ORIGINAL ARTICLE

High human cytomegalovirus DNAemia early post-transplantation associates with irreversible and progressive loss of renal function – a retrospective study

Wouter T. Lollinga[1], Lilli Rurenga-Gard[2], Willem van Doesum[1], Rik van Bergen[1], Arjan Diepstra[3], Judith M. Vonk[4], Annelies Riezebos-Brilman[2], H. G. M. Niesters[2], Willem J. van Son[1], Jacob van den Born[1] & Jan-Stephan Sanders[1]

1 Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
2 Department of Medical Microbiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
3 Division of Pathology, Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
4 Department of Epidemiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Correspondence
Wouter Lollinga MSc, Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, Hanzeplein 1, 9700RB Groningen, The Netherlands.
Tel.: +31 (0)50 363 9330;
fax: +31 (0)50 361 9310;
e-mail: w.t.lollinga@umcg.nl

SUMMARY

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. A total of 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. 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/1.73 m^2 (−12%) over 3 years, and the annual decrease for pronounced infection with high PVL was 2.0 ml/min/1.73 m^2 faster than for noninfected or mildly infected subjects. 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.

Transplant International 2017; 30: 817–826

Key words
estimated glomerular filtration rate, human cytomegalovirus, kidney transplantation, renal function, viral infection

Received: 2 August 2016; Revision requested: 2 October 2016; Accepted: 18 April 2017; Published online: 15 June 2017

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

doi:10.1111/tri.12972

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 in 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 5 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.

Subjects 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

Cytomegalovirus 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 herpesvirus (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′-GCCGATCGTAAAGAG ATGAAGAC; reverse primer; 5′-CTCGTGCGTGTGCTA CGAGA; probe 1, 5′-VIC-AGTGCAGCCCCGACCATC GTTC. Probe 2 (5′-VIC-AGTGCAGCCCCGGCCATCA TTC) 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, California, USA), 200 nM CMV forward primer, 800 nM of CMV reverse primer, 200 nM of CMV probe 1, 100 nM of CMV probe 2, 50 nM PhHV forward, 200 nM PhHV reverse, 100 nM PhHV probe, 5 mg/ml bovine serum albumin and 20 µl of isolated DNA. The ABI Prism 7500 sequence detection system (Life Technologies) 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 s 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 using a low- and high-range positive control calibrated to these standards. CMV monitoring was protocolled at every outpatient visit post-transplantation (weekly in the first month, biweekly in months 2 and 3, and monthly afterward) 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 pretransplantation and >536 IU/ml), at all symptomatic reactivations (CMV IgG-positive pretransplantation 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 3 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 3 months after transplantation ("early"); (i) PVL $\leq$ 536: PVL below the detection limit of 536 IU/ml, (ii) PVL536–6310: PVL of 536–6310 IU/ml, and (iii) PVL > 6310: PVL above 6310 IU/ml. The cutoff 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/1.73 m^2 from the moment of return to dialysis or nephrectomy. Values closest around the intended eGFR measurement were used within specific intervals ($\pm$2, $\pm$3 weeks; $\pm$1.5; $\pm$3; $\pm$5; and $\pm$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 if 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 $P < 0.10$ for 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

Table 1. Subject characteristics, categorized per peak viral load (PVL) category.

