Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Long-term outcome of cytomegalovirus infection in simultaneous pancreas–kidney transplant recipients without ganciclovir prophylaxis

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Keywords
cytomegalovirus, preemptive therapy, simultaneous pancreas–kidney transplantation.

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Received: 27 March 2007
Revision requested: 4 May 2007
Accepted: 21 June 2007

doi:10.1111/j.1432-2277.2007.00526.x

Summary

As cytomegalovirus (CMV) infection frequently occurs in simultaneous pancreas kidney transplantation (SPKT), most centers use general ganciclovir prophylaxis. The aim of the study was to analyze the impact of CMV in a patient cohort with preemptive therapy only. Incidence, course and risk factors of CMV infection were retrospectively analyzed in 94 adult SPK recipients without prophylaxis. Patients with asymptomatic pp65-antigenemia were treated preemptively with intravenous ganciclovir for 14 days. Survival rates after 1, 3, and 5 years were 98%, 97%, and 94% for patients, 96%, 94%, and 88% for renal grafts and 88%, 85%, and 82% for pancreas grafts. CMV infections occurred in 51% of patients and CMV syndrome in 16%. No tissue-invasive disease was observed. Thirty-eight per cent of patients with CMV infection developed a recurrence. Risk factors for CMV in multivariate analysis were the D+/R− constellation, acute rejections, anti-rejection therapy and coronary heart disease. CMV had no impact on patient or graft survival, occurrence of acute or chronic rejection and bacterial infections. Preemptive therapy seems to be safe and effective in SPK recipients, but as the present study was retrospective, prospective randomized studies are needed to confirm our results.

Introduction

Cytomegalovirus (CMV) infection in simultaneous pancreas–kidney transplantation (SPKT) seems to be more frequent than in liver or kidney transplantation because of a high proportion of CMV seronegative recipients, a relatively high number of patients receiving induction therapy with T-cell depleting agents and the presence of diabetes-related diseases in the majority of patients [1]. Owing more specific immunosuppressive regimen, the development of highly sensitive diagnostic tests such as the pp65-antigenemia assay and the quantitative polymerase chain reaction (PCR), and the availability of the potent drugs ganciclovir and valganciclovir, CMV infections have lost much of their threat within the last years. Following recent reports in liver or kidney transplantation, CMV disease rates range between 0% and 12% and CMV related mortality approaches 0% [2–5]. Nevertheless, most centers, especially in the USA, administer some sort of CMV prophylaxis [6]. Several authors even advocate CMV matching to improve outcome [7].

In our liver transplant recipients, we observed that even without prophylaxis, CMV no longer has a negative impact on patient and graft survival and that preemptive CMV therapy nearly abolishes the development of CMV disease. In addition, no correlation between CMV and chronic rejection was found [4,8] and we, therefore, do not use prophylaxis in our SPKT program either. Most available data on CMV after SPKT come from centers, which prefer universal prophylaxis. We analyzed the

incidence and severity of CMV infection and disease as well as the impact of CMV seroconstellation and CMV infection or disease on long-term results after SPKT with preemptive therapy only in a single center experience.

Patients and methods

Patient population

Between April 1995 and June 2000, 99 SPKTs were performed at our center. Five patients were excluded from the analysis: four on account of insufficient data and one 12-year-old patient who was transplanted because of hemolytic-uremic syndrome. Therefore, the records of 94 adult pancreas–kidney transplant recipients (55 male and 39 female patients) were retrospectively analyzed. Six patients had received an isolated kidney transplant with consecutive transplant nephrectomy owing to transplant failure prior to pancreas–kidney transplantation. In all patients, indication for transplantation was diabetes mellitus type 1 with terminal ($n = 83$) or preterminal ($n = 11$) renal insufficiency. All patients had diabetes-related complications: arterial hypertension ($n = 84$), retinopathy ($n = 84$), peripheral neuropathy ($n = 79$), anemia ($n = 37$), hyperparathyroidism ($n = 35$), gastroparesis ($n = 23$), coronary heart disease ($n = 15$), and arterial occlusive disease ($n = 15$). Mean duration of diabetes prior to transplantation was 27.7 ± 8 years and mean recipient age was 41 ± 8 years. Renal replacement therapy consisted of hemodialysis in 63 patients (67%) and peritoneal dialysis in 20 patients (21%). Mean duration of dialysis prior to transplantation was 23 ± 2 months. In addition, 11 patients (12%) were transplanted preemptively shortly before initiation of renal replacement therapy.

