Kidney International, Vol. 66 (2004), pp. 329–337

Impact of early cytomegalovirus infection and disease on long-term recipient and kidney graft survival

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Impact of early cytomegalovirus infection and disease on long-term recipient and kidney graft survival.

Background. The impact of cytomegalovirus (CMV) infection and disease on long-term outcome after kidney transplantation is still unsettled.

Methods. Between 1994 and 1997, 397 consecutive first kidney graft recipients and 74 retransplants were included in the study and followed prospectively until December 31, 2001. CMV infection (CMV pp65 antigenemia) and CMV disease were recorded once weekly during the first 100 days after transplantation. No CMV prophylaxis or preemptive therapy was given. In a multiple Cox proportional hazard model allowing time-dependent covariates, the effects of asymptomatic CMV infection and CMV disease, recipient age and gender, retransplantation, living donor, panel-reactive cytotoxid antibodies, acute rejection, and graft loss were tested on overall mortality beyond 100 days post-transplantation. In a similar analysis, the effect of asymptomatic CMV infection and CMV disease plus other factors were tested on death censored graft loss beyond 100 days.

Results. Median (range) follow up time was 66.6 (<1–86.9) months. The incidence of CMV infection and disease during the first 100 days was 62.8% and 23.4%, respectively. The number of total deaths was 96 (20%), 82 occurred after the first 100 days. Independent risk factors for overall mortality beyond 100 days were asymptomatic CMV infection, RR = 2.90 (95% CI 1.61–5.22) ($P = 0.001$), CMV disease, RR = 2.50 (95% CI 1.31–4.79) ($P = 0.006$), both compared to no infection or disease, recipient age, RR = 1.066 per year (95% CI 1.048–1.084) ($P < 0.001$), and graft loss in the whole study period RR = 7.88 (95% CI 4.75–13.08) ($P < 0.001$). Asymptomatic CMV infection and CMV disease were not independent risk factors for death censored graft loss, but they significantly reduced graft survival uncensored for death, (log rank $P = 0.001$, respectively).

Conclusion. Asymptomatic CMV infection and overt CMV disease during the first 100 days increase the risk of recipient mortality beyond 100 days. This raises the question whether CMV prophylaxis should be given routinely after kidney transplantation.

Key words: cytomegalovirus disease, cytomegalovirus infection, kidney transplantation, kidney graft survival, patient survival.

Received for publication August 19, 2003
and in revised form December 17, 2003
Accepted for publication February 16, 2004

Cytomegalovirus (CMV) is the single most important infectious agent in kidney allograft recipients and is a major source of morbidity [1].

Previously we have investigated the natural history of CMV infection and disease in kidney allograft recipients during the first 100 days post-transplantation [2]. We found that CMV infection and disease are independent risk factors for clinical acute rejection during the first 100 days post-transplantation [3].

Most CMV infection and disease occur during the first 100 days after kidney transplantation. The impact of this early CMV infection and disease on long-term outcome after transplantation remains controversial. Some clinical studies indicate an association of CMV disease with an increased risk of kidney graft loss [4–6]. However, others fail to show such an association [7, 8]. The present study was designed to follow kidney allograft recipients prospectively and evaluate the influence of early CMV infection and disease on long-term recipient and graft survival. First, the objectives were to study recipient overall mortality and death-censored graft loss (deaths with functioning grafts are excluded) beyond 100 days post-transplantation. We also wanted to evaluate uncensored graft loss (death with functioning graft is considered as graft failure) and hospital-treated infectious diseases other than CMV disease beyond 100 days post-transplantation. Our hypothesis was that CMV infection and disease during the first 100 days might have a detrimental effect on overall recipient mortality and death-censored graft loss beyond 100 days post-transplantation.

No patients in this study received CMV prophylaxis or preemptive therapy. Only deferred anti-CMV therapy was given, implying that positive antigenemias were only treated when the patients presented symptoms related to CMV disease.

METHODS

Patients and study design

A cohort of 496 adult recipients received a kidney transplant at our center in the period from October

Table 1. Recipient and donor characteristics

	First transplants $N = 397$	Retransplants $N = 74$	P value
Recipient age *years* mean (range)	50 (17–80)	42 (18–67)	<0.01
Gender *female/male*	126/271	29/45	NS
Living/cadaveric	157/240	15/59	0.002
Donor age *years* mean (range)	45 (0–81)	42 (5–81)	NS
Cold ischemia *hours* mean (range)	9.1 (0–31)	13.1 (<1–28.6)	<0.01
Preemptive transplantation *number* (%)	100 (25)		
Panel-reactive cytotoxic antibodies positivity *number* (%)	15 (4)	19 (26)	<0.01
HLA-AB mismatches mean ± SD	2.1 ± 1.1	1.8 ± 1.1	0.04
HLA-DR mismatches *number* (%)			NS
0	150 (38)	38 (51)	
1	226 (57)	31 (42)	
2	21 (5)	5 (7)	

HLA is human leukocyte antigen. Chi-squared tests were used to compare categorical data and unpaired t tests were used to compare normally distributed data between first transplants and retransplants.

