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Impact of CMV infection on acute rejection and long-term renal allograft function: a systematic analysis in patients with protocol biopsies and indicated biopsies

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Abstract

Background. Higher rates of acute rejection (AR) and reduced graft survival have been reported in patients with cytomegalovirus (CMV) infection, but an association between these factors remains controversial.

Methods. In this study, serial protocol biopsies (PBs) and clinically indicated biopsies (IBs) from a large cohort of renal allograft recipients (n ¼ 594) were analyzed to examine the relation between CMV and AR.

Results. Patients with CMV were more likely to receive IB (85 of the 153 patients; 56%) compared to patients without CMV (138 of 441 patients; 32%; $P = 0.003$). However, this did not translate into a greater number of patients with episodes of acute cellular rejection on histopathology in IBs. Analysis of PBs revealed a significantly higher number of episodes of rejection per patient with CMV infection ($P = 0.04$), but only in a subgroup of patients with triple immunosuppression.

Long-term graft function post-transplantation was analyzed in four different subgroups according to CMV infection and/or AR. Differences in renal function were apparent within the first 6 weeks after transplantation and persisted during follow-up, with the best renal function in patients without AR or CMV, whereas patients with both AR and CMV had the worst ($P < 0.012$ at 1 year; $P < 0.001$ at 2 years). On average, the latter group had significantly older donors and more often delayed graft function.

Conclusions. Our data suggests that the link between CMV and AR is far less significant than previously thought. Outcome in patients with CMV may be more determined by coexisting conditions like high donor age and delayed graft function.

Keywords: acute rejection; CMV; long-term allograft function; protocol biopsies

Introduction

Cytomegalovirus (CMV) infection is a frequent complication in the early post-renal transplant period [1, 2]. It has been associated with increased morbidity [3] and reduced graft survival [4]. Reduced graft survival could be related to an increased rate of acute rejections (ARs) in patients with CMV as suggested by experimental [5, 6] and clinical studies [7–10]. However, the association between CMV and AR remains controversial since some investigators could not confirm this finding [11–13]. Also, it is uncertain whether CMV infection promotes AR [5] or if augmented immunosuppressive therapy in the setting of AR causes CMV infection [12, 14]. In addition, treatment of CMV disease often includes reduction of immunosuppression, which may increase the risk of graft rejection.

The aim of this study is to examine the relation between CMV and AR in a large cohort of patients after renal transplantation. The study focuses on a systematic analysis of serial protocol biopsies (PBs) and biopsies clinically indicated. In addition, this study analyses the association between clinical variables and the long-term allograft outcome in CMV infection after renal transplantation.

Materials and methods

Subjects

A total of 594 patients with a kidney or a combined kidney/pancreas transplantation between 2001 and 2004 were included in this retrospective analysis. All patients were enrolled in the renal transplant PB program as described below. Patients demographics and characteristics are summarized in Table 1.

Induction therapy was given in 88% of all patients (antithymocyte globulin in 6% and interleukin-2 antibodies in 82%). Maintenance therapy with dual immunosuppression consisted of cyclosporine A (CyA) and prednisolone in 200 of 594 patients (33.7%); 227 patients (38.2%) received triple immunosuppression with additional mycophenolate mofetil (MMF). In 167 patients (28.1%), alternative regimens including sirolimus or azathioprine and tacrolimus instead of cyclosporine A were used. A subgroup of patients (340 patients) was created to focus the analysis on patients with triple immunosuppression, which reflects current standards for immunosuppressive therapy [15] (see Table 2).

Standard therapy for first-time acute tubulointerstitial rejection with and without a rise in serum creatinine consisted of pulse methylprednisolone 250–500 mg intravenously given for 3 days. Additionally, in patients

receiving dual immunosuppression with CyA and prednisolone, MMF was added. Patients with cyclosporine A-containing regimens who experienced a second acute tubulointerstitial rejection episode (ARE) or who had the rejection later than 3 months post-transplant were switched to tacrolimus. Patients with a borderline rejection were treated like patients with acute tubulointerstitial rejection if baseline creatinine had increased >25%. Acute vascular rejections were treated with steroid boli (500 mg prednisolone) and a switch from cyclosporine A to tacrolimus. All patients received prophylaxis for Pneumocystis jirovecii with trimethoprim/sulfamethoxazole three times a week for 6 months. Prophylactic antiviral treatment to prevent CMV infection is described below.

PB program

Renal PBs are regularly performed at our transplant center at 6 weeks, 3 and 6 months after kidney or combined kidney/pancreas transplantation [16]. About 45% of patients have additional biopsies to evaluate unexplained allograft dysfunction [clinically indicated biopsy (IB)]. Demographic, clinical and routine laboratory data are concomitantly collected for each patient. The data are entered into a customized database (Oracle Enterprises, version 8.0.5). The institutional review board at Hannover Medical School approved the PB program and informed consent was obtained from each patient.