	PVL ≤ 536 IU	PVL536–6310 IU	PVL > 6310 IU	*P*-value
No. of subjects	151 (57)	57 (22)	56 (21)	
Recipient sex, males	85 (56)	30 (53)	29 (52)	0.80
Recipient age, years	49 [37–59]	56 [46–64]	60 [46–65]	**<0.001**
Recipient race, Caucasian	141 (93)	45 (79)	51 (91)	**0.009**
Graft failure within 3 years	19 (13)	11 (20)	12 (21)	0.22
Return to dialysis	6 (4)	2 (4)	3 (5)	0.87
Nephrectomy	5 (3)	2 (4)	3 (5)	0.79
Death	8 (5)	7 (12)	6 (11)	0.17
Graft failure, time post-Tx, days	496 [18–664]	445 [273–702]	211 [124–569]	0.52
Underlying disease pretransplantation				
Primary glomerular disease/ glomerulonephritis/vasculitis	46 (31)	14 (25)	12 (21)	0.76
Cystic kidney disease	28 (19)	7 (12)	13 (23)	
Dysplasia/hypoplasia	13 (9)	4 (7)	2 (4)	
Renovascular disease/hypertension	15 (10)	7 (12)	9 (16)	
Diabetic nephropathy	13 (8)	7 (12)	4 (7)	
Tubulo-interstitial nephritis	1 (1)	2 (2)	1 (2)	
Urological complications	8 (5)	3 (3)	4 (7)	
Other/unknown causes	27 (17)	13 (13)	11 (20)	
Previous transplantation	19 (13)	9 (13)	5 (11)	0.44
Renal replacement therapy (RRT)				
Preemptive transplantation	77 (51)	42 (74)	41 (73)	**0.007**
Peritoneal dialysis	33 (22)	7 (12)	8 (14)	
Hemodialysis	41 (27)	8 (14)	7 (13)	
Duration of RRT, months	12 [0–38]	28 [5–46]	34 [13–54]	0.34
No. of HLA AB mismatches				
0	26 (17)	6 (12)	10 (18)	0.21
1	34 (23)	10 (18)	16 (29)	
2	52 (35)	26 (47)	21 (38)	
3	24 (16)	12 (21)	9 (16)	
4	13 (9)	2 (4)	0 (0)	
No. of HLA DR mismatches				
0	45 (30)	16 (29)	24 (43)	0.06
1	81 (54)	37 (65)	23 (41)	
2	23 (16)	3 (6)	9 (16)	
Delayed graft function	17 (11)	8 (14)	9 (16)	0.63
BK nephropathy <3 months	1 (1)	0 (0)	0 (0)	0.69
Antibody-mediated rejection <3 months	2 (2)	0 (0)	1 (2)	0.63
T-cell-mediated rejection <3 months	18 (12)	5 (9)	2 (4)	0.19
Rejection, days post-Tx	6 [3–8]	6 [2–64]	26 [10–41]	0.37
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)	185 [167–216]	189 [156–220]	171 [133–205]	0.17
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	0 (0)	1 (2)	1 (2)	0.26
First positive CMV RT-PCR, IU/ml	Not applicable	851 [634–1806]	1832 [882–8596]	**<0.001**
First positive CMV RT-PCR, days post-Tx	Not applicable	33 [22–49]	31 [23–37]	0.19
Peak viral load, IU/ml	Not applicable	2397 [1483–3754]	64 105 [19 490–324 131]	**<0.001**
Peak viral load, days post-Tx	Not applicable	50 [35–65]	48 [39–74]	0.77

Transplant International 2017; 30: 817–826

Table 1. Continued.

	PVL ≤ 536 IU	PVL536–6310 IU	PVL > 6310 IU	*P*-value
Primary infection, *n*	Not applicable	3 (6)	23 (42)	<0.001
Donor sex, males	77 (51)	29 (51)	29 (52)	0.99
Donor age, years	52 [45–60]	50 [39–60]	53 [45–58]	0.35
Transplant source				
Port-mortem	73 (48)	34 (60)	39 (70)	0.14
DBD (remainder DCD)	46 (31)	25 (44)	25 (45)	
Ischemia time, h	7 [3–14]	12 [3–16]	13 [3–17]	**0.01**
CMV serologic status				
D−/R−	56 (37)	0 (0)	0 (0)	**<0.001**
D−/R+	28 (18)	19 (33)	11 (20)	
D+/R−	29 (19)	3 (5)	23 (41)	
D+/R+	34 (23)	31 (55)	21 (37)	
Unknown	4 (3)	4 (7)	1 (2)	

RRT, renal replacement therapy; HLA, human leukocyte antigen; CyA, cyclosporine 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; D−/R−, donor-seronegative, recipient-seronegative.

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. Statistically significant values are represented as bold for clarity.

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

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 two lacked eGFR measurements at the end of follow-up (Fig. 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.

