CLINICAL AND EPIDEMIOLOGICAL STUDY

A stringent preemptive protocol reduces cytomegalovirus disease in the first 6 months after kidney transplantation

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Abstract

Background The optimal strategy to prevent cytomegalovirus (CMV) disease after kidney transplantation continues to be open to debate. The preemptive approach requires regular determination of CMV viremia and prompt initiation of therapy.

Methods We retrospectively compared the incidence of CMV disease during two periods at our center: A first phase (P1, $n = 84$ kidney recipients), during which time the intensity of surveillance was determined by the responsible physician, was compared to a second phase (P2, $n = 74$), when a stringent protocol of CMV surveillance was required for all patients. The preemptive approach was applied for all CMV risk groups; prophylaxis was optional in the case of treatment for rejection or delayed graft function in the intermediate- and high-risk group. Follow-up was truncated at 6 months after transplant surgery. CMV syndrome was differentiated from asymptomatic replication by the presence of at least one systemic symptom, while diagnosis of CMV end-organ disease required histological confirmation.

Results Immunosuppression was similar in the two periods. CMV prophylaxis was used equally (26 %) in both periods. The probability for asymptomatic viremia episodes was not different for patients in P1 and P2 regardless of the prevention strategy. For patients following the preemptive strategy, the probability for CMV disease was increased during P1 ($p = 0.016$), despite fewer PCR assays being performed in phase 2. Protocol violations were only observed during P1.

Conclusions The probability of CMV disease episodes (CMV syndrome and CMV end-organ disease) was substantially reduced using a very stringent protocol. This study highlights the crucial importance of a stringent protocol with optimal adherence by all caregivers if the preemptive strategy is to be successful.

Keywords Cytomegalovirus · CMV disease · Preemptive therapy · Prophylaxis · Adherence

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Introduction

Prevention of cytomegalovirus (CMV) disease after transplantation remains a controversial and contentious issue. It has recently been shown that high-risk (D+/R−) kidney allograft recipients who receive prolonged prophylaxis for 6 months have a lower rate of CMV disease, although late-onset CMV disease still manifests in 16 % of the high-risk recipients [1]. In a similar study performed in high-risk kidney transplant recipients in Europe, 37 % of the investigated patients developed a CMV infection, and the majority were symptomatic after 6 months of valganciclovir prophylaxis [2]. Despite the uncontested benefit of prophylaxis early after transplantation, the problem of late-onset disease remains unresolved. In contrast, the preemptive strategy, which

requires a meticulous adherence to a regular surveillance protocol and prompt initiation of therapy once an infection has been detected, has been reliably associated with a low incidence of late-onset CMV complications.

The aim of the study reported here was to assess the effect of strict enforcement of a preemptive strategy on the incidence of CMV disease by comparing two phases. During the initial phase, a protocol was introduced into daily outpatient care detailing the exact time-points of CMV surveillance after transplantation and the thresholds for the initiation of CMV preemptive therapy for recipients at intermediate risk (recipient CMV-seropositive) and at high risk (recipient CMV-seronegative, donor CMV sero-positive). Implementation of the protocol was left to the treating physician. In the second phase, adherence to the protocol was actively supervised by a staff physician, who had received additional instruction.

Subjects and methods

Patients

We retrospectively assessed the incidence of CMV infection and disease during the first 6 months after transplantation in 158 consecutive kidney transplant recipients at our institution between August 2007 and July 2009. During the initial phase, a protocol was introduced into daily outpatient care recommending time-points of CMV surveillance and thresholds for the initiation of CMV preemptive therapy for intermediate-risk [recipient CMV-seronpositive/donor CMV-seronegative or -positive (R+/D±)] and high-risk [recipient CMV-seronegative/donor CMV-seropositive (R−/D+)] recipients. Between August 2007 and July 2008 (n = 84) the implementation of the surveillance protocol was left to the discretion of the treating physician (P1). In the second phase (P2), between August 2008 and July 2009 (n = 74), a similar, but more detailed preemptive protocol was implemented, as described below, and used as an official guideline; the implementation of this protocol was actively supervised by a staff physician on each patient visit.

Preemptive CMV protocol

The CMV status of the donor and recipient was evaluated before transplantation. CMV monitoring was as follows: for low-risk patients, CMV PCR and serology results were required at months 3 and 6, or upon clinical suspicion for CMV infection; for intermediate-risk and high-risk patients, CMV PCR results were required on a weekly basis during month 1, then every other week for the next 2 months, and thereafter once a month until month 6.