Details of operation and immunosuppression

Pancreas–kidney transplantation was performed using standard techniques with vesical ($n = 18$) or enteral ($n = 76$) drainage of the pancreatic graft [9]. Organ conservation was realized mainly with University of Wisconsin (UW) solution in 80 organs and with HTK-solution in 14 organs. Mean operation time was 246 ± 59 min. During primary hospitalization, a mean of 6 ± 7 packed red cells and 3 ± 7 fresh frozen plasma were transfused. Thirty-five patients (37%) required temporary hemodialysis and nine patients postoperative ventilation after transplantation. The mean number of days on the ventilator was 3 ± 1 and the mean number of days under hemodialysis was 2 ± 4. The transplanted organs were recovered from our explantation team in 29 or shipped in 59 cases; in six patients, the explantation data were not available. The mean cold ischemic time was 600 ± 18 min for the pancreas and 629 ± 18 min for the kidney grafts.

Primary immunosuppression followed different protocols and consisted of tacrolimus ($n = 69$) or cyclosporine ($n = 25$) based quadruple immunosuppression. All patients received steroids, 89 patients received in addition mycophenolate mofetil (MMF) and three patients received azathioprin. Induction therapy was performed with an anti-lymphocyte antibody (ATG/ALG, $n = 84$) or an IL-2 receptor antibody ($n = 10$).

Rejection

If clinically an acute rejection episode was suspected, a percutaneous kidney biopsy was performed and the rejection was classified according to the Banff classification [10]. Rejections were treated with 500 mg of intravenous methylprednisolone for three or five consecutive days. Borderline-rejections were counted as true rejections and also treated. In case of steroid resistant rejections, a re-biopsy was performed and patients were treated with 5 mg/day of OKT 3 monoclonal antibody (Orthoclone®; Cilag, Germany) for 5 days. Pancreas rejections were diagnosed clinically (laboratory parameters).

A CMV-associated rejection was defined as a rejection, which occurred within 4 weeks following CMV infection.

CMV surveillance

Cytomegalovirus serostatus of donors and recipients was determined preoperatively by detection of anti-CMV-IgG and anti-CMV-IgM with commercially available enzyme-linked immunoabsorbent assay (ELISA) (ETI Cytok G, Byk and DiaSorin Diagnostics, Dietzenbach, Germany). CMV-pp65 antigenemia was measured using the APAAP technique (Clonab®; Biotest, Dreieich, Germany) as described previously [11]. Blood was examined weekly during primary hospitalization, every second week thereafter within the first 6 months and once a month until 3 years following transplantation, and also if CMV infection was suspected owing to clinical symptoms or laboratory abnormalities. In case of pp65 antigenemia, the test was repeated twice weekly until it was negative. In some cases, an additional in house CMV PCR was done.

Definition of CMV infection and disease

As in the following, definitions of CMV infection, syndrome and disease were used as suggested by Ljungman *et al.* [12]. Briefly, CMV infection was defined as pp65 antigenemia of at least 0.5 positive cells per 10 000 leukocytes. CMV viral syndrome was defined as CMV infection with CMV specific symptoms (antigenemia plus fever,

leukopenia or thrombocytopenia) and CMV tissue invasive disease as CMV infection plus organ invasion (hepatitis, pneumonia, gastroenteritis or involvement of other organs). In case of suspected organ involvement, respective biopsies were taken and investigated histomorphologically and immunohistochemically for CMV tissue invasion.