1994 to July 1997, 417 first transplantations and 86 retransplantations. The cohort has been described in detail [2]. Multiorgan transplantations were excluded. Excluded from analyses were also four CMV IgG-negative patients receiving transplants from CMV seronegative donors (D− R−) who developed CMV pp65 antigenemia without symptoms of CMV infection, probably due to accidental sample labeling or handling. Six patients received more than one kidney graft during the study period, and only the first transplantation is considered in the statistical analyses.

Adequate data for prospective investigation of CMV infection and disease the first 100 days post-transplantation were obtained for 471 of the patients. The present manuscript deals with a prospective follow up of these 471 patients until December 31, 2001. Of these, 397 were first transplantations and 74 were retransplantations. Donor and recipient demographic characteristics are shown in Table 1.

Information of death-censored graft losses, total deaths, and cardiovascular events and cardiovascular deaths were recorded for all patients over the study period. All infectious diseases requiring hospitalization (except from CMV infections) occurring after the first 100 days post-transplantation were recorded. Data were collected from the Norwegian Renal Registry, and deaths were double-checked with the national census.

Graft losses were censored for death (death with functioning graft is not considered as graft loss). The date of graft loss was defined as the day the patient started dialysis for more than 30 days or was retransplanted. The study was approved by the Datainspectorate, Oslo, Norway, and by the Regional Ethics Committee.

Antimicrobial prophylaxis

Primary prophylaxis against *Pneumocystis carinii* pneumonia with trimethoprim-sulfamethoxazole (or trimethoprim alone when allergy to sulfa was suspected) was given to all the patients for 6 months. Prophylaxis against CMV or other herpes viruses was not given.

Immunosuppressive treatment

Cyclosporin (CsA), steroids, and azathioprine constituted the standard immunosuppressive therapy. All patients received prednisolone and all except two (both retransplant patients) were initially treated with CsA. Patients with stable renal function 1 year after transplantation had the oral prednisolone dose reduced from 10 mg to 7.5 mg or 5 mg once daily.

Among first renal transplants, 362 (91%) received azathioprine versus 60 (81%) in retransplant patients. Mycophenolate mofetil (MMF) was usually given as a substitute to those who did not receive azathioprine. In some cases of rejection or side effects from the standard medication a small percentage of the patients were switched to MMF or tacrolimus during the study. Details of the immunosuppressive regimen have been presented previously [2].

Rejection

Acute rejection episodes were recorded during the first 100 days after transplantation and were treated with boluses of methylprednisolone (clinical rejection episodes).

Rejection episodes were considered steroid resistant if no significant decrease in serum creatinine was observed on the fifth day after start of standard methylprednisolone treatment. In such cases antithymocyte globulin or OKT3 was given. Biopsies were taken in most cases and in all steroid-resistant episodes.

CMV serology

IgG antibodies to CMV were detected by the Abbott Axsym system (Abbott, Chicago, IL, USA). Serum samples for CMV serology were taken immediately prior to transplantation and thereafter together with all specimens for CMV pp65 assay and repeatedly tested for CMV IgG until two consecutive samples tested positive.

CMV pp65 assay

CMV pp65 lower matrix protein was detected in leukocytes from ethylenediaminetetraacetic acid (EDTA)

blood samples. The method was a previously described modification of a reported procedure [9]. CMV infection was defined as a result of one or more cells positive for CMV pp65 antigen per 10^5 leukocytes.

In the first part of the study period screening was performed every second week during the first 100 days after transplantation until suspicion of CMV disease or the CMV pp65 antigen test became positive. Thereafter, the test was performed once or twice a week. Also, with signs of rejection or other adverse events, the test was performed more frequently. In the second half of the study period, screening was performed at least once a week in all patients during the first 100 days post-transplantation.

The results of the routine antigenemia testing were not blinded, and the treating physicians were informed about the results. Retrospective examination of all the patient case records revealed that eight CMV pp65 antigen positive patients received ganciclovir intravenously without fulfilling the definition of CMV disease. These eight patients had actually received preemptive treatment and were excluded from the study.

CMV disease

CMV disease was registered during the first 100 days after transplantation while the patients still were under surveillance at the National Hospital, and the incidence is previously described in detail [2]. The definition of CMV disease was by detection of CMV in a clinical specimen accompanied either by CMV syndrome with fever, muscle pain, leucopenia, and/or thrombocytopenia (other causes excluded), or by organ involvement such as hepatitis, gastrointestinal ulceration, pneumonitis, or retinitis. Leukopenia was defined as a leukocyte count less than 4×10^9/L and thrombocytopenia when the cell count was less than 100×10^9/L in peripheral blood. Hepatitis was defined as a rise in liver enzymes of at least twice the initial values without other known cause. Gastrointestinal CMV ulceration was confirmed by endoscopy and biopsy. Presence of CMV in tissue biopsies was detected by immunohistochemistry or growth of virus in cell cultures.

CMV disease was treated with intravenous ganciclovir, and none of these patients had their immunosuppressive treatment reduced.

Clinical monitoring

After leaving the hospital the patients were seen on an outpatient basis and stayed in a neighborhood residence during the first 100 days post-transplantation. Thereafter, they were under surveillance by their local nephrologist who yearly reported data concerning serum creatinine, dialysis status, medications, infections treated in hospital, blood pressure, adverse events, and deaths to the Norwegian Renal Registry.