Table 1. Demographics and characteristics of all patients

Characteristics	*n*	%
Total number of patients	594	
Recipient mean age ± SD (years)	50 ± 13	
Gender (female/male)	345/249	58/42
Donor mean age ± SD (years)	48 ± 16	
Living/deceased donor	81/513	14/86
Re-transplanted patients	71	12
Kidney-pancreas transplant	47	8
Underlying disease		
Glomerulonephritis/vasculitis	149	25
Interstitial nephritis	55	9
Hypertensive/diabetic nephropathy	85	14
Congenital disease	78	13
Others	21	4
Unknown	206	35
Cold ischemia time (hours)	15 ± 8	
Patients with PRA > 0%[a]	42	7
Sum of HLA mismatches on locus A, B, DR	2.3 ± 1.7	

[a]PRA > 0%: panel reactive antibodies of these 42 patients: 36 ± 27%.

Table 2. Demographics and characteristics of patients with triple immunosuppression[a]

Characteristics	*n*	%
Total number of patients	340	
Recipient mean age ± SD (years)	48 ± 14	
Gender (female/male)	136/204	40/60
Donor mean age ± SD (years)	47 ± 16	
Living/deceased donor	76/264	22/78
Re-transplanted patients	52	15
Kidney-pancreas transplant	46	14
Underlying disease		
Glomerulonephritis/vasculitis	87	26
Interstitial nephritis	24	7
Hypertensive/diabetic nephropathy	65	19
Congenital disease	33	10
Others	13	4
Unknown	118	35
Cold ischemia time (hours)	13 ± 8	
Patients with PRA > 0%[b]	30	9
Sum of HLA mismatches on locus A, B, DR	2.5 ± 1.8	

[a]Compared to the entire group, the subgroup included more living donors (22 versus 14%, $P < 0.01$), more combined pancreas/kidney transplantations (14 versus 8%, $P < 0.01$), had shorter cold ischemia time (13 versus 15 h, $P < 0.01$) and was slightly younger (mean age 48 versus 50 years old, $P = 0.03$).
[b]PRA > 0%: panel reactive antibodies of these 30 patients: 36 ± 25%.

Histological analysis

Biopsies were evaluated according to the updated BANFF classification [17]. Analysis included a total of 1483 PBs (484 biopsies at 6 weeks, 517 and 482 biopsies at 3 and 6 months) and 578 biopsies performed for unexplained graft dysfunction (clinically IBs) within the first year post-transplantation.

Definition of CMV infection

CMV viremia and CMV disease were considered as CMV infection. CMV viremia was defined as any detectable virus by quantitative polymerase chain reaction (PCR) (above the threshold of 600 copies/mL) or positive antigenemia testing (CMVpp65 antigen >2 positive cell/400 000 cells) without clinical symptoms [18, 19]. CMV disease included CMV syndrome (flu-like as fever and myalgia) and organ involvement such as hepatitis, gastrointestinal disease, leukopenia/ thrombocytopenia, etc. similar to the criteria described by Ljungman *et al.* [20] and Preiksaitis *et al.* [21]. CMV colitis was assumed when CMV antigenemia was detected and other causes of diarrhea were excluded. Histologic evidence of tissue invasion was established only in a few of those cases. Hepatitis was defined as rise of hepatic transaminases and cholestatic parameters twice the initial values without other known causes. Leukopenia was defined as a leukocyte count $<4 \times 10^9$/L, thrombocytopenia $<100 \times 10^9$/L or any significant decrease from baseline values with other causes such as drug effects or infection excluded. Recurrence of CMV infection was defined as any episode occurring >28 days after the end of a previous infection, in accordance with previous reports [20].

Virological testing

Viral load testing and IgG and IgM serology were routinely determined at the time of transplantation. CMV antigenemia testing was performed in all patients (CMVpp65) in the first 3 months post-transplantation as part of the routine blood work; initially weekly, then every other week if the patient was clinically stable. In addition, CMV antigenemia testing was performed during and after antiviral treatment for CMV infection.

Viral load testing by PCR was done in patients with unclear results of antigenemia testing. CMV PCR was performed using quantitative PCR assays with a standard thermal cycling profile (lower limit of detection 600 copies/mL) as described elsewhere [18].

Prophylaxis and therapy of CMV infection

Prophylactic treatment with oral ganciclovir or valganciclovir was initiated for 3 months in the setting of CMV IgG-positive donor with a CMV IgG-negative recipient (D+/R−).

Statistical analysis

Categorical variables between the groups and different biopsy time points were compared using chi-square test for two or more samples or Fisher's test for two samples. Long-term allograft function was examined by two-way analysis of variance. All other numerical data were compared with the Kruskal–Wallis test and the Mann–Whitney test. A stepwise negative logistic regression analysis was performed with the variables, which were significantly associated with CMV infection. Nominal regression analysis was used to examine associations of clinical variables with the patient groups with and without CMV and AR. Differences with $P < 0.05$ were considered statistically significant. Values are given as median or mean ± SD, unless otherwise stated. All statistics were done with SPSS version 16.0 (SPSS, Inc., Chicago, IL).

