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ORIGINAL ARTICLE

# The risk of cytomegalovirus recurrence after kidney transplantation

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## Summary

Recurrent cytomegalovirus (CMV) infections commonly occur after kidney transplantation. We studied the impact of secondary prophylaxis and other factors on the risk of CMV recurrence. All kidney transplant recipients between 2004 and 2009 in our institution were analyzed ($N = 254$). Patients with CMV infection were included ($N = 62$). CMV infections were diagnosed with quantitative PCR. CMV D+/R− recipients received 6 months valganciclovir prophylaxis, after which DNAemia was monitored. After treatment, secondary prophylaxis with valganciclovir was given at the clinician's discretion for 2–26 weeks and CMV DNAemia was monitored. Altogether 43 reactivations and 19 primary infections occurred. Antiviral treatment with valganciclovir or ganciclovir was given to 45 patients; 34/62 (55%) patients received secondary prophylaxis for mean 62 days (range 14–180 days). CMV recurrence occurred in 14/43 (33%) seropositive patients and in 4/19 (21%) patients after primary infection. In logistic regression, delayed graft function (OR 3.4) and high viral load (>100 000 copies/ml) at initial diagnosis (OR 5.9) predicted recurrence. Use or length of secondary prophylaxis, CMV serostatus, level of immunosuppression, HLA mismatch, antiviral treatment, or time to clearance of viremia during treatment did not predict recurrence of CMV. CMV recurrences occur commonly despite secondary prophylaxis. High viral load at diagnosis predicted the risk of recurrent CMV infection.

## Introduction

Despite advances in immunosuppressive protocols and antiviral prophylaxis, cytomegalovirus (CMV) remains a major cause of morbidity and costs after organ transplantation and is also associated with long-term consequences [1]. Without prophylaxis, approximately 10–20% kidney transplant recipients suffer from symptomatic CMV infection and asymptomatic activation of the virus is seen in up to 50% recipients [2]. Although antiviral prophylaxis with valganciclovir or oral ganciclovir is effective in preventing CMV infections and prophylaxis is recommended for high-risk recipients for 3–6 months after transplantation [3], late-onset CMV infections commonly occur after completion of prophylaxis [4–7].

Infections caused by CMV can be treated with either oral valganciclovir or with intravenous ganciclovir in case of severe disease [3,8]. Recent consensus guidelines recommend the duration of treatment to be at least 2 weeks or until viral eradication [3]. Secondary prophylaxis is also recommended for 1–3 months after treatment, although not much data support the use of secondary prophylaxis and the optimal duration of secondary prophylaxis is unknown [3].

After treatment of CMV infections, recurrent infections occur in approximately 30% recipients, usually 1–3 months after the first infection episode [9–12]. Most of the recurrences are mild, but also serious infections have been described [13]. Some risk factors for recurrent CMV infections have been recognized, including high

viral load and viral load kinetics during treatment, acute rejection and donor positive/recipient negative (D+/R−) serostatus [9–13]. These risk factors have been recognized mostly in studies including recipients of various solid-organ transplants, with variable degree of immunosuppression and risk of infections. In addition, with the exception of a recent randomized trial of treatment of CMV infections with valganciclovir versus i.v. ganciclovir [8,10], these studies were from the era before valganciclovir. Therefore, not much is known about the risk of CMV recurrence after kidney transplantation in the current era.

The kidney transplant population in Finland is relatively homogenous; all transplantations are performed in one center, transplanted kidneys are well matched, and immunosuppression is relatively conservative. Despite this, CMV infections are common in our population [4,14]. The aim of this study was to investigate the impact of secondary prophylaxis and other potential risk factors (the degree of immunosuppression, HLA match, treatment of CMV infections, and viral load) on the risk of CMV recurrence after kidney transplantation.

## Patients and methods

All Helsinki University Hospital district patients who received a kidney transplant between 2004 and 2009 were retrospectively analyzed ($N = 254$). Patients with a documented CMV infection were included ($N = 62$). Baseline immunosuppression was usually a triple-drug regimen with cyclosporine (CyA), mycophenolate mofetil (MMF), and steroid. In immunologically higher risk patients (long waiting time, poor match, and re-transplantation) CyA was replaced by tacrolimus (Tac) and/or induction therapy with basiliximab was administered. In the majority of patients with stable graft function and especially in patients with problems in glycemic control or osteoporosis, steroids are usually withdrawn slowly during the first or second post-transplant year. Biopsy-proven acute rejections of grade I-II [15,16] were treated with high-dose intravenous corticosteroids and/or conversion of CyA to Tac.