Statistics

Chi-squared tests were used to compare categorical data and unpaired *t* tests were used to compare normally distributed data between first transplants and retransplants. The Cox proportional hazard model was used to estimate the effect of CMV infection, CMV disease, and other covariates on death-censored graft loss, overall mortality, cardiovascular events/deaths, and infection episodes (except from CMV infections) demanding hospitalization beyond 100 days post-transplantation, respectively. For estimating the effect of graft loss in the whole study period on overall mortality and cardiovascular mortality beyond 100 days post-transplantation a Cox proportional hazard model which allows time dependent covariates was used.

The effects of asymptomatic CMV infection, CMV disease, and other factors on the serum creatinine values at the end of follow-up were assessed by univariate and multiple linear regression analysis.

To test the effect of asymptomatic CMV infection and of CMV disease during the first 100 days on graft survival not censored for death beyond 100 days (death with functioning graft considered as graft loss) Kaplan-Meier analysis was used, and statistical significance was estimated using the log-rank test.

The statistical software SPSS (SPSS 11, Inc., Chicago, IL, USA) was used to perform the calculations.

RESULTS

General outcome

The median (range) observation time for all patients was 66.6 (<1 to 86.9) months.

Altogether 96 patients (20.4%) died during follow-up, 82 of the deaths occurred beyond 100 days post-transplantation (Table 2). In the 96 patients who died, median (range) time from transplantation to death was 38.6 (<1 to 79.4) months. Only one death was directly caused by CMV disease in a D+ R+ recipient who developed bleeding CMV duodenal ulcer and subsequently multiple organ failure.

In the whole study period there were 35 cardiovascular deaths with the following causes: myocardial ischemia and infarction ($N = 12$), cardiac failure ($N = 7$), cardiac arrest/sudden death ($N = 9$), fluid overload/pulmonary edema ($N = 1$), and cerebrovascular events ($N = 6$) (except ruptured vascular aneurysm). Twenty-nine of these deaths occurred after 100 days post-transplantation. Median (range) time to cardiovascular deaths was 38.4 (<1 to 79.4) months.

The total number of death censored kidney graft loss was 58 (12%) (Table 2). Median (range) time from transplantation to death censored kidney graft loss was 21.2 (<1 to 81.2) months. Forty-nine (84%) of all graft losses were caused by rejection, in three (5%) cases the graft

Table 2. Mortality, graft losses, and inhospital-treated infections in 471 patients

	First transplants $N = 397$	Retransplants $N = 74$
Death from all causes *number* (%)	84 (21)	12 (16) NS[a]
Cardiovascular deaths *number* (%)	32 (8)	3 (4) NS[a]
Deaths other than cardiovascular *number* (%)	52 (13)	9 (12) NS[a]
Malignant disease	12 (3)	1 (1)
Infectious disease	18 (5)	5 (7)
Other causes	22 (6)	3 (4)
Death-censored kidney graft loss *number* (%)	45 (11)	13 (18) NS[a]
Hospital-treated infectious diseases [other than cytomegalovirus (CMV)] after 100 days post-transplantation	120 (30)	28 (38) NS[a]

[a]Versus first transplants, chi-squared test.

never functioned, in one (2%) case urologic complications caused the graft loss, and finally five (9%) recipients experienced recurrence of the original kidney disease. Eighteen (31%) of these lost the graft function during the first 100 days after transplantation.

Sixteen of the 58 patients with death censored kidney graft loss were retransplanted during the study period, 12 originally in the first transplant group and four in the retransplant group. The number of hospital-treated infectious disease (except from CMV disease) beyond 100 days post-transplantation was 158 (34%).

CMV infection and disease

The overall incidence of CMV infection in all patients during the first 100 days after transplantation was 63%. The relative incidence of primary infection (D+ R−) and CMV reactivation (D± R+) was the same, 69% and 67%, respectively. However, the rate of CMV disease was nearly three times higher in the primary infected group (54% vs. 19%, $P < 0.0001$) [2].

Allograft rejection episodes

Clinical rejection episodes were diagnosed in 59% of the patients and were biopsy verified in 61% of the cases.

Risk factors for overall mortality beyond 100 days posttransplantation

In a Cox regression analysis of all kidney graft recipients surviving the first 100 days ($N = 457$), potential predictors were tested as independent risk factors for overall mortality beyond 100 days post-transplantation. These factors and univariate analyses of overall mortality beyond 100 days postoperatively are shown in Table 3. Independent risk factors for overall mortality beyond 100 days tested in a multiple Cox proportional hazard model are shown in Figure 1: asymptomatic CMV infection (RR = 2.90), CMV disease (RR = 2.50) (both these factors are compared to no CMV infection or disease), patient age (RR = 1.066 per year) (95% CI 1.048 to 1.084, $P < 0.001$) and death censored graft loss in total study period (RR = 7.88).

