<tr>
<td>24 months</td>
<td>-12.26</td>
<td>0.005</td>
<td>51.12 ± 25.74</td>
<td>40.92 ± 19.83</td>
</tr>
<tr>
<td>36 months</td>
<td>-12.36</td>
<td>0.003</td>
<td>51.12 ± 25.74</td>
<td>40.92 ± 19.83</td>
</tr>
</tbody>
</table>

All variables with P<0.10 at any time point between 1 and 36 months in the univariable linear regression were included in the multivariable analysis at 1, 3, 6, 12, 24 and 36 months. The characteristics of additional variables included in the analyses can be found in SDC; Supplementary Table S2.

Table 3. Linear Mixed Effects Model for the development of eGFR from 1-36 months post-transplantation.

<table>
<thead>
<tr>
<th>Change</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>31.00</td>
<td>0.004</td>
</tr>
<tr>
<td>Recipient; PVL&gt;6310</td>
<td>-7.13</td>
<td>0.35</td>
</tr>
<tr>
<td>Recipient; Time post-transplantation, year</td>
<td>1.99</td>
<td>0.001</td>
</tr>
<tr>
<td>Recipient; PVL&gt;6310/time post-transplantation</td>
<td>2.21</td>
<td>0.44</td>
</tr>
</tbody>
</table>

All variables with P<0.10 at any time point between 1 and 36 months from the univariable linear regression were included in the Linear Effects Mixed Model at 1, 3, 6, 12, 24 and 36 months. The estimate for time post-transplantation indicated a 1.99 ml/min/1.73m² change per year (P=0.001) in the PVL>6310 category. The estimate for PVL>6310 is not statistically significant. The estimate for PVL≤6310 is significantly associated with eGFR. It is a higher change per year (P=0.001) in the PVL≤6310 category. Only the peak graft load-related parameters have been displayed for clarity, but the model also included the significantly associated parameters from the univariable analysis: recipient sex and age, recipient underlying disease, ischaemic complications, calcium phosphate precipitation, delayed graft function, cellular rejection ≥3 months, donor living status, donation after brain death and ischemia time.
Because the progression of eGFR over time overlapped between PVL≤536 and PVL536-6310 post-transplantation, these groups were combined into PVL≤6310 for further analyses. The effect of various recipient, donor and transplantation characteristics on renal function was analyzed at every time point using univariable linear regression (SDC; Supplementary Table S1). eGFR was not significantly different at 1 month post-Tx, but was 7.99 (3 months post-transplantation, P=0.012), 8.51 (6 months, P=0.005), 9.83 (12 months, P=0.004), 11.00 (24 months, P=0.003) and 10.88 (36 months, P=0.006) ml/min/1.73m^2 lower for subjects with a PVL>6310 compared to those below the threshold. In available protocol biopsies at one year post-transplantation (n=110), interstitial fibrosis over 10% was more frequent in the high PVL group compared to the low group (Chi-squared test; P=0.04).

**PVL>6310 significantly associated with eGFR irrespective of additional transplantation parameters**

At every time point, PVL>6310 remained associated with eGFR (Table 2) after adjustment for recipient, donor and transplantation characteristics (SDC; Supplementary Table S2) in the multivariable linear models. Subjects with PVL>6310 had a 6.88 (3 months; P=0.03), 7.46 (6 months; P=0.01), 9.34 (12 months; P=0.01), 10.88 (24 months; P=0.005) and 12.26 (36 months; P=0.003) mL/min/1.73m^2 lower renal function compared to subjects with PVL≤6310. Renal function was not significantly different at 1 month post-Tx (P=0.57).

**PVL>6310 related to a more pronounced eGFR decline**

Linear Mixed-Effects Model analysis was performed to determine the association between PVL>6310 and eGFR over time taking into account all the measured time points between 1 and 36 month post-transplantation (Table 3). All the parameters with P<0.10 from the univariable analyses were included. The estimate for time post-transplantation indicated a 1.99 ml/min/1.73m^2 change per year (P=0.001) in the PVL≤6310 category. The estimate for PVL>6310 denotes the difference in eGFR
at baseline between the groups (time = 0, this is an extrapolation since the first measured eGFR was at 1 month post-Tx), which is not statistically significant. The estimate for PVL>6310*time post-transplantation denotes the difference in annual decline in eGFR (the slope of the graph), which is 2.21 ml/min/1.73m²/year faster for PVL>6310 compared to PVL≤6310. In short, the eGFR declined for PVL>6310 subjects compared to PVL≤6310 subjects.

**DISCUSSION**

This study demonstrates the intermediate and long-term progressive loss of renal function after high CMV DNAemia, and its faster annual decline after renal transplantation. A CMV PVL exceeding 6310 IU/ml in the first three months was associated with a lower eGFR between 3 and 36 months independently of recipient, donor and transplantation characteristics. This indicates that recipients do not recover from early CMV-associated renal function loss even at three years post-transplantation. PVL>6310 was not independently associated with eGFR after one month, potentially because CMV-induced damage is time-dependent and only clinically manifests from three months onwards.