Cytomegalovirus infections were diagnosed with a TaqMan-based real-time quantitative plasma PCR [17]. CMV seronegative recipients of an organ from a seropositive donor (D+/R−) received valganciclovir prophylaxis (900 mg once daily or dose adjusted according to renal function) for 6 months after transplantation. After the cessation of prophylaxis, patients were monitored for CMV DNAemia with TaqMan-based real-time quantitative plasma PCR [17] at 2–6 weeks interval for the first 3–6 months and also if CMV disease was suspected (fever, respiratory tract symptoms, leucopenia, thrombocytopenia, hepatopathy, gastroenteritis, and graft dysfunction). The quantitative real-time method used in this study correlates well with the most used commercial CMV-PCR method Cobas Amplicor Monitor [17]. The cut-off level of 2000–5000 copies/ml was found to be optimal for predicting CMV disease [17], as was demonstrated also by others using the Cobas Amplicor Monitor test [18]. D+/R− patients transplanted between 2004 and 2008 were also included in our previous studies of late-onset primary CMV infections [4,19]. In CMV seropositive patients, CMV PCR was performed at weeks 3, 12, 26, and 52 after transplantation and in case of symptoms attributable to CMV (fever, respiratory tract symptoms, leucopenia, thrombocytopenia, hepatopathy, gastroenteritis, and graft dysfunction). No antiviral prophylaxis was routinely given to seropositive patients. CMV infections were treated with either i.v. ganciclovir (severe disease and/or high viral load, 5 mg/kg twice daily or adjusted for renal function according to drug label) or valganciclovir (900 mg twice daily or adjusted for renal function according to drug label), or with reduction of immunosuppression, usually MMF. Occasionally, mild infections with a low viral load were followed until cessation of viremia without interventions. Antiviral treatment was continued until CMV DNAemia disappeared. After eradication of viremia, antiviral treatment was continued as secondary prophylaxis with valganciclovir (900 mg once daily or adjusted for renal function) for 2–26 weeks at clinician's discretion. No secondary prophylaxis was given to patients who were followed without antiviral treatment or were treated only with reduction of immunosuppression. No defined criteria of the length of secondary prophylaxis or when to use secondary prophylaxis existed, but was based on the clinician's discretion. After the cessation of antiviral treatment or secondary prophylaxis, CMV DNAemia was monitored with 2–4 weeks interval for at least 1–2 months.

Baseline and follow-up data were reviewed from the patient files. Baseline data included: baseline renal disease leading to uremia, length and modality of pretransplant dialysis, recipient age and gender, immunosuppressive regimen, cold ischemia time, HLA A, B, and DR mismatch, and delayed graft function as defined by the need of dialysis during the first post-transplant week. Follow-up data included: occurrence of acute rejections, graft function measured by plasma creatinine, and trough levels of CyA and Tac. Data associated with CMV infections included: symptoms associated with CMV infections, duration and modality (valganciclovir or ganciclovir) of treatment, possible reduction of immunosuppression during treatment, time to clearance of viremia, duration of secondary prophylaxis, recurrence of CMV, symptoms associated with recurrence and treatment of recurrence.

All data are expressed as mean ± 1 standard deviation unless otherwise indicated. Statistical significances between the groups were measured with the nonparametric Mann–Whitney's *U*-test and Fisher's exact test. Univariate and multivariate logistic regression was used to estimate risk factors (odds ratio, OR) for recurrent CMV infections. Viral load, modality of treatment of CMV, length of antiviral treatment, the length of secondary prophylaxis, time to clearance of viremia, CMV serostatus, symptoms associated with CMV, immunosuppression (CyA versus Tac), trough levels of CyA or Tac, patient age and gender, renal function, delayed graft function (defined as the need for dialysis during first postoperative week), cold ischemia time and HLA mismatch were included in the analysis of risk factors for CMV recurrence. Multivariate analyses were performed based on the recommendation not to include more than one variable per 10 events in multivariate logistic regression [20]. Graft survival probabilities were estimated with the Kaplan–Meier method and differences between the groups were analyzed with the log-rank test. The calculations were performed with PASW Statistics software (version 18.0.3; IBM Corporation, Somers, NY, USA). *P*-values of <0.05 were considered significant.