Results

Incidence of CMV infection and clinical presentation

A proportion of 153 of the 594 patients (26%) developed a CMV infection within 12 months post-transplantation. The majority of CMV infections occurred within the first

3 months post-transplantation, with a median onset of 63 days and an average duration of 18 days. The incidence of CMV infection varied depending on CMV-IgG serostatus, with seropositive recipients of seropositive organs being the most common group ($P < 0.0001$). This is summarized in Figures 1 and 2.

In 137 patients with CMV infection, the data set regarding CMV antigenemia testing (CMV pp65) was sufficient to analyze the issue of recurrence of CMV infection. Thirty-seven of these 137 patients (27%) had more than one episode of CMV infection in the first year post-transplantation. The fraction of patients with recurrent CMV infection was only numerically higher in the high-risk serogroup D+/R− (14 of 40 patients; 35%) compared to D+/R+ (16 of 67 patients; 24%) and the other two serogroups (D−/R+: 5 of 20; 25%; D−/R−: 2 of 8; 25%; all $P > 0.05$).

All 153 patients with CMV infection had a total of 193 episodes of CMV infection in the first year post-transplantation. CMV disease was observed in 54 of these 193 (28%) episodes. In 14 cases, CMV disease was characterized by more than one manifestation of CMV (flu-like symptoms and/or organ involvement). Patients in the high-risk serogroup D+/R− were more often symptomatic (18 of 41 patients: 44%) than patients with D+/R+ (16 of 71 patients: 23%; $P = 0.02$).

To assess the severity of CMV infection, all results of CMV pp65 antigen testing during the CMV episode were used to calculate the mean and median numbers of positive cells and to determine the highest number of positive cells for each patient (Table 3). Significantly higher CMVpp65 values could be observed in patients with CMV disease compared to those with viremia only. Single versus

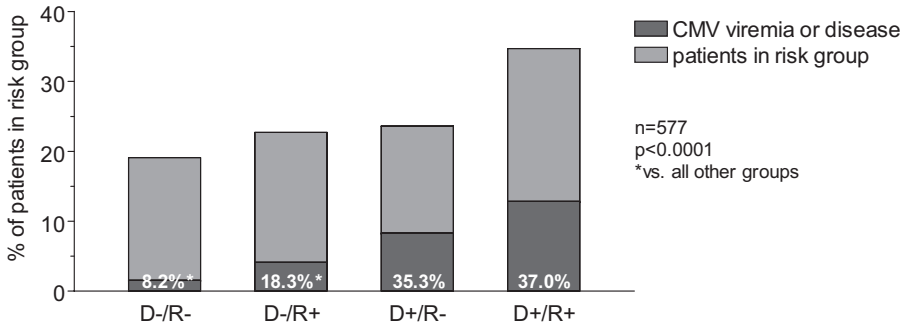

Fig. 1. CMV serostatus and incidence of CMV infection. The total height of the bars illustrates the distribution of patients into the different risk groups according to the serum CMV IgG status of donor (D) and recipient (R). Percentages provided in the bars are indicating the percentages of patients with CMV infection (viremia only or CMV disease) in each risk group.

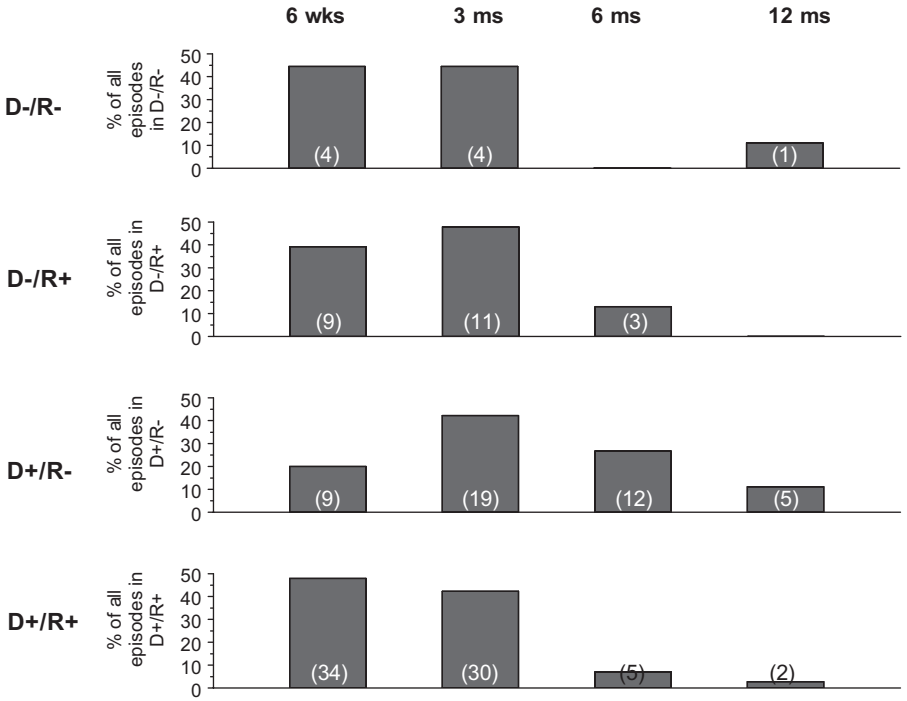

Fig. 2. Time points of CMV infection. Numbers in the bars indicate how many patients had CMV infection within the first 6 weeks (wks), between 6 wks and 3 months (ms), between 3 and 6 ms and between 6 and 12 ms post transplantation according to the serum CMV IgG risk status of donor (D) and recipient (R).

recurrent CMV episodes showed no differences. CMV negative recipients (D+/R−; D−/R−) had higher CMVpp65 values and longer infection episodes compared to CMV-positive patients (D+/R+; D−/R+).