Table 3. Univariate analyses of overall mortality beyond 100 days post-transplantation[a]

Variable	Risk ratio	95% CI	*P* value
Asymptomatic CMV infection[b]	2.81	1.58–4.98	<0.001
CMV disease[b]	2.38	1.25–4.54	0.008
CMV pp65 positive cells × days (AUC)[c]			
1–50	2.74	1.30–5.80	0.008
51–900	1.93	0.98–3.83	0.06
901–5000	3.38	1.74–6.57	<0.001
>5000	3.12	1.59–6.12	0.001
Panel-reactive cytotoxic antibodies	1.16	0.50–2.66	0.7
Patient age	1.066	1.048–1.084	<0.001
Patient gender	1.01	0.64–1.61	1.0
Retransplantation	0.81	0.43–1.52	0.5
Living donor	0.45	0.26–0.75	0.003
Acute clinical rejection first 100 days	1.91	1.18–3.08	<0.01
Death-censored graft loss first 100 days	2.94	1.28–6.75	0.01
Death censored graft loss in total study period[d]	6.32	3.89–10.29	<0.001

Abbreviations are: CMV, cytomegalovirus; AUC, area under the curve.
[a]The statistical test used is a Cox proportional hazard model, which for the variable death-censored graft loss in total study period allows time-dependent covariates.
[b]Recipients with no CMV infection or disease is the reference CMV group.
[c]AUC for CMV pp65 levels measured during the first 100 days after transplantation.
[d]Tested as a time-dependent covariate.

In a similar multiple Cox regression analysis the same potential predictors as above were tested as independent risk factors for overall mortality beyond 100 days, with the exception that death-censored graft loss in the whole study period was replaced by death-censored graft loss during only the first 100 days. The results were essentially the same with respect to CMV infection, CMV disease, and patient age. Death-censored graft loss during the first 100 days did not significantly increase the risk of overall mortality (RR = 2.17) (95% CI 0.90 to 5.22, $P = 0.08$) (see Fig. 1).

CMV infection as a risk factor for overall mortality beyond 100 days was also assessed by evaluating the area under the curve (AUC) for CMV pp65 levels measured during the first 100 days, expressed as CMV pp65-positive cells × days. The AUC was estimated according to the trapezoidal method [10]. AUC was divided into categories with equal number of patients in the groups with CMV pp65 AUC of more than zero: 1 to 50, 51 to 900, 901 to 5000, and >5000. The univariate Cox regression analysis is shown in Table 3. Multiple Cox regression analysis revealed that the same factors as above were independent predictors for overall mortality beyond 100 days (Fig. 2).

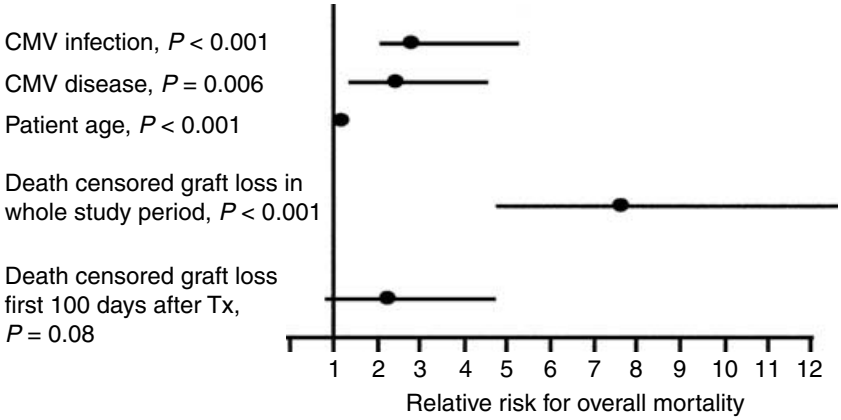

Fig. 1. Independent risk factors for overall mortality beyond 100 days post-transplantation. Relative risks (RR) and 95% CI are indicated. For patient age (RR = 1.066 per year) (95% CI 1.048–1.084). Death-censored graft loss in the whole study period and during the first 100 days were tested in two separate multiple Cox proportional hazard models, allowing time-dependent covariates in the analysis with death-censored graft loss in the whole study period. The risk ratios, confidence intervals, and *P* values for the covariates cytomegalovirus (CMV) infection, CMV disease, and patient age are given from the analysis with death-censored graft loss in the whole study period. The corresponding data with death-censored graft loss during the first 100 days as a covariate are essentially the same.

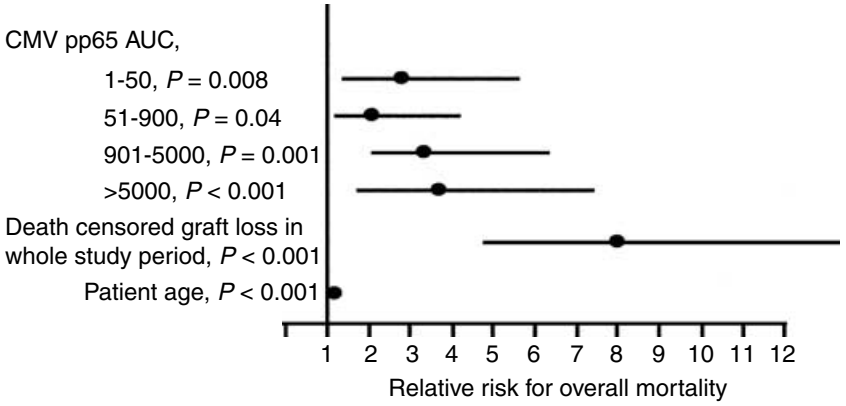

Fig. 2. Indepedent risk factors for overall mortality beyond 100 days post-transplantation. Relative risks (RR) and 95% CI are indicated. For patient age (RR = 1.066 per year) (95% CI 1.048–1.084). The statistical test used is multiple Cox proportional hazard model allowing time-dependent covariates.