## Results

### CMV infections

Of all the patients who received a kidney transplant between 2004 and 2009, 62 suffered from CMV infection after transplantation. Of these 62 patients, 43 were CMV seropositive before transplantation and suffered from CMV reactivation or reinfection, which occurred mean 93 days (range 16–255) after transplantation. Symptomatic reactivation was recorded in 25 patients (58%), whereas 18 infections were asymptomatic. Symptoms included: fever ($N = 15$), gastrointestinal symptoms, such as nausea, vomiting, or diarrhea ($N = 11$), respiratory tract symptoms ($N = 2$), and graft dysfunction ($N = 2$).

Between 2004 and 2009, altogether 48 CMV D+/R− patients received a kidney transplantation, of whom primary infection was detected in 19 patients (19/48, 40%) mean 113 days (range 8–505) after the end of 6 months valganciclovir prophylaxis and mean 292 days (range 186–685) after transplantation. Of the primary infections, only four were asymptomatic. In others, symptoms included: fever ($N = 12$), gastrointestinal symptoms ($N = 6$), respiratory tract symptoms ($N = 4$), and hepatopathy ($N = 1$). Mean viral load at diagnosis of CMV infections was 44 198 copies/ml (range 250–652 000) and mean peak viral load 54 842 copies/ml (range 250–652 000).

### Treatment of CMV infections

Of the 43 patients with CMV reactivation, 10 received treatment with i.v. ganciclovir followed by oral valganciclovir, 16 received treatment only with oral valganciclovir, and three were treated only with reduction or immunosuppression (temporary dose reduction or cessation of mycophenolate). In addition, mycophenolate dose was temporarily reduced in 20 patients treated with antiviral medication. In 14 patients with no symptoms and low-level viremia, infection was carefully followed until viremia subsided without any interventions. Mean duration of antiviral treatment was 22 days (range 7–51). Mean time of clearance of viremia after therapeutic intervention was 22 ± 16 days (ranging from 2 to 80 days). After antiviral treatment, secondary prophylaxis with valganciclovir was given to 16/26 patients with CMV reactivation for mean 52 days (range 14–180). Secondary prophylaxis was given only to those patients who received antiviral therapy for the treatment of CMV infection.

Of the 19 patients with primary CMV infection, nine received treatment with i.v. ganciclovir (followed by oral valganciclovir in four patients), nine were treated with only oral valganciclovir, and one patient was treated only with reduction of immunosuppression (mycophenolate). In addition, mycophenolate dose was temporarily reduced in seven patients treated with antiviral medication. Mean duration of antiviral treatment in patients with primary infection was 19 days (range 14–32). Mean time of clearance of viremia after therapeutic intervention was 24 ± 8 days (ranging from 13 to 40 days). After antiviral treatment, secondary prophylaxis with valganciclovir was given to all 18 patients for mean 72 days (range 14–180).

All patients cleared viremia with simultaneous resolution of symptoms and no clinically suspected cases of antiviral resistance were observed. Viral load at diagnosis and peak viral load were significantly higher in patients who received secondary prophylaxis compared with patients without secondary prophylaxis (75 888 ± 154 126 versus 2366 ± 3099, $P = 0.001$; and 94 468 ± 172 623 versus 2535 ± 3298, $P < 0.001$ respectively). If only patients who received antiviral treatment for CMV infection were included, the differences in viral loads between patients with or without secondary prophylaxis were not statistically significant (75 888 ± 154 126 versus 5431 ± 4390, $P = 0.36$; and 94 468 ± 172 623 versus 5860 ± 4629, $P = 0.14$).

### Recurrence of CMV infections

Recurrence of CMV infection was detected in altogether 18/62 patients (29%); in 14/43 (33%) patients, who were CMV seropositive before transplantation, and in 4/19

(21%) patients seronegative before transplantation. Recurrence occurred mean 32 days after the end of antiviral treatment (range 1–67) or mean 56 days after the last positive CMV PCR in patients not treated with antivirals (range 13–77) (Fig. 1). Mean viral load at diagnosis of the recurrence was 1493 copies/ml (range 260–5800). Majority of the recurrent infections were asymptomatic ($N = 13$). In others, symptoms included fever, gastrointestinal symptoms, and respiratory tract symptoms. In nine patients, recurrent infection was treated with valganciclovir. In one patient, infection was treated with reduction of immunosuppression, and in eight patients with no symptoms and low viral load, infection was carefully followed without interventions until viremia subsided.