It is important to consider that likely not the infection in itself, but the extent of CMV DNAemia affects renal function. A mild CMV DNAemia, defined by us as a PVL below 6310 IU/ml, did not associate with lower renal function at any time point. However, a high CMV DNAemia correlated to a substantially lower eGFR, ranging from 6.88 to 12.26 mL/min/1.73m². eGFR for PVL>6310 decreased from 46.1 to 40.7 mL/min/1.73m² in three years, a 12% decrease. This is a substantial and clinically relevant decrease in eGFR, considering that lowered eGFR one year post-transplantation is predictive for graft failure [14]. The threshold of 6310 IU/ml yields potential use in the clinical setting, as viral loads have previously been used as measure for CMV DNAemia and predicting course of infection [17 – 21]. For example, the initial viral load correlated with CMV PVL and CMV disease in 3873 blood samples from liver, renal and bone marrow transplant recipients (22). Recognized thresholds for DNAemia and initiation of antiviral treatment vary [25–28]. Reischig et al. for instance showed that 21% of subjects demonstrated PVL≥2000 copies/ml in whole blood in the first 100 days after renal transplantation [28]. 2000 copies/ml would translate to 12,098 IU/ml if it would be converted in our assay, a level which 17% of patients exceeded in the first three months.

However, the commutability of benchmark values for initiation and monitoring of treatment remains a challenge and limits straightforward comparison between laboratories and methods [23]. Efforts on further harmonization of CMV quantifications could benefit from the 1st WHO International Standard for CMV [24], intended to limit variability between institutions and stimulate commutability. Discussion on the specific methodology used for quantification remains, for instance on the specimen to be used (e.g. whole blood or plasma) [25]. In this study, DNA was measured in whole blood, due to its sensitivity and quick assay turnaround time.
Our findings suggest that preventing the early peak in CMV viral load may impede the drop in renal function. Unfortunately, the preemptive anti-CMV strategy applied in this cohort did not succeed, considering 56 subjects exceeded the 6310 IU/ml threshold. The scheduling of clinical visits and non-compliance to antiviral medicine may affect this. Nevertheless, the preemptive therapy in this cohort enabled us to study CMV PVL without interference of immediate anti-CMV treatment. Obviously, the act of treating subjects is interfering with the potential rise in PVL, but this is intrinsic to the purpose of treatment, i.e. limiting CMV proliferation. Our institution nowadays applies prophylactic treatment, of which particularly the D+/R- subjects and D+/R+ may benefit, considering their contribution to the PVL>6310 category (41% and 37%).

High PVL early after transplantation was associated with decreased renal function on the intermediate and long-term, even after clearance of active infection. This may be due to CMV latency. The incomplete virus elimination could result in ongoing virus-immune system interactions, causing persistent inflammation which affects tissue injury and remodeling [26]. Subclinical CMV infection was independently associated with GFR decline two years post-transplantation and higher risk for chronic allograft injury [27].

Next to high PVL determining decreased renal function, it is additionally interesting to consider the factors underlying the occurrence of high PVL. The donor kidneys in the PVL>6310 category could have been of worse quality, especially since a higher percentage of these kidneys were derived from postmortem donors and underwent extended duration of ischemia. High PVL is thus associated with decreased eGFR, but it remains intriguing to further explore which factors are inducing the extent of PVL itself.

Although the number of biopsies were limited, our data demonstrate that the proportion of protocol biopsies with >10% interstitial fibrosis was higher in subjects with PVL>6310. This corresponds to earlier findings in which a higher PVL correlated to more interstitial fibrosis in protocol biopsies three months post-transplantation [28]. CMV viremia with PVL>2000 copies/ml associated with three times higher odds for IF/TA, while CMV viremia itself did not. This resembles our finding in that only pronounced infection was related to impaired renal function. It has previously been shown that transplanted and immunosuppressed CMV-infected rats demonstrated vascular intimal thickening 14 days post-transplantation and graft fibrosis six days later [29]. Also, urinary excretion of TGF-β1 was increased in CMV-infected individuals, who additionally displayed increased fibrosis, and a trend towards increased tubular atrophy, in protocol biopsies at six month post-transplantation [30]. Nevertheless, discussion on the role of CMV in fibrosis is ongoing. Serial biopsies demonstrated that the prevalence of IF/TA was higher in recipients with CMV, even before onset of their CMV infection itself [31]. This suggests that donor-related factors or preexisting injury are important during the generation of IF/TA, possibly in combination with CMV infection. No difference in IF/TA between preemptive or prophylactic CMV treatment was observed [32]. All in
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all, the role of CMV in fibrosis remains to be further investigated in the future.

Further expansion of our study design would include recipients undergoing prophylactic treatment, to see whether this can prevent the peak in viral load. However, application of prophylaxis is neither without controversy, since the odds for developing late-onset CMV are higher compared to the preemptive strategy [33]. This frequent and severe CMV disease is less often detected because of more irregular monitoring [34] and therefore potentially inflicts damage without clinicians noticing instantly. On the other hand, the odds of CMV DNAemia were lower for prophylaxis [33], which may be beneficial judging from these findings on peak viral load.

Study limitations

Despite a range of additional factors possibly affecting and explaining a portion of the variance in eGFR, we were specifically interested in determining the association with CMV peak viral load and therefore have focussed on this parameter in our analyses. Due to the retrospective nature of this study, monitoring of CMV DNAemia was not done at identical time points for all subjects. Well-performing subjects regularly do not undergo a protocol biopsy due to its invasive nature, while poor-performing subjects often already underwent a prior indication biopsy (due to e.g. progressive loss of function or suspected rejection). This leads to a selection-bias for subjects with “moderate” transplant function. This study considered PVL in the first three months post-transplantation, in which screening is intensive, but may have missed high PVLs affecting eGFR later after transplantation. Also, additional infections (such as HHV-4, HHV-6 or BKV [35]) may present with CMV after renal transplantation, which is worth further investigating.

Conclusions

In conclusion, high CMV DNAemia early after renal transplantation was associated with significant loss of renal function on the intermediate and long-term, from which subjects did not recover. The severity of infection (high PVL early post-transplantation), more than the infection per se, associated with irreversible and progressive loss of renal function.

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