Clinical factors associated with CMV infection

Several clinical factors including pre-transplant data, donor factors and recipient's comorbidities were explored to identify potential risk factors for CMV infection (see Supplementary Table). Significant differences for patients with CMV infection within the first year post-transplantation were further analyzed in a logistic regression analysis, which revealed that the CMV serostatus is the strongest factor (Table 4).

Clinical variables that changed during the first year post-transplantation like immunosuppression, antibiotic therapies, bacterial infections, etc. were separately analyzed. In patients with CMV infection between 6 weeks and 3 months, antibiotic therapy (given for different indications) was more frequent (30%) compared to patients without CMV (17%), P = 0.054. Notably, different immunosuppressive drug regimens did not show any relation with CMV infection.

Association of CMV infection with acute cellular rejection of renal allografts

Several investigators have reported that more acute rejection episodes occur in patients with CMV infection [7–9], but this association remains controversial [11–13]. Notwithstanding the possible accumulation of ARE in patients with CMV, it also remains to be clarified whether CMV infection precedes ARE or vice versa.

Separate analyses were performed on PBs and on clinically IBs to analyze the association of CMV infection and acute cellular rejection. We felt this distinction was necessary to facilitate comparison of our data with previously performed trials that investigated this issue mainly in patients receiving clinically IBs.

Patients with CMV infection were more likely to receive clinically indicated biopsies (85 of the 153 patients; 56%) compared to patients without CMV infection (138 of 441 patients; 32%; P = 0.003). However, this did not translate into a greater number of patients with episodes of acute cellular rejection on histopathology (Figure 3A). Likewise, analyzing acute rejection episodes in PBs, we could not find a significant difference in patients with and without CMV infection (Figure 3A). The analysis also

Table 3. Severity of CMV infection[a]

CMV pp65 parameters	CMV disease	CMV viremia	Single CMV episode	Recurrent CMV episodes	CMV Ig D+/R+	CMV Ig D+/R−	CMV Ig D−/R+	CMV Ig D−/R−
Mean	26.2*	6.6	9.3	7.3	6.9**	27.4$	3.4***	42.4
Median	7.8*	4.5	5.7	5.2	4.6#	7.5$	3.0	9.0
Maximum	66.0*	9.3	14.0	11.0	8.5**	52.0$	4.6***	183.0
Duration of CMV positivity (days)	12.3	11.5	9.0	15.2	9.2**	19.3$	7.0	17.0

[a]The severity of CMV infection is described by the number of CMV pp65 antigen-positive cells/400 000 cells. For each patient, all results of CMV pp65 antigen testing during the CMV episode were used to calculate the mean and median number of positive cells. In addition, the highest value of positive cells reached during the CMV episode and the duration of CMV antigenemia is given. These results are summarized for different subgroups, such as patients with CMV disease versus viremia only, patients with single compared to recurrent CMV episodes (for definitions see Materials and Methods) and for the four different combinations of CMV IgG-positive and -negative donors and recipients.
*P < 0.05 compared to CMV viremia; **P < 0.02 and #P < 0.05 compared to D+/R−; $P < 0.05 compared to D−/R+; ***P < 0.05 compared to D−/R−.

Table 4. Associations between CMV and clinical variables[a]

Clinical variable	Patients without CMV	Patients with CMV	Univariate analysis; P value	Logistic regression; P value	Odds ratio	95% Confidence interval
Recipient's age (years)	49 ± 13	52 ± 14	0.003			
Pregnancies before Tx (%)	23	33	0.025	0.053	1.6	0.99–2.47
Coronary heart disease (%)	14	23	0.016	0.008	2.0	1.21–3.52
History of myocardial infarction (%)	4.1	9.2	0.022			
Donor's age (years)	46 ± 16	53 ± 16	0.000	0.005	1.02	1.01–1.03
CMV Ig D+/R− (%)	21	29	0,044	0.000	7.0	3.06–16.1
CMV Ig D+/R+ (%)	29	47	0,000	0.000	6.9	3.12–15.3
CMV Ig D−/R+ (%)	24	16	0,031	0.029	2.6	1.10–6.31
CMV Ig D−/R− (%)	24	5	0.000			
Dialysis post-transplantation (%)	30	41	0.012	0.033	1.6	1.04–2.45
Lowest *S*-creatinine within the first 6 weeks after Tx (μmol/L)	141 ± 68	156 ± 90	0.03			