Patients with or without CMV recurrence are characterized in Tables 1 and 2. No differences were observed in the baseline characteristics between patients with or without recurrent CMV infections. The frequencies of delayed graft function and acute rejections were somewhat higher in patients with recurrent CMV infections, but the differences did not reach statistical significance. No significant differences were seen in the intensity of immunosuppression between patients with or without CMV recurrence. Renal function at 12 months was reduced in patients with CMV recurrence ($P = 0.036$). A statistically nonsignificant trend toward reduced renal function was similarly seen at all time points in patients with CMV recurrence. First CMV infection episodes of patients with or without CMV recurrence are characterized in Table 2. Duration of

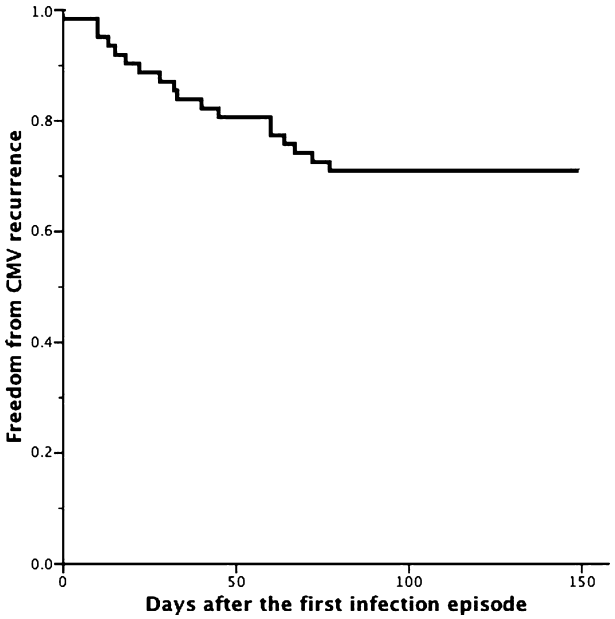


**Figure 1** Occurrence of cytomegalovirus (CMV) recurrence after the first CMV infection episode, either after the end of antiviral medication or after the last positive CMV PCR in patients who did not receive antiviral medication.

**Table 1.** Characteristics of patients with or without recurrent cytomegalovirus infection.

| | No recurrent CMV ($N = 44$) | CMV recurrence ($N = 18$) |
|---|---|---|
| Recipient age | 52 ± 11 | 52 ± 12 |
| Recipient gender (M/F) | 23/21 | 12/6 |
| HLA A, B, and DR mismatch | 2.2 ± 0.9 | 2.1 ± 1.1 |
| Cold ischemia time (hours) | 20 ± 5 | 19 ± 4 |
| Delayed graft function (%) | 11 (25) | 9 (50) |
| Acute rejection (%) | 6 (14) | 5 (28) |
| Cyclosporine trough level at 3 months (μg/l) | 144 ± 46 | 151 ± 37 |
| No. patients on tacrolimus (%) | 8 (18) | 1 (6) |
| Plasma creatinine at 1 month (μM) | 130 ± 71 | 156 ± 61 |
| Plasma creatinine at 12 months (μM)* | 108 ± 35 | 128 ± 41 |
| Plasma creatinine at last follow-up (μM) | 128 ± 86 | 153 ± 73 |
| Patients with D+/R− serostatus (%) | 14 (32) | 4 (22) |
| Length of follow-up (months) | 41 ± 19 | 37 ± 18 |

CMV, cytomegalovirus.
Mean ± SD unless otherwise indicated.
*$P = 0.036$.
All other differences are nonsignificant.