For high-risk patients, serology testing was scheduled at months 3 and 6. CMV therapy was indicated whenever CMV DNA was detectable (high-risk patients) or crossed a threshold of >2,000 copies/ml (intermediate-risk patients). At our center, we use an in-house plasma-based CMV real-time PCR assay.

The protocol allowed primary prophylaxis for the following conditions: (1) treatment with antithymocyte globulin (ATG) for induction; (2) delayed graft function; (3) rejection treatment of any kind. The duration of prophylaxis ranged from 1 to 3 months. Both the use of valganciclovir and valaciclovir (two patients) was counted as prophylaxis. The dosage used was according to the recommendation of the manufacturer, including adjustment for renal insufficiency. Of note, no breakthrough CMV disease was recorded while the patients received prophylaxis. CMV disease was defined in accordance to published definitions [3]. Cases with CMV viremia without clinical symptoms were documented as asymptomatic viremia. Viral syndrome was defined as CMV PCR positivity in the blood and one of the following symptoms with no other explanation: fever, leukopenia, elevated transaminases, or unspecified malaise. End-organ disease was defined as the detection of CMV in the biopsy by immunohistochemistry. CMV PCR positivity in the blood, typical symptoms of end-organ disease (colitis), and no other diagnosis were considered to be signs of viral syndrome. Valganciclovir was the treatment of choice for asymptomatic CMV viremia, while intravenous ganciclovir was used for CMV disease if patients required hospitalization.

The real-time PCR assay for CMV is based on the protocol of Yun et al. [4] The target sequence is the viral glycoprotein B. The linear range of the assay extends from $8 \times 10E2$ to $8 \times 10E7$ IU/ml, and the 95 % confidence interval of the precision within this range is 3/4 0.5 log10. The lower limit of detection is $<8 \times 10E1$ IU/ml. Calibration against the World Health Organization (WHO) standard resulted in a value of 0.4 IU/genome copy.

Intervention

To improve adherence to the protocol, an official written guideline was distributed to all physicians in the nephrology outpatient clinic and regular teaching sessions were held to emphasize the importance of a strict follow-up. The supervising physician reviewed each visit. Each positive CMV PCR result was assessed not only by the treating physician but also discussed with the supervisor.

Rejection

The definition of rejection was based on typical histopathological findings on a kidney biopsy using the Banff

classification, or in few cases in a raising serum creatinine without any other explanation [5]. Rejection treatment consisted of pulses of methylprednisolone, ATG antibody or, rarely, immunoadsorption if patients were not responding.

Statistical analysis

Follow-up was truncated at 6 months for all patients. Fisher's exact, chi-square, and Wilcoxon rank-sum tests were used as appropriate. For the time to event analysis, Kaplan–Meier curves with the log rank-test were used to compare episodes. Analysis was performed separately for patients receiving prophylaxis and patients with a preemptive strategy.

Results

Patient characteristics

Age, gender, cause of end stage kidney failure, gender, and source of organ were comparable between the two phases (Table 1). Percentage of CMV high-risk recipients was higher in phase 1 (24 vs. 12 %), while intermediate-risk recipients accounted for more patients during phase 2 (66 vs. 57 %). The overall percentage of recipients with CMV prophylaxis was 26 % in both phase 1 and in phase 2 (Table 2a).

In total, 46 (55 %) of patients in phase 1 and 45 (61 %) of patients in phase 2 never used any CMV-active drug during the entire follow-up observation.

Asymptomatic viremia

In total, 39 episodes of asymptomatic viremia were detected during phase 1, 31 in recipients without prophylaxis, and eight in patients after stopping prophylaxis. The corresponding numbers were 35 episodes during phase 2, with 30 episodes in patients with a preemptive strategy and five in recipients after completion of prophylaxis (Fig. 1). As expected, the probability of asymptomatic viremia was higher in the population without prophylaxis than in the patients receiving prophylaxis. There was no statistical difference when the probability of asymptomatic viremia was analyzed between phase 1 and 2 in both the preemptive group and the prophylaxis group.