If CMV pp65 antigenemia was detected in a patient who was CMV seronegative before transplantation, this was defined as CMV primary infection, and in case of a preoperatively seropositive patient, this was defined as CMV reactivation. A recurrent infection was defined as a new CMV infection, if it was detected at least 4 weeks after the patient had become negative in the pp65 antigenemia assay.

CMV prophylaxis, preemptive therapy and treatment of disease

None of the patients, including the high-risk patients, received any kind of CMV prophylaxis.

All pp65 antigen (Ag)-positive asymptomatic patients were treated preemptively with intravenous ganciclovir (5 mg/kg bodyweight twice daily or adapted to renal function) for a minimum of 14 days until they became pp65-Ag negative. The same treatment was started in symptomatic patients with CMV syndrome or tissue invasive disease. In these cases, ganciclovir treatment was continued orally (3×1 g/day or adapted to renal function) for another 4 weeks. In addition, in case of CMV infection, immunosuppression was reduced as much as possible.

Laboratory parameters and long-term follow-up

Biochemical and hematological parameters were determined using standard laboratory methods. After primary hospitalization, patients were seen on a regular basis in our outpatient clinic. During the first 6 months, levels of immunosuppressive drugs and laboratory parameters were measured once a week, thereafter once a month. Doppler ultrasound was performed regularly during primary hospitalization and thereafter at least three to four times a year.

Statistical analysis

All values are depicted as mean and standard error of the mean (SEM). Differences between patient groups were analyzed using the Mann–Whitney *U*-test. Actuarial patient and graft survival rates were analyzed using the Kaplan–Meier method. Differences between groups were compared by log-rank test. Patients were followed up until last visit or until death at which time the event was classified as censored. All differences with *P*-values <0.05 were considered significant.

To detect potential risk factors for a CMV infection, an univariate analysis was performed with the following parameters: age, gender, accompanying diseases, type of perfusion solution, organ shipped or explanted by our team, primary immunosuppression, acute rejection episodes, type of rejection therapy (prednisolone, OKT3), serological constellation, postoperative ventilation, intraoperative blood transfusion, cold ischemic time, HLA matching and surgical revisions. Thereafter, a multivariate analysis was performed by binary logistic regression analysis including significant factors from the univariate analysis. Statistical analyses were performed with SPSS 10.0 (SPSS Inc., Chicago, IL).

Results

Patient and graft survival

Patient 1-, 3-, and 5-year survival rates were 98%, 97%, and 94%, respectively (Fig. 1). In four patients, causes of death were lung cancer, myocardial infarction, bacterial sepsis and suicide, and in two patients causes of death remained unclear (3 years after transplantation). All six patients had normal graft function until death. Kidney graft survival rates were 96%, 94%, and 88% after 1, 3, and 5 years, respectively (Fig. 1). Graft failure occurred owing to chronic rejection ($n = 2$), Polyoma virus infection ($n = 1$), Candida peritonitis ($n = 1$), and mycotic aneurysm of the renal artery ($n = 1$). Pancreas graft survival rates were 88%, 85%, and 82% after 1, 3, and 5 years, respectively (Fig. 1). Reasons for graft failure were chronic rejection ($n = 3$), thrombosis of the pancreas ($n = 2$), pancreatitis with hemorrhage ($n = 3$), arterial occlusion ($n = 1$), and Candida peritonitis ($n = 1$). None of the patients with chronic rejection had a CMV infection prior to occurrence of rejection.

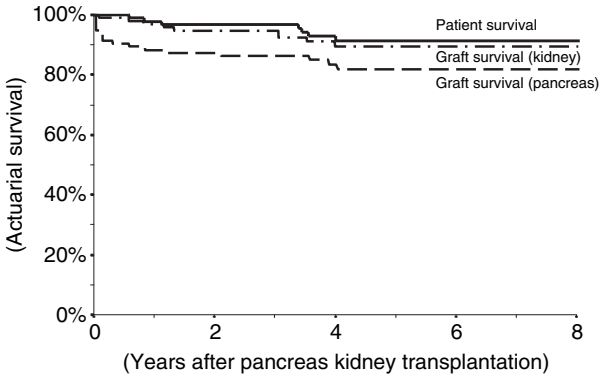

Figure 1 Overall patient and graft survival.

