Transpl Int (2000) 13 [Suppl 1]: S354–S358

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Hyperimmunoglobulin prophylaxis, monitoring and preemptive ganciclovir treatment eliminate the risk of CMV infection to improve patient and renal allograft survival

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Abstract This study was designed to investigate whether the introduction of ganciclovir to clinical use for anti-CMV treatment changes the risk of CMV infection in renal transplant patients. A total of 1545 cases who had received cadaveric renal transplants were divided into two groups: group 1 ($n = 721$) was made up of patients who received their transplants within 6 years before the introduction (1991) of ganciclovir and group 2 ($n = 824$), of individuals transplanted thereafter. Patient and graft survival of CMV D + /R– patients was uni- and multivariately compared with non-CMV D + /R– patients. In CMV D + /R– patients in group 1, survival was significantly lower, and their relative risk for graft loss was 1.32-fold ($P = 0.0483$) that of non-CMV D + / R– patients. In group 2 patient and graft survival was identical regardless of whether the patients were at risk for CMV infection or not. The risk of CMV infection can be eliminated by hyperimmunoglobulin prophylaxis, CMV monitoring and preemptive ganciclovir treatment in renal transplant patients.

Key words Renal transplantation · CMV infection · CMV prophylaxis · Ganciclovir · Long-term results · Multivariate analysis

Introduction

At the ESOT Congress in Rhodes in 1993, we reported that CMV infection after renal transplantation and kidney transplantation of CMV-positive donor organs to CMV-negative recipients (CMV D + /R–) operates as a statistically significant risk factor for chronic renal transplant failure. The analysis was based on 524 cadaveric renal transplantations performed between 1983 and 1993 at our center. Long-term allograft survival (5-year probability) was 21% in patients with post-transplant CMV disease and 11% in CMV D + /R–, respectively.

The aim of the studywas to investigate whether a strictly applied anti-CMV therapy using ganciclovir affects the risk profile for long-term graft function in patients who, after cadaveric kidney transplantation, were considered to be at high risk of CMV infection or had developed CMV antigenemia (syndrome or disease).

The anti-CMV strategies used were reviewed in 1993. Anti-CMV hyperimmunoglobulin has been used since 1983 as anti-CMV-prophylaxis in all known CMV D + /R– transplant cases on postoperative days 1 and 14. Furthermore, any antilymphocyte globulin induction or antirejection treatment course was flanked by pre- and posttreatment anti-CMV hyperimmunoglobulin prophylaxis. Doses used for a single application are 1 ml of Cytotect per kilogram of body weight, or 2 ml/kg of Cytoglobin. In the year 1991 we started to use ganciclovir in the treatment of any signs of CMV syndrome. In the year 1993 be started a post-transplant CMV monitoring program based on weekly CMV-IgM determination. Since 1994 we have followed patients by weekly CMV-pp65 determination (Clonab-Test). Since 1993 ganciclovir therapy has not only been given to patients showing signs of CMV syndrome or disease (presumed disease) but to every patient showing a positive

Table 1 Demographic details of patients and donors in groups 1 and 2

Variable	Group 1 ($n = 721$)	Group 2 ($n = 824$)	P
Male/female	473/248	511/313	ns
Age (years)	44.1 ± 12.9	45.3 ± 13.5	ns
# KTx	1.3 ± 0.6	1.3 ± 0.6	ns
PRA (%)	10.4 ± 23.0	6.9 ± 19.2	0.001
Total HLA mismatch	1.96 ± 1.36	1.93 ± 1.50	ns
HLA-DR mismatch	0.31 ± 0.55	0.45 ± 0.62	< 0.001
CIT (h)	25.5 ± 5.9	23.4 ± 6.6	< 0.001
Donor age (years)	33.3 ± 14.1	40.0 ± 15.6	< 0.001
# ARE (mean)	0.69 ± 0.86	0.74 ± 1.01	ns
Incidence of 1st ARE	45.6%	40.0%	0.027

monitoring test result (preemptive therapy). Test results were considered to be positive when CMV-IgM was > 0.5 U/ml or Clonab ≥ 1/400,000 cells. All patients treated received at least i.v. ganciclovir therapy for 10 days, which was continued until a negative retest result was obtained. Ganciclovir doses used were 10 mg/kg i.v.b.i.d. or less, according to kidney graft function. In patients with CMV disease additional applications of anti-CMV hyperimmunoglobulin were administered.