CMV disease: viral syndrome and end-organ disease

A total of 14 patients developed a viral syndrome attributable to CMV: 12 (14 %) in phase 1 and two (2.7 %) in phase 2. Four patients were diagnosed with end-organ disease, two (2.4 %) in phase 1 and two (2.7 %) in phase 2. Excluding the patients who received prophylaxis, nine CMV disease episodes were recorded during phase 1 and one during phase 2 ($p = 0.016$), when the two clinically relevant endpoints, i.e., viral syndrome and end-organ disease, were combined (Fig. 2). Importantly, using the combined endpoint viral syndrome and end-organ disease, in the high-risk group, three of 11 patients in phase 1 and neither of the two patients in phase two had clinical disease. In the intermediate-risk group, six of 35 patients during phase 1 and one of 38 patients had clinical diseases.

Table 1 Characteristics of patient population

Characteristics of patient population	Phase 1 ($n = 84$ patients)	Phase 2 ($n = 74$ patients)
n	84	74
Age, median (IQR)	51.6 (43.6–60.3)	50.2 (43.5–57.1)
Gender, male (%)	60 (71)	47 (63)
Living donor (%)	28 (33)	22 (30)
AB0 mismatch in living donor (%)	2 (2)	7 (9)
Deceased donor (%)	56 (67)	52 (70)
Causes of terminal kidney failure		
Glomerulonephritis (%)	26 (31)	21 (28)
Diabetic nephropathy (%)	15 (18)	19 (26)
Polycystic kidney disease (%)	13 (15)	8 (11)
Hypertensive nephropathy (%)	4 (5)	4 (5)
Chronic pyelonephritis (%)	3 (4)	2 (3)
Hemolytic uremic syndrome (%)	2 (2)	1 (1)
Other (%)	10 (12)	7 (9)
Unknown etiology (%)	11 (13)	12 (16)

IQR Interquartile range

Table 2 Cytomegalovirus risk groups and prevention strategies

a Prevention strategies	Phase 1[a] ($n = 84$ patients)	Phase 2[a] ($n = 74$ patients)	p value
CMV risk, n (% of all patients, $n = 158$)			0.17[b]
Low	16 (19)	16 (22)	
Intermediate	48 (57)	49 (66)	
High	20 (24)	9 (12)	
Preemptive strategy, n (% of patients receiving preemptive therapy, $n = 117$)			0.047[c]
Low	16 (26)	15 (27)	
Intermediate	35 (56)	38 (70)	
High	11 (18)	2 (4)	
Prophylaxis, n (%, patients under prophylaxis, $n = 41$)			0.74[c]
Low	0 (0)	1 (6)	
Intermediate	13 (59)	11 (58)	
High	9 (41)	7 (37)	
Number of patients who did not receive drugs for CMV throughout study, n (%)	46 (55)	45 (61)	0.59[b]
b CMV diseases patients receiving preemptive strategy (total patients in the respective risk group)			
Low	0 (16)	0 (15)	
Intermediate	6 (35)	1 (38)	
High	3 (11)	0 (2)	

CMV Cytomegalovirus

[a] Phase 1 (P1), treatment phase during which time the intensity of surveillance was determined by the responsible physician; Phase 2 (P2), when a stringent protocol of CMV surveillance was required for all patients (preemptive protocol)

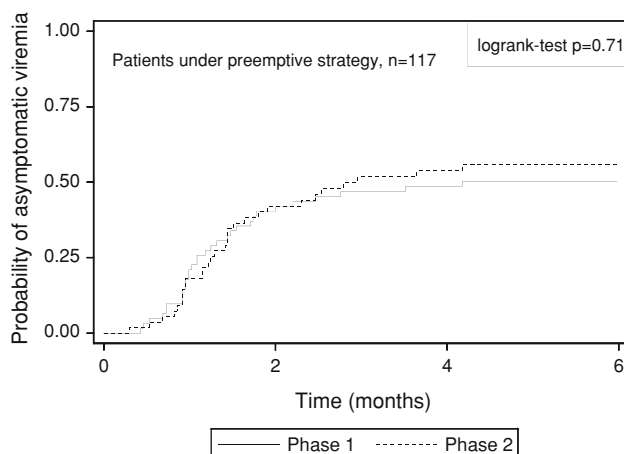[b] Chi-square test

[c] Fisher's exact test

Fig. 1 Probability of asymptomatic cytomegalovirus (CMV) viremia after transplantation in patients followed by the preemptive strategy. During the study, 31/62 patients (phase 1) and 30/55 patients (phase 2) experienced asymptomatic CMV viremia ($p = 0.71$)