Rejection

In total, 69 patients (73%) experienced at least one acute rejection episode; 63 patients in the kidney and six in the pancreas. In 52 patients (75%) the rejection was successfully treated with steroids and 17 rejection episodes (25%) were steroid-resistant and required OKT3-therapy.

CMV infection

CMV seroconstellation

The distribution of donor and recipient CMV serostatus was: D+R+ in 27 patients (29%), D+R− in 18 patients (19%), D−R+ in 18 patients (19%) and D−R− in 31 patients (33%).

Incidence and timing of infection

In total, 48 out of 94 patients (51%) developed CMV infection during follow-up; 33 of these infections were asymptomatic and 15 (16%) presented as CMV syndrome; no tissue-invasive disease was observed. Out of these 15 patients, 12 had received a CMV seropositive and three a seronegative organ.

Fifty-one per cent of the infections occurred within the first month and 100% within 6 months after transplantation. The distribution of CMV infections in the different seroconstellations is shown in Fig. 2. As expected, the highest incidence of CMV infections as well as CMV syndrome was seen in the Donor positive groups. In addition, patients with preoperative negative CMV serology showed higher pp65-Ag positive cell counts and a slower reduction under therapy (Fig. 3).

Impact of CMV on patient and graft survival

The occurrence of CMV infection had no significant negative impact on patient or graft survival, although there was a trend toward a lower survival especially for the pancreas (Figs 4 and 5). Likewise, there were no differences regarding patient and graft survival in the patients with different CMV seroconstellations.

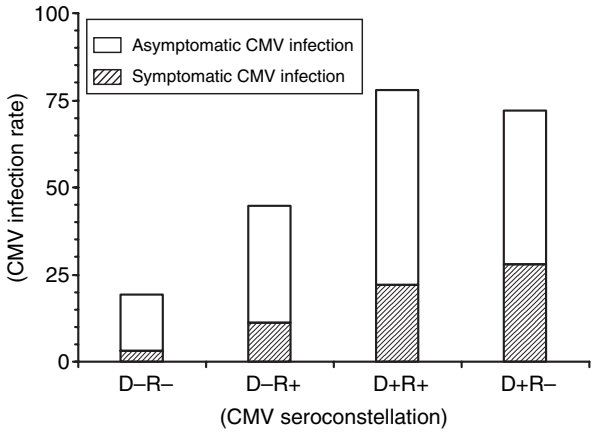

Figure 2 Symptomatic/asymptomatic cytomegalovirus (CMV) infection rate and CMV seroconstellation.

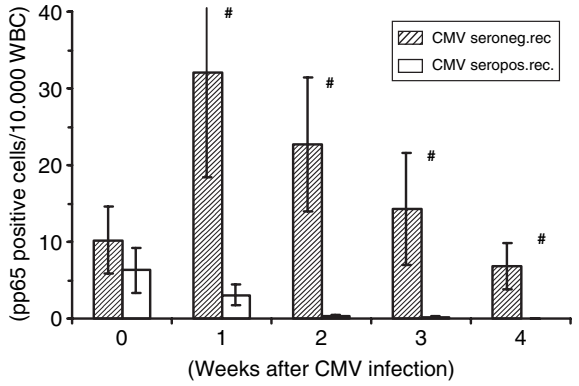

Figure 3 Quantitative pp65 positive cells in cytomegalovirus (CMV) seropositive and seronegative recipients (#$P < 0.05$).