Risk factors for cardiovascular mortality beyond 100 days post-transplantation

Separate univariate and multiple Cox regression analyses were performed for cardiovascular mortality beyond 100 days post-transplantation. Univariate analyses are shown in Table 4. Independent risk factors for cardiovascular mortality after 100 days in multiple analyses were patient age and death-censored graft loss in the whole study period, while CMV disease and acute clinical rejection the first 100 days were risk factors of borderline significance (see Fig. 3).

In a separate multiple Cox regression analysis of non cardiovascular mortality beyond 100 days ($N = 54$), recipient age, and death-censored graft loss still remained independent predictors in addition to asymptomatic CMV infection (compared to no infection or disease). For asymptomatic CMV infection (RR = 3.67) (95% CI 1.73–7.78, $P = 0.001$), CMV disease did not reach statistical significance (RR = 2.11) (95% CI 0.87–5.11, $P = 0.1$).

Risk factors for death-censored kidney graft loss beyond 100 days post-transplantation

A number of potential predictors were tested as independent risk factors for death-censored graft loss beyond 100 days post-transplantation. The factors were asymptomatic CMV infection and CMV disease (both compared to no CMV infection or disease), clinical rejection during the first 100 days, human leukocyte antigen region D, sublocus R (HLA-DR) zero vs. one and two mismatches, HLA-AB mismatches, panel-reactive cytotoxic antibodies, donor age, retransplantation, living donor, female gender, serum creatinine, and antihypertensive medication at 100 days after transplantation. These

Table 4. Univariate analyses of cardiovascular mortality beyond 100 days post-transplantation[a]

Variable	Risk ratio	95% CI	*P* value
Asymptomatic CMV infection[b]	1.64	0.63–4.23	0.3
CMV disease[b]	2.73	1.06–7.04	0.04
Panel-reactive cytotoxic antibodies	0.53	0.07–3.91	0.5
Recipient age	1.080	1.048–1.112	<0.001
Retransplantation	0.60	0.18–1.97	0.4
Living donor	0.4	0.17–1.01	0.05
Female gender	0.66	0.28–1.53	0.3
Acute clinical rejection first 100 days	4.65	1.62–13.35	0.004
Death-censored kidney graft loss in the whole study period	8.42	3.88–18.26	<0.001

CMV is cytomegalovirus.
[a]The statistical test used is a Cox proportional hazard model, for the variable death censored graft loss a Cox proportional hazard model allowing time-dependent cofactors is used.
[b]Recipients with no CMV infection or disease is the reference CMV group.

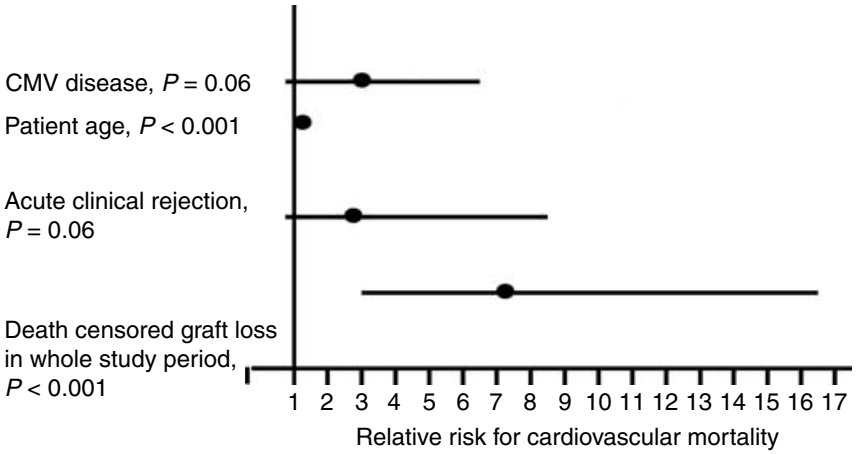

Fig. 3. Relative risk (RR) and 95% CI for cardiovascular mortality after 100 days post-transplantation. For patient age (RR = 1.085 per year) (95% CI 1.052–1.118). The statistical test used is a multiple Cox hazard model allowing time-dependent covariates.

factors were tested in a univariate Cox proportional hazard model (Table 5).

The same factors were tested in a multiple Cox proportional hazard model. Independent risk factors for death-censored kidney graft loss beyond 100 days post-transplantation were HLA-AB mismatches (RR = 1.49) (95% CI 1.09–2.04, $P = 0.01$), donor age (RR = 1.028 per year) (95% CI 1.003–1.053, $P = 0.03$), and serum creatinine at 100 days after transplantation (RR = 1.006) (95% CI 1.000–1.011, $P = 0.007$). Clinical rejection episodes were of borderline statistical significance (RR = 2.29) (95% CI 0.98–5.38, $P = 0.06$).