**Table 2.** Characteristics of first cytomegalovirus (CMV) infection episode in patients with or without CMV recurrence.

| | No recurrent CMV ($N = 44$) | CMV recurrence ($N = 18$) |
|---|---|---|
| Time of infection (days after transplantation) | 164 ± 133 | 130 ± 84 |
| Patients with fever during CMV infection (%) | 21 (48) | 6 (33) |
| Patients treated with antiviral medication (%) | 29 (66) | 14 (78) |
| Duration of antiviral treatment (days)* | 19 ± 6 | 26 ± 12 |
| Patients treated with mycophenolate dose reduction (%) | 23 (52) | 9 (50) |
| No. patients receiving secondary prophylaxis (%) | 22 (50) | 12 (67) |
| Length of secondary prophylaxis (days) | 73 ± 54 | 44 ± 22 |
| Viral load at diagnosis (copies/ml) | 18 951 ± 57 764 | 110 471 ± 200 572 |
| Peak viral load (copies/ml) | 24 524 ± 66 163 | 134 427 ± 224 293 |
| Clearance of viremia (days) | 23 ± 16 | 21 ± 9 |

CMV, cytomegalovirus.
Mean ± SD unless otherwise indicated.
*$P = 0.03$.
All other differences are nonsignificant.

**Table 3.** Main characteristics of (a) seronegative recipients of an organ from a seropositive donor (D+/R–) and (b) seropositive patients with or without cytomegalovirus recurrence.

| (a) D+/R– patients | No recurrent CMV (*N* = 15) | CMV recurrence (*N* = 4) |
|---|---|---|
| Recipient age | 48 ± 11 | 44 ± 4 |
| Delayed graft function (%)* | 4 (27) | 4 (100) |
| Time of infection (days after transplantation) | 303 ± 139 | 263 ± 58 |
| Patients treated with antiviral medication (%) | 14 (93) | 4 (100) |
| Duration of antiviral treatment (days)** | 18 ± 5 | 25 ± 6 |
| No. patients receiving secondary prophylaxis (%) | 14 (93) | 4 (100) |
| Length of secondary prophylaxis (days) | 84 ± 50 | 48 ± 29 |
| Viral load at diagnosis (copies/ml) | 41 060 ± 92 835 | 48 650 ± 92 648 |
| Clearance of viremia (days) | 21 ± 6 | 27 ± 5 |
| **(b) Seropositive patients** | **No recurrent CMV (*N* = 29)** | **CMV recurrence (*N* = 14)** |
| Recipient age | 54 ± 11 | 54 ± 13 |
| Delayed graft function (%) | 7 (24) | 5 (36) |
| Time of infection (days after transplantation) | 99 ± 63 | 92 ± 37 |
| Patients treated with antiviral medication (%) | 16 (55) | 10 (71) |
| Duration of antiviral treatment (days) | 20 ± 7 | 27 ± 13 |
| No. patients receiving secondary prophylaxis (%) | 8 (28) | 8 (57) |
| Length of secondary prophylaxis (days) | 58 ± 57 | 42 ± 20 |
| Viral load at diagnosis (copies/ml)*** | 7896 ± 23 173 | 131 078 ± 225 086 |
| Clearance of viremia (days) | 24 ± 19 | 19 ± 9 |

CMV, cytomegalovirus.
Mean ± SD unless otherwise indicated.
*$P$ = 0.02, **$P$ = 0.03, ***$P$ = 0.02.
All other differences are nonsignificant.

antiviral treatment was significantly longer in patients with recurrent CMV ($P$ = 0.03). No other significant differences were seen in the time of CMV infections, symptoms associated with infections or treatment of CMV infections. A trend toward higher peak viral load and higher viral load at diagnosis was seen in patients with CMV recurrences, but the difference did not reach statistical significance. Graft survival did not differ between patients with or without CMV recurrence (data not shown). D+/R– and seropositive patients with or without CMV recurrence are characterized briefly in Table 3. In D+/R– patients the frequency of delayed graft function was significantly higher ($P$ = 0.02) and duration of antiviral therapy significantly longer ($P$ = 0.03) in patients with recurrent CMV. In seropositive patients, viral load at diagnosis was significantly higher in patients with CMV recurrence ($P$ = 0.02). No other significant differences were found. A comparison of D+/R– and seropositive patients with recurrent CMV is shown in Table 4. As expected, the occurrence of first CMV infection episode was later after transplantation in D+/R– patients compared with seropositive patients ($P$ = 0.001). No other statistically significant differences were recorded.