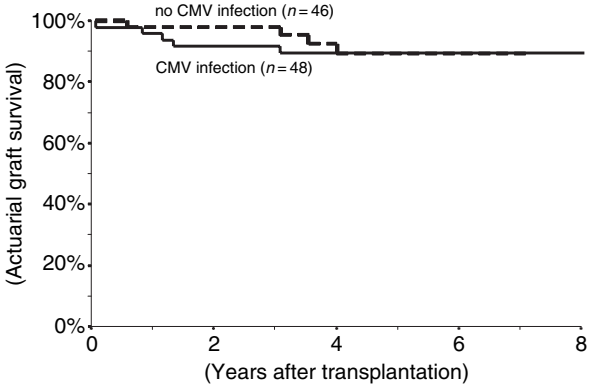

Figure 4 Renal graft survival related to cytomegalovirus (CMV).

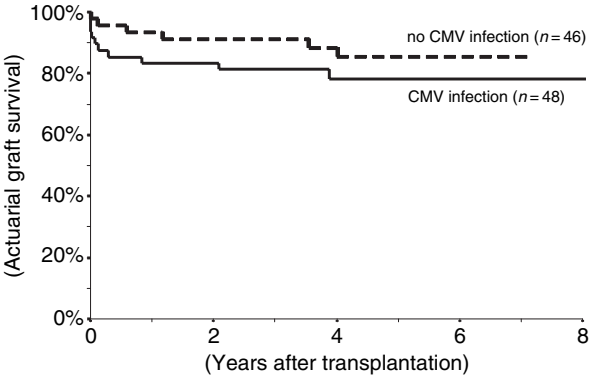

Figure 5 Pancreas graft survival related to cytomegalovirus (CMV) ($P = 0.21$).

Impact of immunosuppression on CMV infection
Cytomegalovirus infection rates were similar under tacrolimus and cyclosporine (49% and 56%, respectively), but the time-point of infection was significantly earlier under tacrolimus (mean 5 ± 4 weeks post-transplantation) than under cyclosporine (mean 8 ± 6 weeks post-transplantation). The severity of infection was also comparable. Likewise, there was no significant difference in CMV infection rates between patients under azathioprin and MMF.

Course of CMV infection
All 48 patients with CMV infection received antiviral treatment. Thirty-three asymptomatic patients were treated preemptively, remained symptomless and became pp65-Ag negative. The 15 patients who had CMV syndrome at the time of CMV antigenemia, were also effectively treated and did not develop tissue-invasive disease.

Timing of acute rejection and CMV infection
Thirty-nine of the 69 patients (57%) with acute rejection episodes developed CMV infection; 11 patients before and 28 patients after the rejection episode. Mean interval between rejection and CMV infection was 23 ± 30 days. In contrast, 36% of the patients without rejection had CMV infections. This difference was not statistically significant. From the 17 patients who required OKT3-treatment, 10 (59%) developed CMV infection (one CMV syndrome, nine asymptomatic infections). In contrast, 49% of patients without OKT3-treatment had CMV infections. CMV infection rates in the different serological constellations with and without OKT3-treatment were comparable: 25% and 19%, respectively, in the D−R− group; 40% and 46%, respectively, in the D−R+ group; and 67% and 73%, respectively, in the D+R− group. All of the five D+R+ patients who received OKT3 developed asymptomatic CMV infections compared to 16/22 (72%) D+R+ patients without OKT3 treatment. These differences were also not statistically significant.

Risk factors for CMV infection
In an univariate analysis, the following parameters were significant risk factors for CMV infection: high risk seroconstellation (D+R−), pretransplant coronary heart disease, acute rejection episode and anti-rejection therapy (Fig. 6). The multivariate analysis confirmed these four parameters.

Recurrent CMV infection
Thirty-eight per cent (n = 18) of the patients with CMV infection experienced at least one CMV recurrence after a mean interval of 71 days (8–969 days) following the first episode. These 18 CMV recurrent infections were preceded by asymptomatic CMV infections in 13 patients and by CMV syndromes in five patients. The overall incidence of recurrence was 39% in asymptomatic CMV infections and 35% in CMV syndromes. The highest incidence of recurrent CMV infection was observed in the D+R− constellation (n = 7, 54%), followed by D+R+ (n = 9, 43%) and D−R+ (n = 2, 25%). Only one D−R− patient developed a recurrence (17%). Rejection episodes or type of immunosuppression were no risk factors for recurrent CMV. On the other hand, CMV recurrence had no influence on patient or organ survival.