Fig. 2 Probability of CMV disease (viral syndrome and CMV end-organ disease) in patients receiving the preemptive strategy. In total, nine of 62 patients (phase 1) and one of 55 patients (phase 2) experienced a CMV disease episode ($p = 0.016$)

Median CMV PCR load was 8,900 copies/ml (range 1,970–85,000 copies/ml) at the time of start of therapy (phase 1, only preemptive patients); in phase 2, the only pati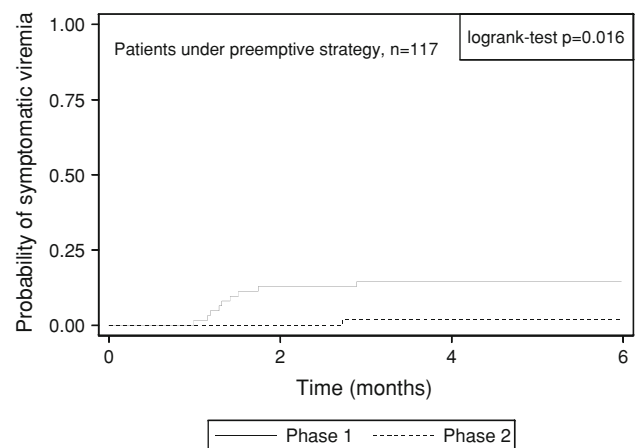ent with a CMV episode presented with low viremia but clinical symptoms of colitis. During phase 1, therapy was started according to the guidelines in five of the nine CMV patients, while there was a delay of 9.5 days (range 3–16 days) in the four remaining patients. In phase 2, the

one patient was treated according to protocol. Among the high-risk patients, seroconversion was seen in three patients (15 %; phase 1) and one patient (8 %, phase 2).

CMV surveillance

The mean number of CMV PCR determinations per patient was assessed in the preemptive group and compared to the number required by the surveillance protocol. Surprisingly, only 91 (intermediate-risk recipients) and 59 % (high-risk recipients) of the required CMV PCR determinations were performed during phase 2, a lower proportion than in phase 1 (131 and 99 %, respectively) (Fig. 3a, b). Only regular CMV PCR determinations obtained before any type of event (asymptomatic viremia or CMV disease) and corrected for missing time were included in the analysis.
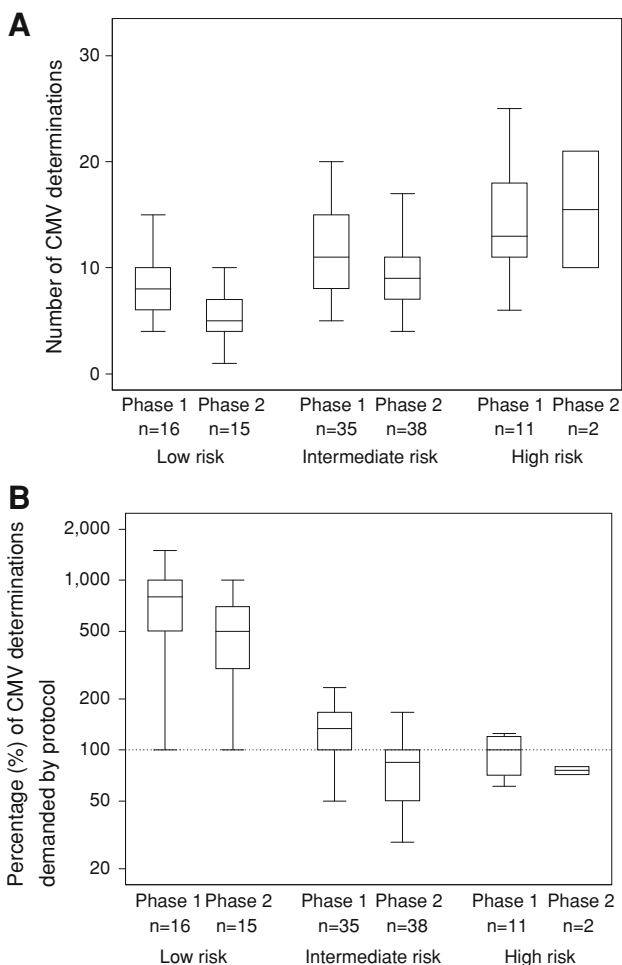

Fig. 3 a Mean number of CMV PCR determinations performed during phase 1 and phase 2, respectively. Only determinations in patients followed by the preemptive strategy and before the occurrence of a CMV event are analyzed and corrected for missing time. b Percentage of CMV PCR determinations according to CMV risk stratification and phase

Immunosuppressive treatment

Immunosuppresive treatment was analyzed during the first week after transplantation and at 4 weeks after transplantation (Table 3).