In univariate logistic regression shown in Table 5, a high viral load (>100 000 copies/ml) at diagnosis of first CMV infection episode predicted the risk of recurrent CMV infection [odds ratio (OR) 5.9, $P$ = 0.03]. Similarly, delayed graft function predicted the risk of recurrent CMV infection (OR 3.4, $P$ = 0.04). Also longer duration

**Table 4.** Comparison of seronegative recipients of an organ from a seropositive donor (D+/R–) and seropositive patients with cytomegalovirus recurrence.

| | Seropositive patients (*N* = 14) | D+/R– patients (*N* = 4) |
|---|---|---|
| Recipient age | 54 ± 13 | 44 ± 4 |
| Delayed graft function (%) | 5 (36) | 4 (100) |
| Time of infection (days after transplantation)* | 92 ± 37 | 263 ± 58 |
| Patients treated with antiviral medication (%) | 10 (71) | 4 (100) |
| Duration of antiviral treatment (days) | 27 ± 13 | 25 ± 6 |
| No. patients receiving secondary prophylaxis (%) | 8 (57) | 4 (100) |
| Length of secondary prophylaxis (days) | 42 ± 20 | 48 ± 29 |
| Viral load at diagnosis (copies/ml) | 131 078 ± 225 086 | 48 650 ± 92 648 |
| Clearance of viremia (days) | 19 ± 9 | 27 ± 5 |

*$P$ = 0.001.
All other differences are nonsignificant.

**Table 5.** Univariate logistic regression analysis of the risk of cytomegalovirus recurrence.

| Variable | Odds ratio (OR) | 95% confidence interval | *P* |
|---|---|---|---|
| HLA A, B, and DR mismatch | 0.86 | 0.46–1.59 | 0.63 |
| Delayed graft function | 3.38 | 1.05–10.9 | 0.04 |
| Acute rejection | 2.44 | 0.64–9.34 | 0.19 |
| Plasma creatinine at 1 month | 1.01 | 0.99–1.01 | 0.20 |
| Cyclosporine trough level at 3 months | 1.00 | 0.99–1.02 | 0.57 |
| D+/R− serostatus | 0.55 | 0.15–1.98 | 0.36 |
| Treatment of CMV with mycophenolate reduction | 0.83 | 0.27–2.60 | 0.75 |
| Treatment of CMV with antivirals | 0.83 | 0.22–3.11 | 0.78 |
| Duration of antiviral treatment (days) | 1.11 | 1.02–1.22 | 0.02 |
| Clearance of viremia (days) | 0.99 | 0.95–1.04 | 0.66 |
| A high viral load (>100 000 copies/ml) at diagnosis | 5.91 | 1.22–28.69 | 0.03 |
| A high peak viral load (>100 000 copies/ml) | 4.32 | 0.99–18.90 | 0.05 |
| Fever as a symptom of CMV infection | 0.55 | 0.17–1.72 | 0.30 |
| Use of secondary prophylaxis | 2.00 | 0.64–6.28 | 0.24 |
| Length of secondary prophylaxis (days) | 0.98 | 0.96–1.01 | 0.10 |

CMV, cytomegalovirus.

of antiviral treatment was associated with increased risk of CMV recurrence (OR 1.11 per 1 day increase in the duration of treatment, $P = 0.02$). Other factors were not associated with the risk of recurrent CMV infections. A trend toward increased risk of CMV recurrence in patients with a high peak viral load during the first CMV infection episode was recorded, but the difference did not reach statistical significance ($P = 0.05$).

The low number or events allowed only two variables to be included in multivariate analyses, but the high viral load at diagnosis and delayed graft function were both independent risk factors when analyzed together in a multivariate model [OR 8.48, 95% confidence interval (CI) 1.36–52.67, $P = 0.02$; and OR 6.25, 95% CI 1.53–25.53, $P = 0.01$ respectively]. Similarly the risk associated with a high viral load at diagnosis was independent of CMV serostatus, use of secondary prophylaxis, or use of antivirals in the treatment of CMV infection, or other factors included in the analyses. However, the risk associated with the longer duration of antiviral treatment was not significant after adjustment with viral load at diagnosis (OR 1.12, 95% CI 0.99–1.25, $P = 0.06$). To increase the statistical power of our analyses, CMV seropositive and D+/R− patients were all included in the initial analyses. When seropositive and D+/R− patients were analyzed separately, no significant risk factors were identified in the D+/R− group in univariate logistic regression analyses (data not shown). In seropositive patients, only high viral load (>100 000 copies/ml) at diagnosis was significantly associated with the risk of CMV recurrence (OR 13.0, 95% CI 1.27–133.64, $P = 0.03$); no other significant risk factors were identified (data not shown). As secondary prophylaxis was only given to patients who were treated with antiviral medication during the infection, the risk of recurrence associated with the use or length of secondary prophylaxis was also analyzed separately in these patients with similar negative results in both D+/R− and seropositive patients (data not shown).