Peak viral load was determined in the first 3 months post-transplantation, because intensive monitoring 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 (2397 vs. 64 105 IU/ml). Additional recipient and donor characteristics are described 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

To determine the effect of PVL on renal function, eGFR was compared between PVL ≤ 536, PVL536–6310, and PVL > 6310 (Fig. 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

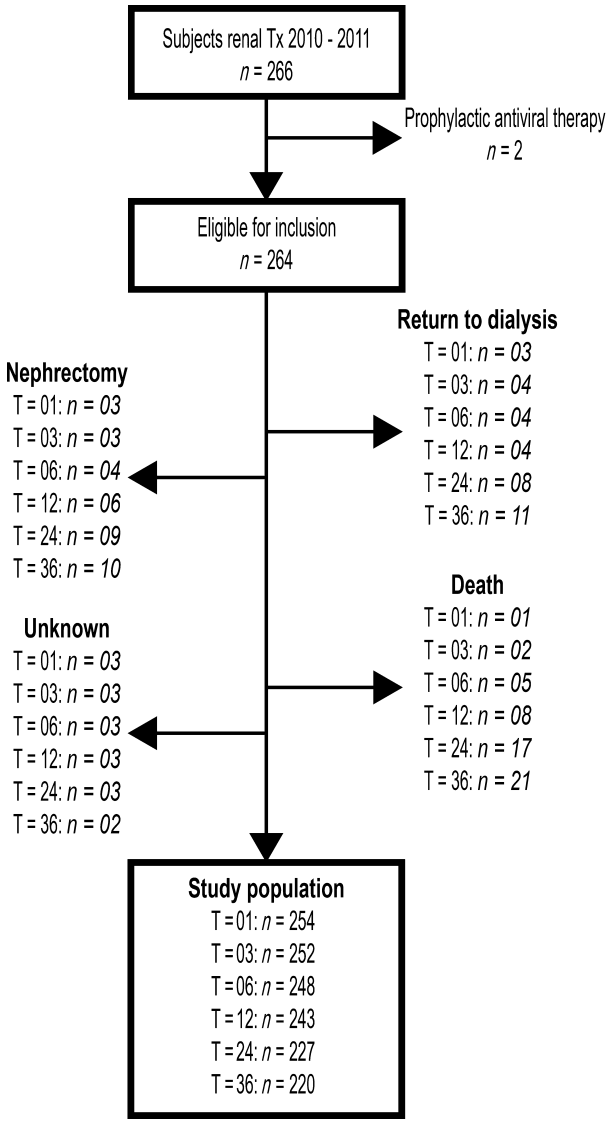

Figure 1 Subject selection flowchart. Of the 266 initially selected transplant recipients, two were excluded for receiving immediate prophylactic antiviral treatment. Between 254 and 220 subjects were eligible for analysis between 1 and 36 months post-transplantation, comprising the study population. Subjects were classified to the initial event that occurred after transplantation. Renal Tx = renal transplantation. T = 01: 1 month post-transplantation.

not differ immediately after transplantation. eGFR for PVL > 6310 declined from 46.1 to 40.7 ml/min/1.73 m^2 in 3 years, a substantial and clinically relevant 12% decrease (Fig. 2).

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; Table S1). eGFR was

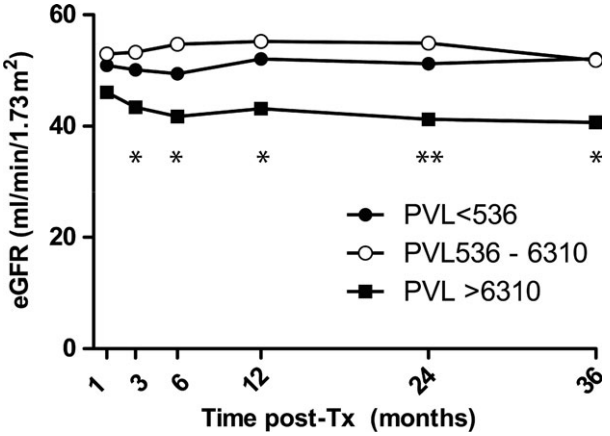

Figure 2 Recipient Estimated Glomerular Filtration Rate (eGFR) post-transplantation, categorized per category of peak viral load (PVL). Comparisons of eGFR at 1, 3, 6, 12, 24, and 36 months post-transplantation demonstrated significantly decreased eGFR for PVL > 6310 (Kruskal–Wallis; $P = 0.02$, $P = 0.01$, $P = 0.02$, $P = 0.006$, and $P = 0.01$), but not at 1 month ($P = 0.20$). eGFR for PVL ≤ 536 and PVL536–6310 was not significantly different at any time point. *$P < 0.05$, **$P < 0.01$.

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/1.73 m^2 lower for subjects with a PVL > 6310 compared to those below the threshold. In available protocol biopsies at 1 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; 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/1.73 m^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

Table 2. Multivariable linear regression for eGFR at various months post-transplantation.