This analysis was repeated with HLA-DR plus AB mismatches (0 to 6) as one continuous variable replacing HLA-DR and HLA-AB mismatches as two separate variables. The results were essentially the same, for HLA-DR plus AB mismatches (RR = 1.43) (95% CI 1.11–1.83, $P = 0.006$) (data not shown).

CMV pp65 AUC was not found to be an independent risk factor for death-censored graft loss beyond 100 days (data not shown).

Table 5. Univariate analyses of death-censored kidney graft loss beyond 100 days post-transplantation[a]

Variable	Risk ratio	95% CI	*P* value
Asymptomatic CMV infection[b]	1.24	0.58–2.65	0.6
CMV disease[b]	1.99	0.91–4.37	0.09
Clinical rejection episodes first 100 days	3.81	1.69–8.62	0.001
HLA-DR one mismatch[c]	1.56	0.79–3.11	0.2
HLA-DR two mismatches[c]	1.88	0.53–6.66	0.3
HLA-AB mismatches	1.43	1.07–1.92	0.02
Panel-reactive cytotoxic antibodies	1.90	0.67–5.34	0.2
Donor age	1.046	1.023–1.070	<0.001
Retransplantation	1.63	0.77–3.42	0.2
Living donor	0.85	0.44–1.63	0.6
Female gender	0.98	0.51–1.91	1.0
Serum creatinine at 100 days after transplantation	1.009	1.005–1.013	<0.001
Medication for hypertension at 100 days after transplantation	0.94	0.48–1.82	0.9

Abbreviations are: HLA, human leukocyte antigen; CMV, cytomegalovirus.
[a]The statistical test used is a Cox proportional hazard model.
[b]Recipients with no CMV infection or disease is the reference CMV group.
[c]HLA zero mismatches is the reference group.

Risk factors for impaired long-term graft function

Analyses of the effects of asymptomatic CMV infection, CMV disease, and other factors on long-term graft function were also performed. Serum creatinine was registered at the turn of each year and the last value was used for analysis of long-term graft function. In patients who died during follow-up the last available serum creatinine was used. In cases with censored graft loss, the last available serum creatinine before graft loss is used in the analyses. Univariate analyses of serum creatinine at the end of follow-up are shown in Table 6, and the multiple analysis is shown in Table 7. The statistical tests used are linear regression. The results were essentially the same as for uncensored graft loss. Asymptomatic CMV infection and CMV disease were not independent risk factors for serum creatinine at the end of follow-up or for uncensored graft loss.

Graft survival uncensored for death

With death considered as a graft loss, overall graft survival from transplantation through the whole study period was 73%. In patients with functioning graft at 100 days post-transplantation, asymptomatic CMV infection during the first 100 days was associated with 74% graft survival compared with 86% graft survival in patients with no CMV infection or disease (log-rank $P = 0.001$) (patients with CMV disease, death, or graft loss during the first 100 days were excluded from analysis). Similarly, CMV disease during the first 100 days was associated with 71% graft survival compared to 86% graft survival in those with no CMV infection or disease (patients with asymptomatic CMV infection, death, or graft loss were excluded from analysis, log-rank $P = 0.001$). Kaplan-Meier survival curves for those with CMV infection and those with CMV disease are shown in Figure 4A and B, respectively.

Table 6. Univariate analyses of serum creatinine at the end of follow up[a]

Variable	Estimates	95% CI for estimates	SE	*P* value
Asymptomatic CMV infection[b]	8.87	−13.50–31.27	11.39	0.4
CMV disease[b]	49.40	23.25–75.56	13.31	<0.001
Acute clinical rejection first 100 days	55.57	36.12–75.03	9.90	<0.001
HLA DR 0 vs. one and two mismatches	25.26	5.08–45.43	10.26	0.01
HLA AB mismatches	4.48	−4.65–13.60	4.64	0.3
Panel-reactive cytotoxic antibodies	−13.55	−54.42–27.31	20.79	0.5
Donor age	2.46	1.90–3.03	0.29	<0.001
Retransplantation	20.10	−7.11–47.31	13.84	0.1
Living donor	−10.76	−31.34–9.82	10.47	0.3
Female gender	−22.60	−43.77–−1.42	10.77	0.04
Serum creatinine at 100 days after transplantation	1.01	0.85–1.18	0.09	<0.001
Medication for hypertension at 100 days after transplantation	1.88	−19.52–23.27	10.89	0.9

Abbreviations are: CMV, cytomegalovirus; HLA, human leukocyte antigen.
[a]The statistical test used is linear regression.
[b]The effect of asymptomatic CMV infection and CMV disease on serum creatinine are performed in the same analysis.

Risk factors for hospital-treated infections other than CMV beyond 100 days post-transplantation

In univariate and multiple Cox proportional hazard models a number of variables were tested as independent risk factors for hospital-treated infectious disease (with the exception from CMV infections) beyond 100 days post-transplantation. The variables were CMV infection (CMV disease excluded) and CMV disease during the first 100 days after transplantation, acute clinical rejection episodes during the first 100 days after transplantation, HLA-DR one and two mismatches, HLA-AB mismatches, panel-reactive cytotoxic antibodies, donor age, recipient age, retransplantation, living donor, female gender of recipient, serum creatinine, and hypertension medication at 100 days after transplantation. None of these variables came out as independent risk factors for hospital-treated infections.

