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ORIGINAL ARTICLE

Factors associated with the development of cytomegalovirus infection following solid organ transplantation

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Abstract

Background: Infection with cytomegalovirus (CMV) remains a potentially serious complication in transplant patients. In this study we explored the risk factors for CMV infection in the 12 months following a solid organ transplantation (n = 242) in patients monitored for CMV infection from 2004 to 2007. *Methods:* CMV infection was defined as 2 consecutive quantifiable CMV-polymerase chain reaction (PCR) values or 1 measurement of >3000 copies/ml. Data describing pre- and post-transplantation variables were extracted from electronic health records. Time to CMV infection was investigated using Cox proportional hazards analysis. *Results:* Overall, 31% (75/242) of solid organ transplant patients developed CMV infection: 4/8 (50.0%) heart, 15/43 (34.9%) liver, 30/89 (33.7%) lung and 26/102 (25.5%) kidney transplant patients. The risk of CMV infection according to donor (D)/recipient (R) CMV serostatus (positive + or negative−) was highest for D+/R−(adjusted hazard ratio 2.6, 95% confidence interval 1.6–4.2) vs D+/R+, and was reduced for D−/R+(adjusted hazard ratio 0.2, 95% confidence interval 0.2–0.8) vs D+/R+. *Conclusion:* Positive donor CMV-serostatus is a major risk factor for CMV-infection in CMV-naïve recipients, but also in recipients with positive CMV-serostatus. Conversely, if donor is CMV serostatus is negative, the risk of CMV infection is low, irrespective of recipients CMV-serostatus. These findings suggest poorer immune function towards donor-induced strains of CMV versus recipient own latent strains.

Keywords: *CMV infection, transplantation, serostatus*

Introduction

Post-transplantation cytomegalovirus (CMV) infection remains a potentially serious complication of immunosuppression, with the risk of progression to CMV disease, and is associated with increased morbidity and mortality and reduced graft survival [1,2].

There is currently no consensus on how best to prevent CMV disease after transplantation and a number of different prevention strategies exist [3,4]. Chemoprophylaxis or pre-emptive treatment of emerging infections using ganciclovir, valganciclovir or foscarnet in mono- or dual-therapy combinations has widely been applied and the various combinations appear to be equally effective. In addition CMV immune globulin is also used in lung and/or paediatric transplant recipients in some centres. Alternatively, it is possible to use a deferral strategy and treat CMV disease as it emerges; however this strategy is linked to increased morbidity and mortality [3–6]. The choice of which approach to take may be dependent on individual patient risk factors or it may be hospital- or department-dependent, where it is broadly applied to a population of patients without any type of stratification.

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Pathogenesis and risk factors for CMV disease are well described. There is an increased risk of CMV disease with increasing viral load, primary infection (in transplantation of a seropositive organ to a seronegative recipient), with the use of anti-thymocyte globulin, muromonab-CD3, and alemtuzumab, in patients with high Tacrolimus (TAC)/Cyclosporin A (CsA) levels, and through augmented immunosuppression with increasing doses of methylprednisolone for episodes of acute rejection [1,3,7]. However, relatively few studies have addressed combinations of risk factors predisposing to CMV infection. Recent evidence suggests that CMV infection, even in the absence of CMV disease, can cause diminished graft function, increased graft fibrosis, reduction of graft survival and, in stem cell transplant patients, a higher risk of graft-versus-host reactions [8–10].

Therefore, we explored risk factors for CMV infection within 12 months of transplantation in a cohort of solid organ transplant patients from a single hospital.

Patients and methods

Study design

Data were extracted retrospectively from electronic health records of solid organ transplant recipients. Written approval was obtained from the Danish National Board of Health. A search was done of the Rigshospitalet microbiology database and consecutive patients who had undergone a transplantation between 2004 and 2007 and had a minimum of 3 CMV-polymerase chain reaction (PCR) measurements in the 12 months following the transplantation were included in these analyses. Baseline was considered to be the time of the transplantation. Patients with fewer than 3 CMV-PCR measurements were excluded in order to secure the ability to evaluate the endpoint and avoid negative outcomes due to a lack of performed CMV-PCRs. Patients with unknown donor and/or recipient CMV serostatus were excluded from the multivariate analysis.