In phase 1, 13 (15 %) patients received an induction treatment [5 with ATG, 6 with basiliximab, and 2 with rituximab (ABO-incompatible kidneys)]; in phase 2, 18 (24 %) patients had an induction therapy (7 with ATG, 4 with basiliximab, and 7 with rituximab). The initial drug therapy for phase 1 patients was cyclosporine (47 patients, 56 %), tacrolimus (21, 25 %), and a non-calcineurin inhibitor (CNI)-based treatment (13, 15 %). In phase 2, fewer patients were initially treated with cyclosporine (22, 30 %) as their first CNI, while 45 (61 %) patients were initially started on tacrolimus and six (8 %) patients with other drugs.

Regular triple immunosuppressive maintenance was used in which an antimetabolite, CNI, and steroid drugs were combined. Four weeks after transplantation, in phase 1, 44 (52 %) patients were receiving cyclosporine, 39 (46 %) patients had tacrolimus, and one (1 %) patient received neither. In phase 2, 22 (30 %) patients were receiving cyclosporine, 49 (66 %) patients had tacrolimus, and three (4 %) patients had another non-CNI-based regimen.

Rejections, transplant failure, reduced graft function, and death

No difference was observed between phases in terms of rejections [45 (phase 1) vs. 42 % (phase 2); $p = 0.67$]. Fourteen patients (17 %) in phase 1 and 12 patients (16 %) in phase 2 had a calculated glomerular filtration rate (GFR) of <30 ml/min at the end of the 6-month observation period ($p = 0.94$). The number of transplant failures was slightly lower in phase 2 [8 (phase 1) vs. 4 % (phase 2); $p = 0.34$]. No CMV-related deaths were observed (Table 4).

Discussion

The prevention of CMV disease after solid organ transplantation has considerably reduced morbidity and mortality and is a cornerstone of every transplant program regardless of the organ transplanted. The availability of efficient drugs against CMV has led to different prevention strategies. Prophylaxis is given to all patients at risk, usually for a defined period. Preemptive therapy uses a marker, such as CMV PCR or pp65 assay results, to detect replication before the onset of disease. Therapy is initiated in order to prevent clinical disease. Both strategies have their

Table 3 Induction and maintenance immunosuppressive treatment at weeks 0 and 4

Immunosuppressive treatment	Phase 1 ($n = 84$ patients)	Phase 2 ($n = 74$ patients)	p value
Induction treatment, n (%)			
Antithymocyte globulin	5 (6)	7 (9)	0.55[a]
Basiliximab (%)	6 (7)	4 (5)	0.75[a]
Rituximab	2 (2)	7 (9)	0.084[a]
Maintenance treatment at 0 and 4 weeks after transplant (0/4), n (%)			
Cyclosporine	47 (56)/44 (52)	22 (30)/22 (30)	0.001/0.004[b]
Tacrolimus	21 (25)/39 (46)	45 (61)/49 (66)	<0.001/0.012[b]
Other immunosuppressive drug	13 (15)/1 (1)	6 (8)/3 (4)	0.22/0.051[a]

[a] Chi-square test

[b] Fisher's exact test

Table 4 Rejection, graft function, and death

	Phase 1 ($n = 84$ patients)	Phase 2 ($n = 74$ patients)	p value
One or more graft rejection, n (%)	38 (45)	31 (42)	0.67[b]
GFR[a] <30 ml/min at month six, n (%)	14 (17)	12 (16)	0.94[b]
Graft failure, n (%)	7 (8)	3 (4)	0.34[c]
Deaths, n (%) (none was CMV-related)	1 (1)	4 (5)	0.19[c]

[a] Glomerular filtration rate; calculated according to the (Modification of Diet in Renal Disease (MDRD) formula