Table 7. Independent risk factors of serum creatinine at the end of follow-up[a]

Variable	Estimates	95% CI for estimates	SE	*P* value
Acute clinical rejection first 100 days	12.86	−5.48–31.20	9.33	0.2
HLA DR zero vs. one and two mismatches	17.78	0.32–35.25	8.89	0.05
Donor age	1.42	0.88–1.96	0.28	<0.001
Serum creatinine at 100 days after transplantation	0.78	0.60–0.97	0.09	<0.001

HLA is human leukocyte antigen.
[a]The statistical test used is multiple linear regression. Acute clinical rejection did not reach statistical significance.

DISCUSSION

In this prospective study of 471 consecutive renal transplant recipients, both asymptomatic CMV infection and CMV disease compared to no CMV infection during the first 100 days after transplantation were independent risk factors for overall mortality beyond 100 days. To our knowledge the present study is the first to show that early asymptomatic CMV infection is an independent risk factor for recipient death. Our data are in accordance with two previous reports of a detrimental effect of CMV disease on renal transplant recipient survival [8, 11]. These studies comprised fewer patients and the immunosuppressive protocol (including antilymphocyte globulin induction) differed from the present study. Moreover, in one of these studies the CMV diagnosis was based on serology and urinary culture, which are less sensitive and less specific tests than those available today [11]. CMV viremia is also found to be associated with death in pediatric lung transplant recipients [12]. Moreover, after liver transplantation CMV disease is found to be a significant risk factor for patient death [13].

The AUC for CMV pp65 is a way to evaluate the "overall" viral load considering both duration and load of antigenemia in one parameter. The AUC concept has suggested advantages over quantification of viral load in predicting the clinical role for CMV antigenemia [10]. However, there were essentially no differences between the quartiles of CMV pp65 AUC as risk factors for recipient death beyond 100 days post-transplantation in the present study.

A possible causal association has been suggested between CMV and atherosclerosis. Such a mechanism could be a plausible explanation of increased mortality in the CMV infection and disease groups. Several authors have reported an association between CMV antibodies and coronary restenosis after atherectomy, but others have not found any association [14–17]. However, there is little epidemiologic proof of any links between CMV infection and classic native atherosclerois [18–20]. Indeed, in univariate analysis CMV disease was found to be an independent predictor of cardiovascular mortality beyond 100 days post-transplantation while asymptomatic CMV infection did not reach statistical significance. Although CMV disease was a risk factor of only borderline significance in multiple analysis this study was not powered to show an effect of CMV disease or asymptomatic CMV infection on cardiovascular death. On the other hand,