[a]Pre-transplant data, donor factors and recipient's co-morbidities were compared between the patient groups with and without CMV infection in univariate analyses to identify potential risk factors for CMV infection (for a complete list of examined factors, see Supplementary table). Significant factors of these univariate analyses are shown in the table and were further analyzed in a stepwise negative logistic regression analysis, which revealed that the CMV serostatus is the strongest factor for CMV infection. Tx, transplantation; D/R, donor/recipient.

looked at the average number of rejection per patient (Figure 3B). There was only a numerical trend that patients with CMV had slightly more episodes of rejection per patient (AR in PBs: 0.22 episodes per patient without CMV versus 0.28 episodes per patient with CMV; in clinically IBs: 0.18 episodes per patient versus 0.22 episodes per patient).

Our large cohort of 594 patients received different immunosuppressive regimens reflecting variability in immunosuppressive regimens in different centers across the world. We therefore looked at a subgroup of patients receiving only triple immunosuppression according to current standards for immunosuppressive therapy [15]. Interestingly, the percentage of patients having a CMV infection remained the same (25.6% in subgroup versus 25.8% in 'all patients'). There were four characteristics, which distinguished these two groups. The subgroup included more living donors (22 versus 14%, $P < 0.01$), more combined pancreas/kidney transplantations (14 versus 8%, $P < 0.01$), had shorter cold ischemia time (13 versus 15 h, $P < 0.01$) and was slightly younger (mean age 48 versus 50 years old, $P = 0.03$).

We performed the same statistical analysis we applied to the entire group of 594 patients. Figure 4 summarizes the findings of this subgroup analysis. Analyzing PBs, an insignificantly higher proportion of patients in the CMV group had borderline rejection (BL) and AR and less patients had only BL ($P = 0.063$). This trend translated into more episodes of rejections (Banff type Ia-IIb) per patient with CMV infection in PBs ($P = 0.04$).

We could not find a significant difference regarding the number of rejection episodes per patient in patients with and without CMV infection who received clinically IBs. Since previous studies have analyzed data from clinically IBs and not from PBs, we believe that this particular analysis of clinically IBs is directly comparable with analyses reported so far in the literature.

Further subgroup analysis showed that patients with recurrent CMV infection were no more likely to develop AR than those with a single CMV episode. Also, patients with clinical CMV disease were no more likely to develop AR than patients with viremia only (data not shown). Both analyses were performed looking at two separate groups of PBs and clinically IBs.

In order to analyze the timely relationship between CMV and acute tubulointerstitial rejection, we again looked separately at PBs and clinically IBs. We found both scenarios, i.e. CMV infection preceding AR and CMV infection after treatment of rejection episodes, almost equally present. This is illustrated in Figure 5, which shows a Gaussian distribution of the rejection episodes with regards to the timely relationship to the occurrence of CMV infection. The small shift of the angular point of the Gaussian distribution suggests that slightly more CMV infection episodes may occur before an AR compared to CMV infections occurring after AR in patients with PBs

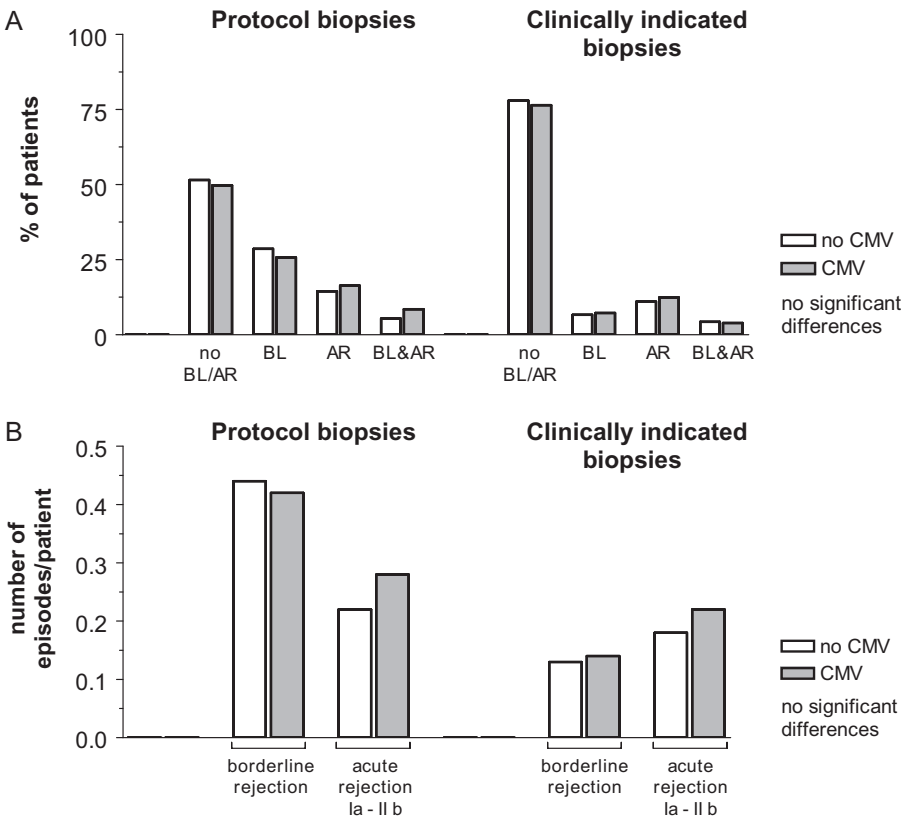

Fig. 3. Borderline rejection (BL) and acute cellular rejection in PBs and IBs from patients with and without CMV. (**A**) Proportion of patients with rejection episodes. (**B**) Average number of rejection episodes per patient.