## Discussion

In this retrospective analysis of CMV infections in a well-matched kidney transplant population with a relatively conservative immunosuppression, recurrent CMV infections occurred commonly. Most of the recurrences were mild and asymptomatic. A high viral load at diagnosis of first CMV infection and delayed graft function after transplant operation were the only significant independent risk factors for recurrent CMV DNAemia, whereas CMV serostatus was not associated with the risk of CMV infections.

Although CMV infections are common after organ transplantation, not very much is known about the risk of CMV recurrence after kidney transplantation in the current era of valganciclovir. In previous studies including recipients of all solid-organ transplants, the risk of recurrent CMV disease is estimated between 15% and 23% [10,12] and the risk of recurrent CMV infection between 30% and 50% [9,10,13]. However, the level of immunosuppression and also the risk of infections differ between solid-organ transplants and the type of transplant is shown to affect also the risk of CMV recurrence [10]. In our material including only kidney transplant recipients, recurrent CMV infections were seen in 29% of patients. In our material, CMV was not monitored frequently in asymptomatic patients and probably some asymptomatic infections of short course were missed. In accordance with previous findings, recurrences in our study occurred

1–3 months after the first infection episode. Interestingly, the timing of recurrent infections after the first infection episode were similar in D+/R− patients, in whom primary infections occurred late, almost 10 months after transplantation because of 6 months valganciclovir prophylaxis. Similarly, primary CMV infections in D+/R− patients occurred mean 113 days after the end of prophylaxis, whereas first infection episode in seropositive patients occurred mean 93 days after transplantation. These finding suggests that longer antiviral prophylaxis delays but does not prevent primary infections or recurrences after treatment of infections and that CMV should be carefully monitored in these high-risk patients, also after treatment of late-onset primary infections. Most of the recurrences were virologic recurrences without any symptoms. Symptomatic recurrence was seen in 8% of patients. The symptoms attributable to CMV infections were not exactly defined in our material and probably did not meet the criteria of CMV disease in multicenter studies [8,21], making it difficult to compare the frequency of CMV disease in our material with that seen in other studies. Although according to international definitions of CMV infections in transplant recipients, recurrent infection is defined only as ''new detection of CMV infection in a patient who has had previously documented CMV infection'' without defining any symptoms [21], the clinical relevance of asymptomatic recurrences or recurrences with only mild symptoms is not known.

Several risk factors have been identified for CMV recurrence, including high viral load during the first infection episode and viral load kinetics during infection [9,10,12,13], D+/R− serostatus [9,22], acute rejection [11], and increased age [13]. Also we identified high viral load at diagnosis of first infection episode as a risk factor for recurrent CMV DNAemia. In univariate analysis, longer duration of treatment was associated with increased risk of CMV recurrence. This effect was not independent after adjustment with viral load, suggesting that the longer duration of antiviral treatment only reflects more severe CMV infection with higher viral load, and therefore longer antiviral treatment. We failed to confirm other risk factors described in the previous studies, most importantly D+/R− serostatus. However, D+/R− patients in our material received valganciclovir prophylaxis for 6 months after transplantation, which probably has an impact on the frequency of CMV recurrence after primary infection. Despite 6 months prophylaxis, late-onset primary infections are common in our transplant population [4]. Although effective antiviral prophylaxis might prevent the development of adequate CMV immunity [23], prolonging prophylaxis to 6 months delays the onset of CMV infections to a period with less intense immunosuppression (often more than 1 year after transplantation). This may enable stronger host immune responses, as in these high-risk patients, the level of CMV-specific immune response and also the level of innate immune response has been associated with the risk of late-onset disease [24,25]. Although no data about the immune responses of the patients in this study were available, this delayed onset of primary infections might possibly explain the finding that D+/R− serostatus was not identified as a risk for recurrent CMV in our study. As a result of low number of patients with CMV recurrence in our study, D+/R− and seropositive patients were all included in the analysis. Although the biology and timing of CMV infections are different in primary infections compared with reactivations or reinfections in seropositive patients and also the risk of recurrence may be different, the similar incidence of recurrences in both patient groups suggests that these groups may be analyzed together to increase the power of our analyses. Also previous studies about the risk of CMV recurrences have included all patients in their analyses [9–13]. When analyzed separately, only high viral load at diagnosis was associated with higher risk of CMV recurrence in seropositive patients, but no risk factors were found in D+/R− patients. The numbers of patients in these subgroup analyses may have been too small to detect significant differences. On the other hand, viral load may not be a significant risk factor in D+/R− patients, as these recurrences occurred much later after transplantation because of 6 months antiviral prophylaxis. In accordance with previous reports [26], the degree of immunosuppression was not associated with the risk of CMV recurrence in our study. The frequency of Tac use (versus CyA) was slightly lower (although not significantly) in patients with CMV recurrence, as has been described previously [26]. In our material, delayed graft function was a significant risk factor for CMV recurrence. No previous studies have associated delayed graft function with the risk of recurrent CMV infections. Delayed graft function is common in our kidney transplant population, probably because of long cold ischemia times resulting from long geographic distances and is seen in approximately 30% patients [4]. The mechanism of how delayed graft function might increase the risk of CMV recurrence is unclear and deserves to be studied further.