The following data were gathered: demographics, type of transplantation (heart, liver, kidney and lung), donor (D)/recipient (R) CMV serostatus (positive + or negative−) at time of transplantation, CMV-PCR levels, start and stop dates and dosages of all anti-CMV drugs (defined as any dose of cidofovir, foscarnet, ganciclovir and valganciclovir) and all immunosuppressive drugs (list of drugs used during the study period in at least 1 patient: anti-thymocyte globulin, basiliximab, daclizumab, rituximab, ciclosporin, tacrolimus, azathioprine, mycophenolic acid, mycophenolate mofetil, everolimus, sirolimus, methylprednisolone and prednisolone).

Each department used a specific immunosuppressive protocol, hence 4 different regimes were used. Universal or selective anti-CMV chemoprophylaxis was used depending on the department. Overall from our material, 166/258 (64.3%) patients were provided with anti-CMV chemoprophylaxis in the year (y) following the transplantation. Chemoprophylaxis consisted of either valganciclovir or a short course of intravenous ganciclovir followed by valganciclovir for all 166 patients.

Surveillance for CMV infection combined with diagnostics in the case of a suspected CMV infection was the guideline and clinical standard of care in all departments.

Detection of CMV-DNA was performed in the Virology Laboratory of the Clinical Microbiology Department at Rigshospitalet. The analysis was done on ethylenediaminetetraacetic acid (EDTA)-blood using the Cobas Amplicor monitor test [11].

Due to the centralization of surgical transplant procedures to Rigshospitalet, it is possible that a number of patients were discharged to their local regional outpatient clinic following the transplantation and thus were screened for CMV at other laboratories.

Endpoint definition

CMV infection was considered to be present if a patient had 2 consecutive CMV-PCR measurements above the lower limit of detection –300 copies/ml (i.e. a quantifiable CMV-PCR level) or alternatively 1 CMV-PCR of >3000 copies/ml, whichever came first. This definition was chosen in order to secure a reproducible and biologically relevant endpoint.

Statistical analysis

Cox proportional hazards models were used to assess the time from transplantation (baseline) until the endpoint, and Kaplan–Meier plots were produced. Patients were censored at the time of death, the end of the patient's individual follow-up period or a y after the transplantation, whichever came first. Time-fixed and time-updated variables were explored in the Cox models. The following time-fixed variables were considered for inclusion in the Cox models: type of transplantation, donor/recipient CMV serostatus, gender, age, prior transplantation and y of transplantation. The following time-updated variables were included in the Cox models: use of anti-CMV drugs and use of immunosuppressive drugs. Statistical analyses were performed using STATA (Stata Statistical Software version 10.1, 2001; StataCorp., College Station, TX, USA). All reported p-values are 2-sided using a level of significance of 0.05.

Results

Patients

Between 2004 and 2007 a total of 546 patients underwent solid organ transplantation. A total of 258 (47%) patients were routinely screened for post-transplantation CMV infection at Rigshospitalet; 20% of heart transplant patients, 29% of liver transplant patients, 78% of lung transplant patients and 50% of kidney transplant patients. A total of 288 patients were not routinely screened for post-transplantation CMV infection at Rigshospitalet, had fewer than 3 CMV-PCR measurements in the y following the transplantation and thus were excluded. The characteristics of patients are listed in Table I.

A total of 242 patients had full baseline data describing donor/recipient CMV serostatus available and thus were included in the multivariate analysis; 16 patients were excluded from this analysis due to unknown donor and/or recipient CMV serostatus.

Endpoints

In the 12 months from baseline, 86 (33%) patients developed CMV infection. Among the 242 patients included in the multivariate analysis, 75 (31%) patients developed CMV infection.

CMV-PCR measurements

There were no significant differences in the number of CMV-PCR measurements in patients where donor and recipient pre-transplantation CMV serostatus was known. Among the 4 groups, D+/R+ had a median (IQR) of 9 (5, 18) measurements, D+/R− had 12 (7, 21), D−/R+ had 11 (5.5, 16.5) and D−/R− had 11 (5, 16) measurements in the y following transplantation ($p = 0.32$).