Multivariable regression, sequential time points (month)	PVL > 6310	95% CI Lower bound	95% CI Upper bound	*P*-value	Mean eGFR PVL ≤ 6310 (ml/min/1.73 m^2)	Standard deviation	Mean eGFR PVL > 6310 (ml/min/1.73 m^2)	Standard deviation
1	−1.81	−8.02	4.41	0.57	51.95	22.76	46.73	25.32
3	−6.88	−12.94	−0.82	**0.03**	52.34	21.27	44.25	19.28
6	−7.46	−13.30	−1.61	**0.01**	51.26	20.44	42.55	16.32
12	−9.34	−16.09	−2.60	**0.01**	53.09	22.60	43.00	18.98
24	−10.88	−18.36	−3.40	**0.005**	52.15	24.56	40.92	19.83
36	−12.26	−20.20	−4.32	**0.003**	51.12	25.74	40.08	21.67

All variables with $P < 0.10$ at any time point between 1 and 36 months from 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; Table S2. Statistically significant values are represented as bold for clarity.

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/1.73 m^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/1.73 m^2/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 3 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 3 years post-transplantation. PVL > 6310 was not independently associated

Table 3. Linear mixed-effects model for the development of eGFR from 1 to 36 months post-transplantation.

LME eGFR over time		95% CI Lower bound	95% CI Upper bound	*P*
Intercept	31.00	10.35	51.65	**0.004**
Recipient; PVL > 6310	−7.13	−14.61	0.35	0.06
Recipient; time post-transplantation, year	1.99	1.05	2.92	**<0.001**
Recipient; PVL > 6310*time post-transplantation	−2.21	−3.97	−0.44	**0.01**

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 mixed-effects model at 1, 3, 6, 12, 24, and 36 months. The estimate for time post-transplantation indicated a 1.99 ml/min/1.73 m^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 a extrapolation, 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/1.73 m^2/year faster for PVL > 6310 compared to PVL ≤ 6310.PVL: Peak viral load. Only the peak viral 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; urological complications, Caucasian race, renal replacement therapy; preemptive transplantation, delayed graft function, cellular rejection <3 months, donor living status, donation after brain death, and ischemia time. Statistically significant values are represented as bold for clarity.

with eGFR after 1 month, potentially because CMV-induced damage is time-dependent and only clinically manifests from 3 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 with a substantially lower eGFR, ranging from 6.88 to 12.26 ml/min/1.73 m^2. The cutoff value of 6310 IU/ml was selected because this represents the median peak viral load in our cohort. eGFR for PVL > 6310 decreased from 46.1 to 40.7 ml/min/1.73 m^2 in 3 years, a 12% decrease. This is a substantial and clinically relevant decrease in eGFR, considering that lowered eGFR 1 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 [15–19]. 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 [20]. Recognized thresholds for DNAemia and initiation of antiviral treatment vary [21–24]. Reischig *et al.* [22] for instance showed that 21% of subjects demonstrated PVL ≥ 2000 copies/ml in whole blood in the first 100 days after renal transplantation. 2000 copies/ml would translate to 12 098 IU/ml for our assay, a level which 17% of patients exceeded in the first 3 months.

Nevertheless, the commutability of benchmark values for initiation and monitoring of treatment remains a challenge and limits straightforward comparison between laboratories and methods [25]. Efforts on further harmonization of CMV quantifications could benefit from the 1st WHO International Standard for CMV [20], 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) [26]. 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 noncompliance 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, that is, 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 [27]. Subclinical CMV infection was independently associated with GFR decline 2 years post-transplantation and higher risk for chronic allograft injury [21].

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 as a higher percentage of these kidneys were derived from postmortem donors and underwent longer ischemia time. 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 was 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 with more interstitial fibrosis in protocol biopsies 3 months post-transplantation [22]. 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 been previously shown that transplanted and immunosuppressed CMV-infected rats demonstrated vascular intimal thickening 14 days post-transplantation and graft fibrosis 6 days later [23]. Also, urinary excretion of TGF-β1 was increased in CMV-infected individuals, who additionally displayed increased fibrosis, and a trend toward increased tubular atrophy, in protocol biopsies at 6 month post-transplantation [24]. 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 [28]. 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 in biopsies [29]. All in 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, as the odds for developing late-onset CMV are higher compared to the preemptive strategy [30]. This frequent and severe CMV disease is less often detected because of more irregular monitoring [31] and therefore potentially inflicts damage without clinicians noticing instantly. On the other hand, the odds of CMV DNAemia were lower for prophylaxis [30], which may be beneficial judging from our 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 3 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 [32]) may present with CMV after renal transplantation, which is worth further investigating.