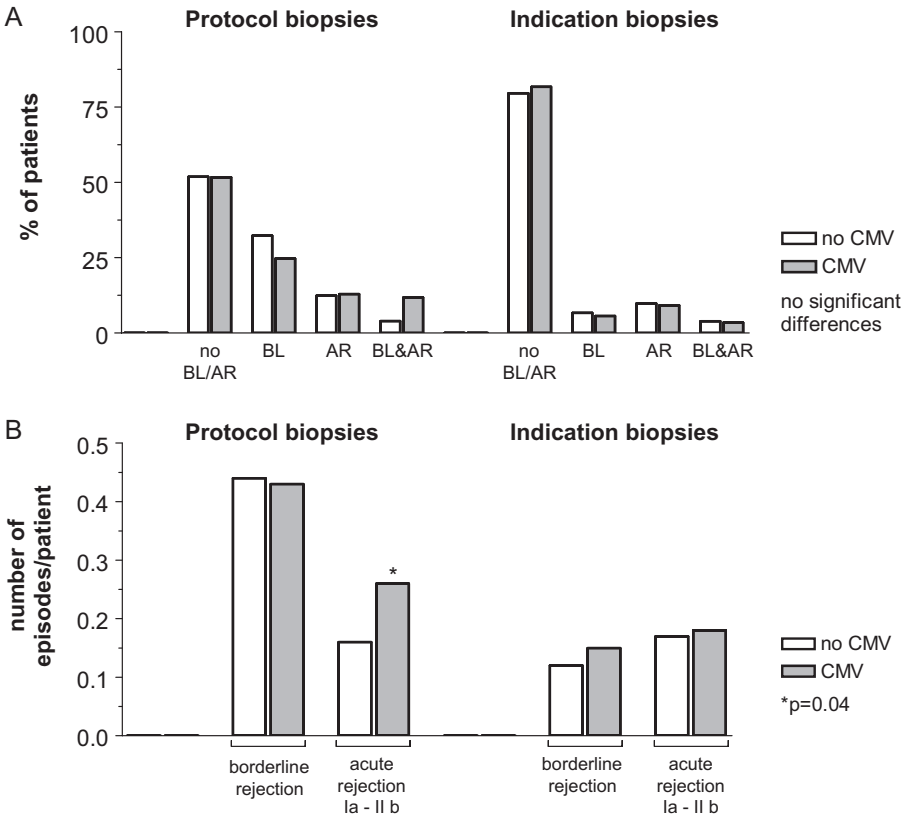

Fig. 4. Borderline rejection (BL) and acute cellular rejection in PBs and IBs in the subgroup of patients ($n = 340$) with and without CMV on a triple immunosuppressive therapy. (**A**) Proportion of patients with rejection episodes. Compared to patients without CMV, a trend was observed for patients with CMV, showing more patients with BL&AR and less patients with only BL in multiple protocol biopsies (P = 0.063). (**B**) Average number of rejection episodes per patient.

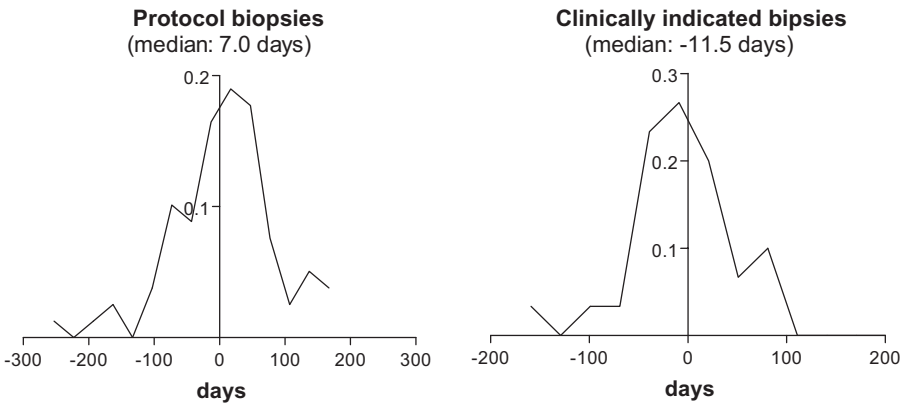

Fig. 5. Timely relationship between acute rejection episodes and CMV infection. The interval between onset of CMV infection and the biopsy with AR was calculated (days). Positive values represent CMV infections occurring before AR and negative values indicate cases with CMV infection occurring after the rejection.

and vice versa in patients receiving clinically indicated biopsies.