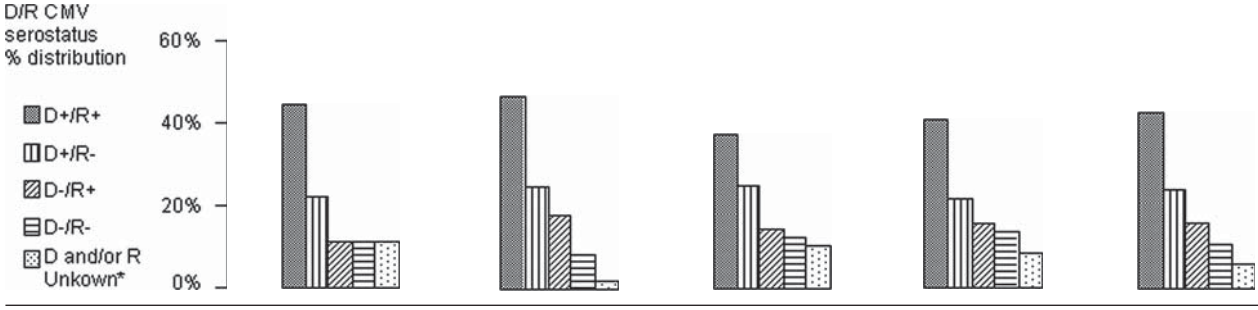
Risk factors

The results of the unadjusted and multivariable Cox analysis investigating risk factors for the development of CMV infection following solid organ transplantation are shown in Table II. The risk of CMV infection varied according to the type of transplantation: of those with a heart, liver, lung and kidney transplantation, 50.0%, 34.9%, 33.7% and 25.5%, respectively, had CMV infection in the y following the transplantation. The cumulative probabilities of CMV infection varied accordingly: of those with a heart, liver, lung and kidney transplantation, 82.2% (95% confidence interval (CI) 46.2–99.2%), 45.7% (95% CI 31.3–63.2%), 38.1.0% (95% CI 29.1–48.7%) and 32.4% (95% CI 23.1–44.2%), respectively, had CMV infection in the y following the transplantation (Figure 1).

The risk of CMV infection also varied according to donor/recipient serostatus prior to the transplantation. Of those with D+/R−, D+/R+, D−/R+ and D−/R−, 62.3%, 28.8%, 9.8% and 3.5%, respectively, had CMV infection in the y following the transplantation. The risk of CMV infection relative to D+/R+ (reference group) was increased for

Table I. Baseline characteristics of the 258 patients who underwent transplantations between 2004 and 2007 and had a minimum of 3 CMV-PCR measurements in the 12 months following the transplantation.

	Type of transplant				
	Heart ($n = 9$)	Kidney ($n = 104$)	Liver ($n = 48$)	Lung ($n = 97$)	Total ($n = 258$)
Gender, *n* (%)					
Female	2 (22.2)	44 (42.3)	25 (52.1)	52 (53.6)	123 (47.7)
Male	7 (77.8)	60 (57.7)	23 (47.9)	45 (46.4)	135 (52.3)
Age at transplantation, median y (IQR)	52 (41, 57)	40 (31, 54)	40 (11, 54)	51 (35, 58)	46 (30, 56)
Y of transplantation, median y (IQR)	2006 (2005, 2007)	2006 (2006, 2007)	2006 (2005, 2007)	2006 (2005, 2007)	2006 (2005, 2007)



CMV, cytomegalovirus; PCR, polymerase chain reaction; IQR, interquartile range.

Table II. Risk factors associated with CMV infection in the 12 months following solid organ transplantation.[a]

		Unadjusted			Multivariable		
	No. with event	HR	95% CI	*p*-Value	HR	95% CI	*p*-Value
Time-fixed variables							
Type of transplant							
Lung	30 (33.7%)	Ref.	–	0.15	Ref.	–	0.06
Heart	4 (50.0%)	2.3	0.8–6.6		1.9	0.6–5.9	
Liver	15 (34.9%)	1.7	0.9–3.2		2.2	1.1–4.6	
Kidney	26 (25.5%)	0.9	0.6–1.6		0.9	0.4–2.0	
Gender							
Female	33 (29.0%)	Ref.	–	0.44	Ref.	–	0.47
Male	42 (32.8%)	1.2	0.8–1.9		0.8	0.5–1.4	
Prior transplantation							
No	74 (31.0%)	Ref.	–	0.95	Ref.	–	0.12
Yes	1 (33.3%)	1.1	0.1–7.6		6.1	0.6–60.2	
CMV antibody status at transplant							
D+/R+	32 (28.8%)	Ref.	–	< 0.000	Ref.	–	< 0.000
D−/R+	4 (9.8%)	0.3	0.1–0.9	1	0.2	0.1–0.8	1
D+/R−	38 (62.3%)	2.5	1.6–4.0		2.6	1.6–4.2	
D−/R−	1 (3.5%)	0.1	0.0–0.7		0.1	0.0–0.5	
Age at baseline (per 5 y older)	–	1.01	0.9–1.1	0.87	1.02	0.9–1.1	0.61
Y of transplant (per additional y)	–	1.3	1.1–1.7	0.01	1.3	0.8–1.9	0.25
Anti-CMV drugs used at baseline							
No	58 (29.0%)	Ref.	–	0.38	Ref.	–	0.44
Yes	17 (40.5%)	1.3	0.7–2.2		1.4	0.6–3.6	
Immunosuppressive drugs used at baseline							
No	33 (31.1%)	Ref.	–	0.57	Ref.	–	0.11
Yes	42 (30.9%)	1.1	0.7–1.8		0.5	0.3–1.1	
Time updated variable							
Anti-CMV drugs used[b]	–	0.6	0.3–1.3	0.20	0.3	0.1–0.6	0.001