Conclusion

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.

Authorship

WTL, LR-G, WD, RB, AR-B, WJS, JB, J-SS: Substantial contributions to the conception or design of the work. WTL, LR-G, WD, RB, AD, JMV, AR-B, HGMN, WJS, JB, J-SS: Substantial contributions to the acquisition, analysis, or interpretation of data for the work. WTL, LR-G, WD, RB, AD, JMV, AR-B, HGMN, WJS, JB, J-SS: Drafting the work or revising it critically for important intellectual content.

Funding

This study was financially supported by the Graduate School of Medical Sciences.

Conflict of interest

The results presented in this paper have not been published previously in whole or part, except in abstract form. The authors declare no conflict of interest.

Acknowledgements

We would like to express our gratitude toward A. Hernandez Segura for support with the figures.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Univariable linear regression for eGFR at various months post-transplantation.

Table S2. Multivariable linear regression for eGFR, variable specification at eGFR06.

REFERENCES

1. Fishman JA. Overview: cytomegalovirus and the herpesviruses in transplantation. *Am J Transplant* 2013; **13**: 1.
2. Sagedal S, Nordal KP, Hartmann A, *et al.* The impact of cytomegalovirus infection and disease on rejection episodes in renal allograft recipients. *Am J Transplant* 2002; **2**: 850.
3. O'Connor CM, Murphy EA. A myeloid progenitor cell line capable of supporting human cytomegalovirus latency and reactivation, resulting in infectious progeny. *J Virol* 2012; **86**: 9854.
4. Söderberg-Nauclér C, Fish KN, Nelson JA. Reactivation of latent human