Long-term outcome in patients with and without CMV and AR

Long-term allograft function was assessed by the creatinine clearance applying the Cockroft–Gault formula. In addition, individual changes of clearance over time were calculated as percentage change for each patient relating the best creatinine clearance within the first 6 weeks post-transplantation to the following time points ('delta creatinine clearance'). The creatinine clearance was significantly higher after 1 and 2 years in patients without CMV (median values: 55.5 and 53.4 mL/min), compared to patients with CMV infection (median values: 49.4 and 43.2 mL/min; $P < 0.01$ for both time points). The delta creatinine clearance between those two groups was not significantly different after 1 and 2 years

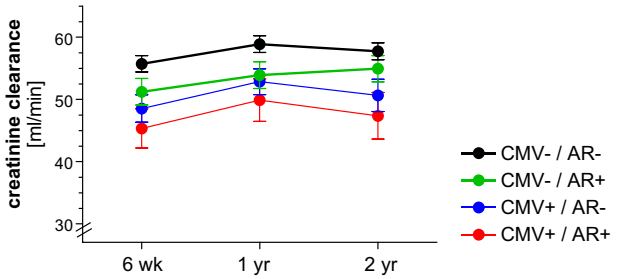

Fig. 6. Long-term allograft function in patients with and without CMV infection and AR. Four groups were created depending on a positive (+) or negative (-) history of CMV infection and AR during the first post transplant year. Creatinine clearance was calculated by the Cockroft–Gault formula at 6 weeks, 1 and 2 years after transplantation. Groups were significantly different (two-way analysis of variance: $P < 0.0001$), whereas no time-dependent changes were found ($P = 0.08$). Post testing by the Mann–Whitney *U*-test revealed significant differences at all three time points between CMV−/AR− and the other three groups. No significant differences were detected among the other three groups by the post test. Values represent means ± SEMs.

(delta after 1 year: −6.4 versus −6.6%, after 2 years: −9.4 versus −10.7%) indicating that patients in the CMV group had an inferior graft function early on after transplantation.

We even analyzed long-term functional outcome in subgroups depending if patients had a history of CMV infection and/or AR (Figure 6). For this analysis, patients with rejection episodes in protocol and clinically IBs were combined. Differences in renal allograft function were found early on after transplantation at 6 weeks and persisted at 1 and 2 years post-transplantation ($P < 0.0001$). The group without CMV infection and AR had the best function, whereas patients with CMV infection and AR had the worst function. Comparison of the individual clearance changes over time between the four groups showed that patients with CMV and AR tended to have a greater decline of allograft function (−7.7 and −18% at 1 and 2 years), compared to patients without CMV and AR (−6.2 and −9.6% at 1 and 2 years; $P = 0.064$).

Differences in the allograft function between the four groups were discernible early in the post-transplant course particularly between the CMV−/AR− and the CMV+/AR+ patients. Therefore, we looked into potential pre-existing influencing factors for the allograft function by multinominal regression analysis such as donor age and gender, cold ischemia time, delayed graft function, donor's and recipient's CMV serostatus. Besides the donor's and recipient's CMV serostatus, two factors were significantly different: Firstly, patients with CMV and AR had grafts from donors that were up to 11.5 years older. Secondly, the rate of delayed graft function in CMV+/AR+ patients was much higher than in CMV−/AR− patients, particularly in female patients (44.4 versus 22.8%) (Table 5).

Finally, we examined mortality and graft loss of patients with and without CMV infection. In the 153 patients with CMV infection, 5 patients (3.2%) had a graft loss and 5 patients (3.2%) died during the first year. These numbers were not significantly different ($P = 0.39$) from the 441 patients without CMV, which showed 7 graft losses (1.6%) and 8 deaths (1.8%). Also, no differences were found for graft loss and death after the second year of transplantation ($P = 0.37$).

Table 5. Analysis of factors[a] with potential effect on long-term allograft function in the different patients groups formed depending on a history of CMV and AR

	Donor age (years)		Rate of delayed graft function (%)	
	(Male recipients)	(Female recipients)	(Male recipients)	(Female recipients)
CMV−/AR−	44.4 ± 16.9	47.2 ± 14.2	27.7	22.8
CMV−/AR+	45.1 ± 15.9	49.4 ± 15.7	35.2	27.1
CMV+/AR−	51.5 ± 14.1	53.0 ± 16.8	30.8	38.0
CMV+/AR+	55.9 ± 14.6	52.7 ± 16.7	32.3	44.4

[a]Nominal regression analysis was separately performed in male and female patients because of the inclusion of the factor pregnancy in females. Donor age differed significantly between the four patient groups ($P < 0.01$ in male patients; $P = 0.037$ in female patients). Delayed graft function was significantly different in females only ($P = 0.05$).