CMV, cytomegalovirus; HR, hazard ratio; CI, confidence interval; D+, donor positive; D−, donor negative; R+, recipient positive; R−, recipient negative.

[a]CMV infection was defined to be present if a patient had 2 consecutive quantifiable CMV-PCR measurements or 1 CMV-PCR of >3000 copies/ml.

[b]Anti-CMV drugs were defined as: any dose of cidofovir, foscarnet, ganciclovir and valganciclovir.

D+/R− and reduced for D−/R+ and D−/R−; adjusted hazard ratios ranged from 0.1 to 2.6 (global $p < 0.0001$) (Table II and Figure 2).

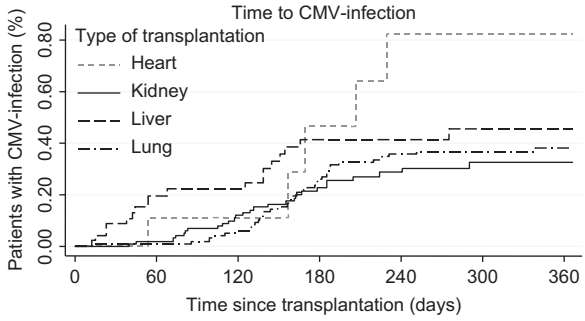
The use of chemoprophylaxis in the 4 types of solid organ transplantation was as follows: heart 66.7%, liver 47.9%, lung 67.0% and kidney 69.2%. Among the 166 patients given chemoprophylaxis, 7 (4.2%) developed CMV infection while using the medication, and an additional 47 (28.3%) developed CMV infection after cessation of prophylaxis. Among the 92 solid organ transplant recipients not given anti-CMV chemoprophylaxis, 32 (34.8%) developed CMV infection during follow-up. In the multivariable analysis, the risk of developing CMV infection was reduced by 70% (95% CI 40–90%) when using anti-CMV prophylaxis in a time-updated analysis (Table II).

Discussion

In our study CMV infection affected 31% of patients in the 12 months following a solid organ transplantation. Variations in infection rates were seen according to type of solid organ transplantation. Positive donor serostatus was associated with an increased risk of CMV infection in recipients with both negative and positive serostatus. Antiviral drugs were found to reduce the risk of CMV infection, but only during the period of active use.

A 31% risk of CMV infection in the y following a transplantation is consistent with previous reports of the risk of post-transplantation CMV infection [12,13]. While it was not possible to assess how many of the CMV-infected patients subsequently developed CMV disease in our study, a recently published meta-analysis has found the average risk of post-transplantation CMV disease to be similar at 30% [3].

In our study the risk of CMV infection was highest after heart, liver and lung transplantation, while the lowest risk was seen after kidney transplantation. The number of heart transplant patients in our study was low and as a result there was reduced



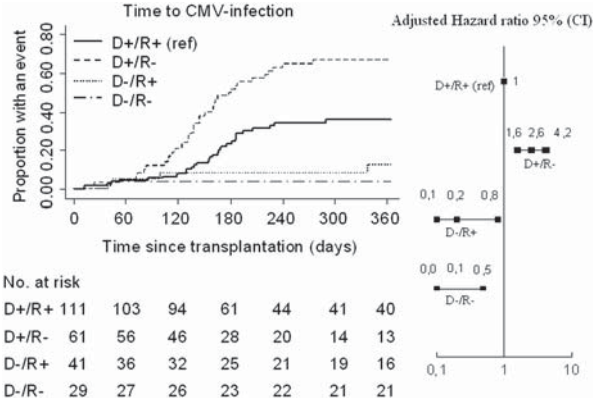
No. at Risk

Heart	9	8	5	3	1	1	1
Kidney	104	99	85	54	40	34	32
Liver	48	34	30	17	15	11	10
Lung	97	95	90	70	57	54	51