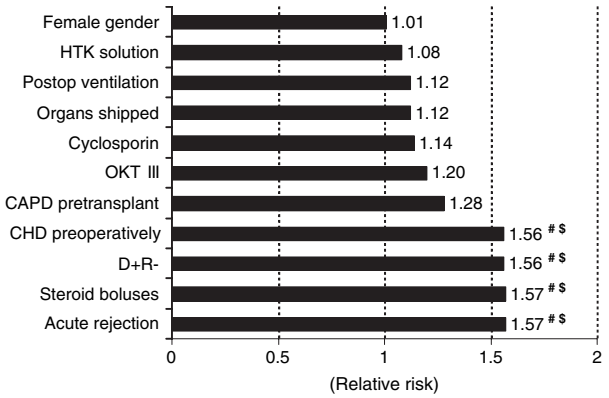

Figure 6 Relative risk of cytomegalovirus (CMV) infection (#$P < 0.05$ by univariate analysis; \$$P < 0.05$ by multivariate analysis).

Other infections

In total, 82 patients (87%) had at least one bacterial infection throughout the follow-up period. The majority of these infections were urinary tract infections. CMV was not a risk factor for a bacterial infection.

Discussion

In this retrospective analysis of 94 SPKT without ganciclovir prophylaxis and with preemptive therapy only, several observations were quite surprising and need to be discussed. First of all, the incidence of CMV disease was comparable to most other studies in SPKT patients, which used high dose acyclovir, ganciclovir or valganciclovir prophylaxis. Sixteen per cent of our patients developed CMV disease, exclusively CMV syndrome and no tissue-invasive disease. Kaufman *et al.* [13] reported a 17% incidence of CMV disease including 5% tissue invasive disease under a similar immunosuppressive regimen and a sequential intravenous and oral ganciclovir prophylaxis for 3 months. In another report, the same prophylactic regimen was associated with CMV disease in 10% of all SPKT recipients and 44% of the D+R− patients under quadruple immunosuppression [14]. In a study of

298 SPKT, the CMV disease rate was 13.4% despite a 3-month course of oral ganciclovir [15]. The time-point of CMV in the mentioned studies was much later than in our patients. In most cases, it occurred after cessation of prophylaxis, and the authors observed that the prophylaxis delayed the onset of CMV disease but often had no impact on its incidence and severity. Two studies had much lower CMV disease rates. Axelrod *et al.* [16] could reduce CMV infection and disease using prednisolone-free immunosuppression and intravenous or oral ganciclovir prophylaxis, but despite low overall infection rates, nearly 8% of patients had tissue-invasive disease. Only one out of 161 patients developed CMV disease, namely hepatitis, under tacrolimus, prednisolone and mycophenolate mofetil and using a 3-month course of oral valganciclovir [17]. These data are exceptional not only with regard to CMV but also because of the low rate of acute rejections, which was only 4%. Moreover, only two patients died within the follow-up period. It remains unclear, why CMV rates in this study remained so low compared to other studies under ganciclovir. One reason could be the predominance of kidney transplant recipients and the relatively low number of SPKT.

The major limitation of the present study is its retrospective design and the relatively small number of patients. Unfortunately, prospective randomized studies comparing general prophylaxis with preemptive therapy in solid organ transplantation do not exist. In kidney and liver transplantation, preemptive therapy, as well as general prophylaxis, were both able to reduce CMV disease significantly compared to no prophylaxis or therapy [18]. So far, there are very few data on preemptive treatment in SPKT. In a Dutch study, preemptive therapy with both oral valganciclovir and intravenous ganciclovir was able to reduce CMV viral load and to prevent the development of CMV disease in 57 renal and SPKT [19] From our experience, it also seems that general prophylaxis is not superior to preemptive therapy in reducing CMV disease. However, the data should be reevaluated by a prospective, randomized trial.