[b] Fisher's exact test

[c] Chi-square test

clear benefits and dangers. The current guidelines prefer prophylaxis, at least for the high-risk constellation of a CMV-seronegative recipient of an organ from a CMV-seropositive donor [6]. The ease of prophylaxis treatment and the documented benefits in term of mortality is counterbalanced by the potential side effects, such as cytopenia and the occurrence of late-onset CMV disease. A longer period of prophylaxis (6 months) has been shown to further reduce late-onset CMV disease [1], but increased costs are an issue. Important considerations are the indirect effects of CMV on transplant-related endpoints, such as rejection and long-term graft function, which are linked to CMV by circumstantial evidence. Some studies show an advantage of prophylaxis over preemption for these indirect effects [7], while others do not [8, 9]. The major hurdles for the preemptive strategy are the logistics necessary not only to ensure timely assessment of biomarkers, but also to take appropriate and prompt action upon receipt of the result. At our center, we became aware that while CMV PCR determinations were performed regularly, taking action upon learning of a positive result was often delayed. For the two periods studied, CMV PCR results were automatically transmitted to the electronic patient file, where they had to be looked up actively, as compared to the time before, where they were communicated by telephone and were therefore less likely to be missed. After adopting a stringent control mechanism, we analyzed the incidence of CMV events before and after implementation and found a significant decrease of CMV disease events even though CMV viremia was assessed less often. The overall incidence of CMV disease was significantly reduced from 14 cases (17 %) in phase 1 to four cases (5 %) in phase 2. Importantly, this reduction held true even when only the patients without prophylaxis were analyzed separately [9 (11 %) in phase 1 vs. 1 (1 %) in phase 2], demonstrating a strong impact of strict adherence to the protocol.

Retrospective analyses are always prone to systematic biases. However, no new immunosuppressive strategy was introduced during the observation period, and the characteristics of the patient population were comparable between phase 1 and 2. There was an imbalance of more patients receiving an ABO-incompatible living donor kidney transplant in phase 2, but the small number precluded any statistical analysis. Importantly, the imbalance in the percentage of high-risk patients between phase 1 and 2 in the group followed preemptively did not account for the differences seen in CMV diseases. Of the two patients at risk in phase 2, none had a CMV disease episode, while three of 11 patients had a disease episode, which was fewer than expected. However, while only one of 38 patients at intermediate risk showed a clinical episode of CMV disease in phase 2 compared to six of 35 patients during phase 1. Taken together, we believe that it is very unlikely that other systemic factors were responsible for the difference seen in CMV disease endpoints between the two phases.

Prophylaxis was given to 26 % of the patients in both groups. The duration of the treatment varied from 1 to 3 months, which explains why asymptomatic viremia was detected before month 3 in some patients. No CMV viremia or CMV disease occurred in patients receiving prophylaxis.

The observation period was too short and the numbers to small to make a statement on common endpoints, such as graft rejection, GFR, graft failure, or death.

The overall rate of patients with end-organ disease was low [2 (2 %) in phase 1 vs. 2 (3 %) in phase 2]. A number of studies have shown that prophylaxis is cost-effective; however, the rate of asymptomatic viremia and CMV disease was higher in those studies than in our study [10]. We are currently performing a cost analysis to determine the impact of our low incidence of CMV disease on overall costs. Notably, over 55 % of all patients never were treated with any kind of drug directed against CMV.

A surprising finding is that despite the better outcome during phase 2, fewer CMV PCR determinations were performed in phase 2 than in phase 1. The difference is small when the absolute number of PCR determinations performed is compared (7 vs. 5). Only viremia assessed prior to any detection of CMV was counted as a PCR determination, as the frequency of assessments was altered by the detection of CMV. The mean number of CMV PCR determinations performed before the occurrence of a clinical endpoint (viral syndrome or end-organ disease) was lower in phase 1 (6 determinations) than in phase 2 (8 determinations). Due to the low number of events, no statistical significance was reached. However, this observation is contrary to the mean number of PCR determinations performed overall. The same is true when the days between the last positive CMV PCR and any clinical endpoint were calculated. Of all patients who should have started therapy according to the guidelines, none were missed during phase 2, while four patients during phase 1 (preemptive group) did not receive the appropriate treatment, with a median delay of start of therapy of 9.5 days. This delay may explain in part the difference in outcome observed. Our patients all live close to the transplant center, and the follow-up is performed at the transplant center within the first year. These circumstances certainly facilitate a uniform approach and a meticulous adherence to the preemptive protocol.

At our center, preemptive therapy is a safe option, but requires a close adherence to the protocol. A constant investment in teaching and surveillance is necessary to maintain a high compliance with the preemptive protocol.

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