Figure 1. Kaplan–Meier plot of time to CMV infection following solid organ transplantation according to type of transplant. CMV infection was defined to be present if a patient had 2 consecutive quantifiable CMV-PCR measurements or 1 CMV-PCR of >3000 copies/ml.

power for comparisons according to type of solid organ transplantation. In studies comparing the risk of CMV disease following various types of solid organ transplantation, recipients of lung and liver transplants are reported as having the highest risk and kidney transplant recipients the lowest risk [13–15].

All serostatus matches in our study were tested with CMV-PCR measurements at similar rates, indicating that routine surveillance for CMV infection was carried out for all included patients. In our material the majority of grafts came from CMV-serostatus positive donors, which was expected considering the serostatus distribution in the background population, while the least frequently encountered match was negative/negative. We found an increased risk of CMV infection in CMV-naïve recipients who



No. at risk

D+/R+	111	103	94	61	44	41	40
D+/R-	61	56	46	28	20	14	13
D-/R+	41	36	32	25	21	19	16
D-/R-	29	27	26	23	22	21	21

Figure 2. Time to and risk of CMV infection according to donor (D)/recipient (R) CMV serostatus (positive + or negative −) in 242 solid organ transplantation patients. CMV infection was defined to be present if a patient had 2 consecutive quantifiable CMV-PCR measurements or 1 CMV-PCR of >3000 copies/ml.

received a CMV-positive organ, which is consistent with previous reports [2,13]. However, there was vast variation in risk of CMV infection among recipients who were CMV serostatus-positive at the time of transplantation, with an 80% higher risk if the donor was serostatus positive vs negative. These findings suggest less immunologic competence towards new donor-induced CMV strains compared with the recipient's weakened immune system to maintain control of his/her own latent strain. But they may also reflect poor viral control in a transplanted graft.

Previous studies have shown that CMV infection in D+/R+ is more often a donor-derived 're-infection' rather than a reactivation of a latent strain [16]. In addition, in a recent study of CMV infection following kidney transplantation the infection rate in D+/R+ was 1.72 times that of D−/R+, representing an odds ratio of 2.2 ($p = 0.006$), findings that also support the validity of our observations [16,17].

Consistent with the literature [13], we found that anti-CMV chemoprophylaxis reduced the risk of CMV infection while actively used. However, the 12-month CMV infection prevalence rate was comparable in patients given and not given chemoprophylaxis, suggesting that chemoprophylaxis merely postpones the onset of this event relative to the time of transplantation, rather than preventing it. Results from several studies support these findings, commonly referring to the observations as so-called delayed onset CMV infection and disease [13,18]. Further prolongation of the prophylaxis period to 6 months in a group of D+/R− kidney transplant recipients has recently been found not to prevent infection either, but simply to delay the time of onset even further [19].

There are several limitations to this study; most importantly the retrospective nature of the study may have made it impossible to capture all of the data accurately. A major limitation is that routinely screening for CMV was only performed in 20–78% of the patients depending on the type of transplantation. The single centre setting may have limited the variations in post-transplant therapy.

In summary our results suggest that individual patient risk factors for CMV infection can be identified and potentially be useful for stratification of antiviral intervention.

Most notable, positive donor CMV serostatus was found to be a major risk factor for CMV infection regardless of recipient serostatus, while the risk of CMV infection following transplantation of a donor negative organ was small. Determination of CMV donor and recipient status should be standard in modern transplant medicine and could be used to stratify intervention based on risk. Thus the pre-emptive strategy could be used in donor-negative transplantations, while prophylaxis for at least 3

and maybe as long as 6 months could be used following all donor-positive transplantations. Such actions could potentially rationalize the choice of strategic antiviral approach and prove to reduce the risk of post-transplantation CMV infection in high-risk patients, while reducing the drug burden as a result of unnecessary chemoprophylaxis in low-risk patients.

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Declaration of interest: All authors declare no conflict of interest.

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