The largest material reporting recurrent CMV infections is from the multicenter VICTOR- trial, in which 321 solid-organ transplant recipients with CMV infection were randomized to receive either i.v. ganciclovir or oral valganciclovir for 21 days for the treatment of CMV infections, followed by valganciclovir (prophylaxis dose) for 28 days [8,10]. In a multivariate logistic regression model, the only independent risk factor for CMV recurrence was failure to eradicate virus by day 21 [10].

Analysis of the risk of CMV recurrence from this study is limited by the study design; all patients received the same course of antiviral treatment despite failure to eradicate the virus by day 21 or day 49, whereas the current guidelines recommend continuing treatment of CMV for at least 14 days or until the eradication of the virus. In addition, recipients of all solid-organ transplants were included in VICTOR- trial, despite marked differences in immunosuppression and risk of CMV infections depending on the type of transplant. Our study is limited by the nonrandomized and retrospective design and also by the lower number of patients in our analyses, but compared with VICTOR- trial, our study describes the current clinical practice of CMV infections in our relatively homogenous material including only kidney transplant recipients.

Secondary prophylaxis is commonly used after treatment of CMV infections to prevent recurrent infections and current guidelines recommend secondary prophylaxis for 1–3 months after treatment of infection [3]. There is not much evidence for or against the use of secondary prophylaxis and the optimal duration of secondary prophylaxis is currently unknown. In our study, secondary prophylaxis was used in 79% patients treated with antivirals and in 55% of all patients included in our study. The use of secondary prophylaxis was not associated with a lower risk of CMV recurrence, but the length of secondary prophylaxis was somewhat shorter in patients with CMV recurrence, although the difference was not statistically significant. In addition, both peak viral load and viral load at diagnosis were higher in patients who received secondary prophylaxis, suggesting that longer secondary prophylaxis may be beneficial. On the other hand, our data could also suggest that the benefit of secondary prophylaxis may not be so considerable that secondary prophylaxis could be recommended for all patients after the treatment of CMV infection, especially as valganciclovir is not without side-effects. No defined criteria were used to assess the need for secondary prophylaxis after infections and the severity of infections may have affected the decision to use secondary prophylaxis, which may have biased our analysis of the impact of secondary prophylaxis. In addition, seropositive and seronegative patients with different biology of CMV infections were analyzed together, limiting our analyses. Therefore, no firm conclusions about the usefulness of secondary prophylaxis can be drawn based on these data. Although not ongoing at the moment to our knowledge, larger randomized multicenter studies are needed to confirm the usefulness of secondary prophylaxis. On the other hand, the use of antiviral treatment was not associated with the risk of CMV recurrence, suggesting that mild infections may be treated and followed also without antiviral medication. However, no definite recommendations can be made without further studies.

In conclusion, we identified high viral load as a risk factor for recurrent CMV infection after kidney transplantation. The usefulness and optimal duration of secondary prophylaxis warrants further studies.

## Authorship

IH: primary researcher, data collection and analysis and preparation of manuscript. IL: study design, clinical virology, interpretation of results and preparation of manuscript. PK: study design, clinical nephrology, interpretation of results, preparation of manuscript and head of the project.

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