cytomegalovirus by allogeneic stimulation of blood cells from healthy donors. *Cell* 1997; **91**: 119.
5. Ross SA, Novak Z, Pati S, Boppana SB. Overview of the diagnosis of cytomegalovirus infection. *Infect Disord Drug Targets* 2011; **11**: 466.
6. Hart A, Smith JM, Skeans MA, *et al.* OPTN/SRTR 2014 annual data report. *Kidney Am J Transplant* 2016; **16**(Suppl. 2): 11.
7. Gondos A, Döhler B, Brenner H, Opelz G. Kidney graft survival in Europe and the United States: strikingly different long-term outcomes. *Transplantation* 2013; **95**: 267.
8. van Son WJ, The TH. Cytomegalovirus infection after organ transplantation: an update with special emphasis on renal transplantation. *Transpl Int* 1989; **2**: 147.
9. van Ree RM, de Vries APJ, Zelle DM, *et al.* Latent cytomegalovirus infection is an independent risk factor for late graft failure in renal transplant recipients. *Med Sci Monit* 2011; **17**: CR609.
10. Schnitzler MA, Lowell JA, Hardinger KL, Boxerman SB, Bailey TC, Brennan DC. The association of cytomegalovirus sero-pairing with outcomes and costs following cadaveric renal transplantation prior to the introduction of oral ganciclovir CMV prophylaxis. *Am J Transplant* 2003; **3**: 445.
11. Sagedal S, Hartmann A, Nordal KP, *et al.* Impact of early cytomegalovirus infection and disease on long-term recipient and kidney graft survival. *Kidney Int* 2004; **66**: 329.
12. Owers DS, Webster AC, Strippoli GFM, Kable K, Hodson EM. Pre-emptive treatment for cytomegalovirus viraemia to prevent cytomegalovirus disease in solid organ transplant recipients. *Cochrane Database Syst Rev* 2013; **2**: CD005133.
13. Dzabic M, Rahbar A, Yaiw K-C, *et al.* Intragraft cytomegalovirus protein expression is associated with reduced renal allograft survival. *Clin Infect Dis* 2011; **53**: 969.
14. Park WD, Larson TS, Griffin MD, Stegall MD. Identification and characterization of kidney transplants with good glomerular filtration rate at 1 year but subsequent progressive loss of renal function. *Transplantation* 2012; **94**: 931.
15. Razonable RR, Hayden RT. Clinical utility of viral load in management of cytomegalovirus infection after solid organ transplantation. *Clin Microbiol Rev* 2013; **26**: 703.
16. Cope AV, Sweny P, Sabin C, Rees L, Griffiths PD, Emery VC. Quantity of cytomegalovirus viruria is a major risk factor for cytomegalovirus disease after renal transplantation. *J Med Virol* 1997; **52**: 200.
17. Fox JC, Kidd IM, Griffiths PD, Sweny P, Emery VC. Longitudinal analysis of cytomegalovirus load in renal transplant recipients using a quantitative polymerase chain reaction: correlation with disease. *J Gen Virol* 1995; **76**(Pt 2): 309.
18. Hassan-Walker AF, Kidd IM, Sabin C, Sweny P, Griffiths PD, Emery VC. Quantity of human cytomegalovirus (CMV) DNAemia as a risk factor for CMV disease in renal allograft recipients: relationship with donor/recipient CMV serostatus, receipt of augmented methylprednisolone and antithymocyte globulin (ATG). *J Med Virol* 1999; **58**: 182.
19. Sia IG, Wilson JA, Groettum CM, Espy MJ, Smith TF, Paya CV. Cytomegalovirus (CMV) DNA load predicts relapsing CMV infection after solid organ transplantation. *J Infect Dis* 2000; **181**: 717.
20. Fryer JF, Heath AB, Anderson R, Minor PD, Collaborative study to evaluate the proposed 1st [first] WHO international standard for human cytomegalovirus (HCMV) for nucleic acid amplification (NAT)-based assays/Biologicals. 2016 Jul; **44**: 242.
21. Smith JM, Corey L, Bittner R, *et al.* Subclinical viremia increases risk for chronic allograft injury in pediatric renal transplantation. *J Am Soc Nephrol* 2010; **21**: 1579.
22. Reischig T, Jindra P, Hes O, Bouda M, Kormunda S, Treska V. Effect of cytomegalovirus viremia on subclinical rejection or interstitial fibrosis and tubular atrophy in protocol biopsy at 3 months in renal allograft recipients managed by preemptive therapy or antiviral prophylaxis. *Transplantation* 2009; **87**: 436.
23. Lautenschlager I, Soots A, Krogerus L, *et al.* Effect of cytomegalovirus on an experimental model of chronic renal allograft rejection under triple-drug treatment in the rat. *Transplantation* 1997; **64**: 391.
24. Helanterä I, Koskinen P, Finne P, *et al.* Persistent cytomegalovirus infection in kidney allografts is associated with inferior graft function and survival. *Transpl Int* 2006; **19**: 893.
25. Hayden RT, Preiksaitis J, Tong Y, *et al.* Commutability of the first World Health Organization international standard for human cytomegalovirus. *J Clin Microbiol* 2015; **53**: 3325.
26. Kraft CS, Armstrong WS, Caliendo AM. Interpreting quantitative cytomegalovirus DNA testing: understanding the laboratory perspective. *Clin Infect Dis* 2012; **54**: 1793.
27. Reinke P, Prösch S, Kern F, Volk HD. Mechanisms of human cytomegalovirus (HCMV) (re)activation and its impact on organ transplant patients. *Transpl Infect Dis* 1999; **1**: 157.
28. Erdbruegger U, Scheffner I, Mengel M, Schwarz A, Haller H, Gwinner W. Long-term impact of CMV infection on the allograft and on patient survival in renal transplant patients with protocol biopsies. *Am J Physiol Renal Physiol* 2015; **309**: F925–32.
29. Spinner ML, Saab G, Casabar E, Bowman LJ, Storch GA, Brennan DC. Impact of prophylactic versus preemptive valganciclovir on long-term renal allograft outcomes. *Transplantation* 2010; **90**: 412.
30. Florescu DF, Qiu F, Schmidt CM, Kalil AC. A direct and indirect comparison meta-analysis on the efficacy of cytomegalovirus preventive strategies in solid organ transplant. *Clin Infect Dis* 2014; **58**: 785.
31. De Keyzer K, Van Laecke S, Peeters P, Vanholder R. Human cytomegalovirus and kidney transplantation: a clinician's update. *Am J Kidney Dis* 2011; **58**: 118.
32. Weikert BC, Blumberg EA. Viral infection after renal transplantation: surveillance and management. *Clin J Am Soc Nephrol* 2008; **3**(Suppl. 2): S76.