Likewise, CMV recurrence rates were not higher than in other trials using CMV prophylaxis. Humar *et al.* also reported CMV recurrence in 40% of patients. Parallel to our study, recurrence occurred relatively late after the first episode and had no impact on patient survival [20].

In the present study, we wanted to focus on the long-term impact of CMV in SPKT recipients and, as valganciclovir was not available during the study period, intravenous and oral ganciclovir were used for preemptive therapy. Valganciclovir has an improved oral absorption compared to ganciclovir [21]. Data on this drug in SPKT recipients are very rare. In one study including kidney, pancreas–kidney and pancreas transplant recipients, prophylaxis with valganciclovir was able to nearly abolish CMV infection but this finding is still to be confirmed [17]. We now use valganciclovir for preemptive therapy and hope that the rate of CMV disease will be further reduced.

As expected and in accordance with most other studies [13,22], the high risk serological constellation (D+R−), a previous acute rejection and a rejection therapy were risk factors for the occurrence of CMV.

Compared to similar trials, rejection rates were quite high and one quarter of the patients even had steroid-resistant rejection requiring OKT3-treatment [14,15,23]. This finding may result from an aggressive biopsy regimen and due to the fact that even borderline-rejections were counted as rejections and treated accordingly. In addition, since patients in Germany stay longer in hospital than in USA, the follow-up was closer and more borderline-rejections were detected and treated. Despite the high rejection rates, patient and graft survival were comparable or even better than in other trials [13,14,16,23,24]. This could indeed be a result of the liberal policy toward biopsy and rejection therapy.

Cytomegalovirus seroconstellation and CMV infection had no impact on patient and graft survival. The role of CMV for the survival after SPKT is discussed controversially in the literature. Kaufman *et al.* [13] observed that CMV increased mortality, but had no influence on graft survival. This is astonishing as only one of their patients died due to CMV. In most other studies, CMV did not decrease patient or organ survival [14,15,25,26]. Conflicting results were published by Stratta *et al.* [7] who found that a seronegative recipient status was a risk factor for patient and kidney survival. On the other hand, the lack of CMV sero-pairing was no risk factor for graft loss or rejection [7].

The relationship between rejection and CMV is also a point of discussion. Especially in kidney transplantation, CMV is thought to trigger chronic rejection. In this series, CMV did not induce acute or chronic rejection. Likewise, CMV was no risk factor for bacterial infections. Similar results were published by most other study groups [7,13,14,25]. Only one group reported a higher rate of rejections and other bacterial infections in the D+R− patients [15].

The incidence of CMV infection in the D−R− group was higher than in other studies [13–15,22,25]. We assume that CMV was transmitted via blood products in these patients although only so-called CMV depleted blood products were transfused.

Cytomegalovirus infection rates were not different under tacrolimus or cyclosporine, but infections under tacrolimus occurred earlier. In the Euro SPK study, 34% of patients developed CMV both in the tacrolimus and

cyclosporine-arm. A difference between the time-point of infection was, however, not reported [25].

In conclusion, as CMV infection had no impact on patient and graft survival, and no tissue invasive disease occurred, preemptive CMV therapy seems to be safe and effective in SPKT. The preemptive strategy could help avoid development of drug resistance and leucopenia, which are important side effects of prolonged ganciclovir administration [27,28]. As the present study was retrospective and the patient number relatively small, randomized, controlled studies to compare this approach with general prophylaxis are urgently needed to confirm our results.

Authorship

NR: designed study, wrote paper, collected and analyzed data; DS: designed study, wrote paper, collected and analyzed data; AK: designed study, corrected paper, analyzed data; SK: collected and analyzed data; JP: analyzed data; UF: analyzed data; PN: analyzed data.

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