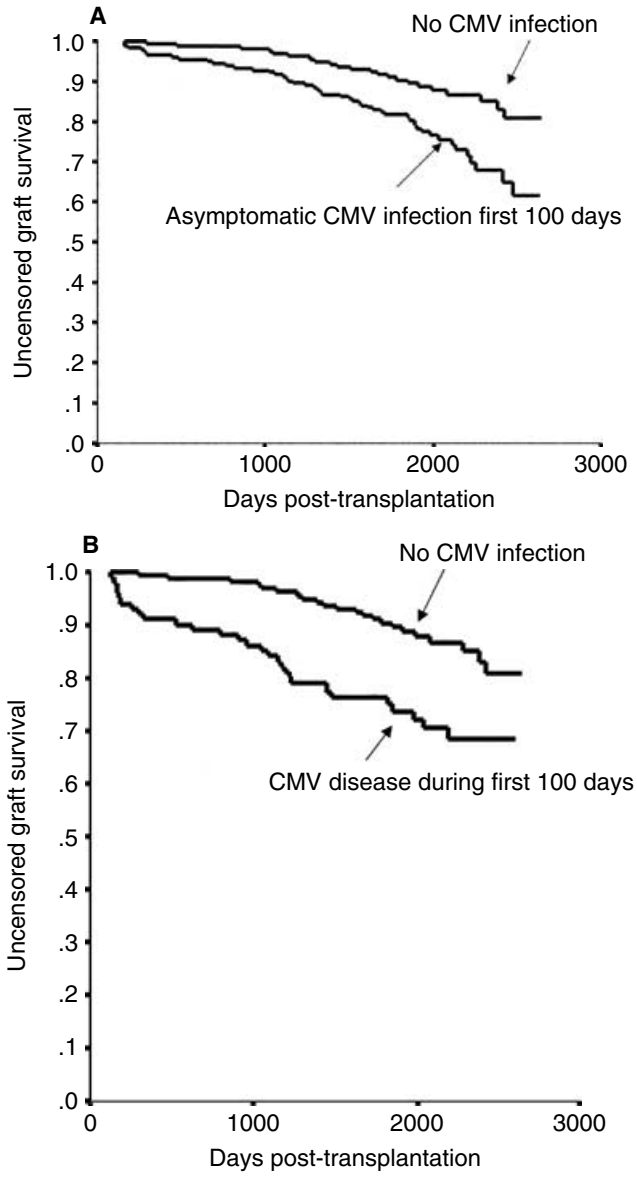

Fig. 4. Kaplan-Meier analysis of graft survival not censored for death beyond 100 days post-transplantation. (*A*) According to asymptomatic cytomegalovirus (CMV) infection during the first 100 days ($N = 182$) or no CMV infection ($N = 159$). Patients with CMV disease during the first 100 days post-transplantation are excluded from analysis ($N = 110$). Excluded are also patients with graft loss or death during the first 100 days ($N = 29$). Nine of these patients also suffered from CMV disease. The difference is significant (log-rank $P = 0.001$). (*B*) According to CMV disease occurring during the first 100 days ($N = 101$) or no CMV disease or infection ($N = 159$). Patients with asymptomatic CMV infection during the first 100 days are excluded from analysis ($N = 186$). Four of these patients also suffered from graft loss or death during the first 100 days. Excluded are also patients with graft loss or death during the first 100 days ($N = 29$). The difference is significant (log-rank $P = 0.001$).

multiple analyses revealed that asymptomatic CMV infection was an independent predictor of non-cardiovascular mortality. This indicates that also factors other than atherosclerosis might be involved with the increased mortality caused by CMV in this study.

Ganciclovir-treated CMV disease is reported to be an independent predictor of post-transplant diabetes mellitus in renal transplant recipients [21]. This raises the question whether occurrence of diabetes mellitus contributes to the increased mortality. Unfortunately, oral glucose tolerance test for surveillance of post-transplant diabetes mellitus was not introduced as a routine in our center at that time and such a hypothesis remains speculative. Nevertheless, the important finding of the present study is the excess of overall mortality associated with CMV infection and disease.

The present study revealed no detrimental effect of early CMV infection or disease on death-censored graft loss. There are only a few other studies investigating CMV disease as risk factor for death-censored graft loss. One example is a study of Humar et al [5], showing that CMV disease is a significant risk factor for death-censored graft loss, but only in the presence of acute rejection. However, when analyzing graft survival not censored for death (death with functioning graft considered as graft failure) in the present study, both asymptomatic CMV infection and CMV disease significantly reduced the risk of graft survival in Kaplan-Meier analyses. This is in accordance with other reports of CMV disease as a risk factor for graft loss [4]. Schnitzler et al [6] found a detrimental effect of CMV disease on kidney graft survival, but only in recipients with zero HLA-DR matches. In that study oral ganciclovir prophylaxis was given to all patients (except D− R−). On the other hand, no increased risk of graft failure was found in the CMV risk group (D+ and/or R+) in a small study of 84 renal graft recipients, where those in the CMV risk group received ganciclovir intravenously prophylactically when treated with antilymphocyte globulins due to acute rejection [7]. Eriksson et al [8] did not find any effect of CMV disease on graft survival in another small study of 85 renal transplant recipients of whom only two received ganciclovir prophylactically, but in that study graft survival is not clearly defined. It is obviously important to define graft loss censored or uncensored to make comparisons meaningful. In our study CMV seems to be associated with increased risk for death rather than isolated effects on the graft.

In multiple analyses where recipient age is one of the cofactors it seems most appropriate to use death-censored graft loss, as was done in the present study. The findings that donor age, serum creatinine and number of HLA-AB plus DR mismatches came out as independent risk factors for graft loss are in accordance with previous reports [8, 22, 23]. Acute clinical rejection significantly increased the risk of death-censored graft loss in univariate analysis, in accordance with reports by Opelz [22].

Preemptive therapy has been appointed the treatment of choice, at least in CMV seropositive recipients [24]. The same author advises prophylaxis in seronegative recipients of seropositive donors. With preemptive therapy

CMV disease is reduced [25, 26]. Moreover, a somewhat small retrospective study indicated that preemptive therapy against CMV may reduce uncensored graft loss [27]. Beneficial effects of preemptive therapy on graft and patient survival are also found in a larger, retrospective study [28]. However, the two groups in that study are not directly comparable. Also, the incidence of CMV infection was low in that study.

It is possible that a preemptive therapy would have modified the impact of CMV infection on long-term mortality in our study. Evidently, preemptive therapy may also reduce the AUC of CMV pp65 antigen. Obviously, a potential beneficial effect of preemptive treatment on graft and patient survival needs further study.

CMV is known to predispose to superinfection with a variety of microbial agents [1]. The present study could not reveal any effect of CMV infection or disease during the first 100 days on later hospital-treated infections.

CONCLUSION

The present study confirms that both asymptomatic CMV infection and overt CMV disease during the first 100 days post-transplantation are independent risk factors for overall recipient death beyond 100 days. Asymptomatic CMV infection or disease had no detrimental effect on death censored graft loss, but significantly reduced uncensored graft survival (Kaplan-Meier). Whether CMV prophylaxis or preemptive CMV therapy given routinely after kidney transplantation would improve patient survival remains to be shown.

ACKNOWLEDGMENTS

The study was financially supported by grants from The Research council of Norway and Hofmann-La Roche, Norway.

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