Discussion

CMV infection is a frequent complication in the early post-renal transplant period [1]. In addition to CMV-related morbidity and mortality, CMV has been associated with a negative impact on the graft function and with increased rates of AR, but data from previous clinical observations have been inconsistent and are considered controversial [3, 4]. We have utilized our large cohort of patients with clinically IBs/PBs and functional data to explore the association between CMV and AR in renal transplantation.

We have been able to confirm previous observations that most CMV infections occur in the first 6 months post-transplant [22], and that disease onset is delayed in patients receiving CMV prophylaxis in the early post-transplant period (Figure 1). Additionally, we confirm previous observations that patients with the serostatus D+/R− or D+/R+ have more often CMV infection [22], with higher rates of recurrent and symptomatic CMV episodes in the D+/D− group. This could indicate a benefit of extended CMV prophylaxis in patients with D+/R− serostatus and possible efficacy of CMV prophylaxis in patients with D+/R+ serostatus to avoid CMV-related morbidity [23].

With the inclusion of >40 clinical variables in our analyses, we have been able to identify additional potential risk factors for CMV infection that have not been previously described. The need of dialysis post-transplantation, higher donor's age and recipients' pregnancies prior to transplantation were factors associated with a higher incidence of CMV infection. Nonetheless, regression analysis identified CMV serostatus as the most important risk factor for CMV infection.

Former studies reported MMF and anti-lymphocyte induction therapy as significant risk factors for CMV infection [24–26]. Interestingly, more recent studies have suggested a protective effect of MMF in this setting [27, 28]. In our patient cohort, the choice of immunosuppressive regimen including induction therapy was not related to the incidence of CMV infection.

Several previous studies have postulated an association between CMV infections and AR, but the results are inconsistent [7–9, 10–13, 29]. These inconsistencies may derive

from the fact that ARE were not always confirmed by biopsy [7] and that small patient cohorts were analyzed in some of the studies. In our reasonably large cohort of patients, we did not find a significant association between CMV and AR in clinically IBs. Even more, the analysis of PBs that may pick up clinically silent AR did only reveal a relation to CMV in a subgroup of patients with triple immunosuppression.

Studies have suggested that patients with clinically symptomatic or recurrent CMV infection are especially vulnerable having a higher frequency of AR. However, detailed analysis of our clinical data did not confirm this link as the same frequency of AR was observed in patients with asymptomatic CMV viremia or a single episode of CMV infection.

Another matter of controversy is the time course of CMV infection and AR. It is unclear whether CMV infection precedes AR or vice versa [4, 12, 14]. A case can be made for both scenarios. Immunosuppressive therapy is often reduced when the diagnosis of CMV infection is established, which may subsequently promote AR [5]. Also, CMV infection has been associated with upregulation of cytokines, adhesion molecules and increased expression of MHC class II surface markers that may result in AR [5]. On the other hand, acute rejection episodes require escalation of immunosuppressive therapy, which may promote CMV infection. We found that both scenarios—AR preceding CMV infection and CMV infection occurring before AR—occurred with almost identical frequencies in our patient cohort. One possible explanation for this observation is that both pathophysiologic entities are similarly relevant. Alternatively, one could postulate that there is no causal link between CMV infection and AR.

Finally, it is important to know whether CMV is related to worse long-term outcome. Similar to others [4, 23, 30, 31], overall outcome was inferior in patients with CMV infection. However, we and others show that a more detailed examination is necessary. Boratynska *et al.* [30] reported that patients with both CMV infection and AR had the worst renal function. The potentiation of deleterious effects of AR and CMV disease was also suggested by Humar *et al.* [31] and similarly, by Nett *et al.* [4]. In the report of McLaughlin [32] patients with the CMV serostatus D+R− had an increased risk of allograft loss; yet, multivariate analysis identified only delayed graft function and AR but not CMV as independent factors. In our analysis, the four groups with and without CMV/AR were clearly different in the long-term outcome and with regards to donor age and rates of delayed graft function. The worst renal function at 1 and 2 years occurred in the subgroup of 21 patients who encountered both CMV infection and AR. Yet, we propose that the strong differences in donor age and rate of delayed graft function between patient groups with and without CMV/AR can explain the observed outcomes adequately. In favor of this hypothesis is the fact that the worse graft function in patients with CMV and AR was observed early on after transplantation.

There are possible limitations to this study. The study design is retrospective. Nevertheless, we utilize a large cohort of patients that can outweigh the deficits a retrospective study carries. Less than half of the patients received a more 'traditional' immunosuppressive regimen with two immunosuppressive drugs. This might better reflect the variability in immunosuppressive regimens in different centers across the world. More than 50% of the patient receive immunosuppressive protocols according to current standards.

In conclusion, our data suggest that the link between CMV infection and ARs is far less significant than previously thought. Also, outcome in patients with CMV infection may be more determined by coexisting conditions like high donor age and delayed graft function, which are more prevalent in cases with CMV. Nonetheless, antiviral prophylaxis may be beneficial in patients with D+/R− and D+/R+ serostatus to minimize CMV-related morbidity in the early post-transplant period.

Supplementary data

Supplementary data is available online at http://ndt.oxfordjournals.org.

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Conflict of interest statement. None declared.

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