Materials

A total of 1545 cases of cadaveric renal transplantation performed between January 1985 and December 1997 with known CMV serology of donor and recipient at the time of transplantation were analyzed retrospectively. Patients in group 1 ($n = 721$) received transplants before the use of ganciclovir, and patients in group 2 ($n = 824$) received transplants later, when we had started using ganciclovir for anti-CMV therapy. Demographic characteristics of the patients in both group are shown in Table 1.

For Cox proportional hazards regression we dichotomized the variables of potential influence. The distributions of potential risk factors in both groups are given in Table 2: in group 1 there were 129 CMV D + /R- (17.9%), and 59 (8.2%) were diagnosed with CMV infection, syndrome or disease, while 553 had no apparent CMV problem. Most ($n = 47$) CMV infections had been diagnosed clinically, suggesting that these patients had already had distinct symptoms of CMV disease at the time of diagnosis. In group 2 there were 218 (26.5%) CMV D + /R-: 195 (23.7%) were diagnosed with CMV, and 486 had no apparent CMV problem. Most ($n = 119$) of the CMV infections had been diagnosed as CMV antigenemia by the Clonab-test in the CMV monitoring program. In 65 patients a CMV-IgM was found and in 11 patients the diagnosis was made clinically. Thus, most of the CMV diagnoses in group 2 were established very early, before the patients showed any symptoms of CMV. Thus, ganciclovir therapy was preemptive in most cases.

Methods

Data analysis was performed separately in groups 1 and 2. CMV D + /R- patients were compared with patients with the other possible CMV constellations (D- and D + /R +) in the same group. Patients with the diagnosis of CMV after the transplant were compared with those who were free of CMV. For comparison of survival we used the Kaplan-Meier method. To estimate whether the influence of risk factors on survival was significant we used Cox proportional hazard regression [backward elimination, P(in) 0.05, P(out) 0.10].

Results

CMV D + /R- patients

When comparing transplant cases in group 1 in terms of CMV D + /R- versus other CMV constellations (D- and D + /R +), we found most of the covariables were comparable, with the exception of lower mean PRA 6.8 + 19.4% ($P = 0.026$), less mean total mismatches 1.74 + 1.35 ($P = 0.043$) and less HLA-DR mismatches 0.21 + 0.50 ($P = 0.014$) in the CMV D + /R- constellation subgroup. In group 2 covariables are comparable, with the exception of lower mean number of KTx 1.23 + 0.46 ($P = 0.009$) and older donors 42.0 + 14.8 ($P = 0.034$) in the CMV D + /R- subgroup.

Table 2 Distribution of potential risk factors in groups 1 and 2

Variable	Group 1 ($n = 721$)		Group 2 ($n = 824$)		P
CMV D+/R- (yes/no)	129	17.9%	218	26.5%	< 0.001
Diagnosis of CMV (yes/no)	59	8.2%	195	23.7%	< 0.001
Re-KTx (yes/no)	154	21.4%	208	25.2%	< 0.1
Immunized > 50% (yes/no)	70	9.7%	46	5.6%	< 0.01
Mismatch HLA DR > 0 (yes/no)	192	26.6%	310	37.6%	< 0.001
Old donors > 50 years (yes/no)	96	13.3%	227	27.5%	< 0.001
CIT > 30 h (yes/no)	137	19.0%	114	13.8%	< 0.01
Delayed graft function (yes/no)	369	51.2%	323	39.2%	< 0.001
Incidence of 1st ARE (yes/no)	329	45.6%	330	40.0%	< 0.05
ALGs use in ARE (yes/no)	146	20.3%	216	26.2%	< 0.01
CsA based immunosuppressive therapy (yes/no)	721	100%	719	87.3%	< 0.001

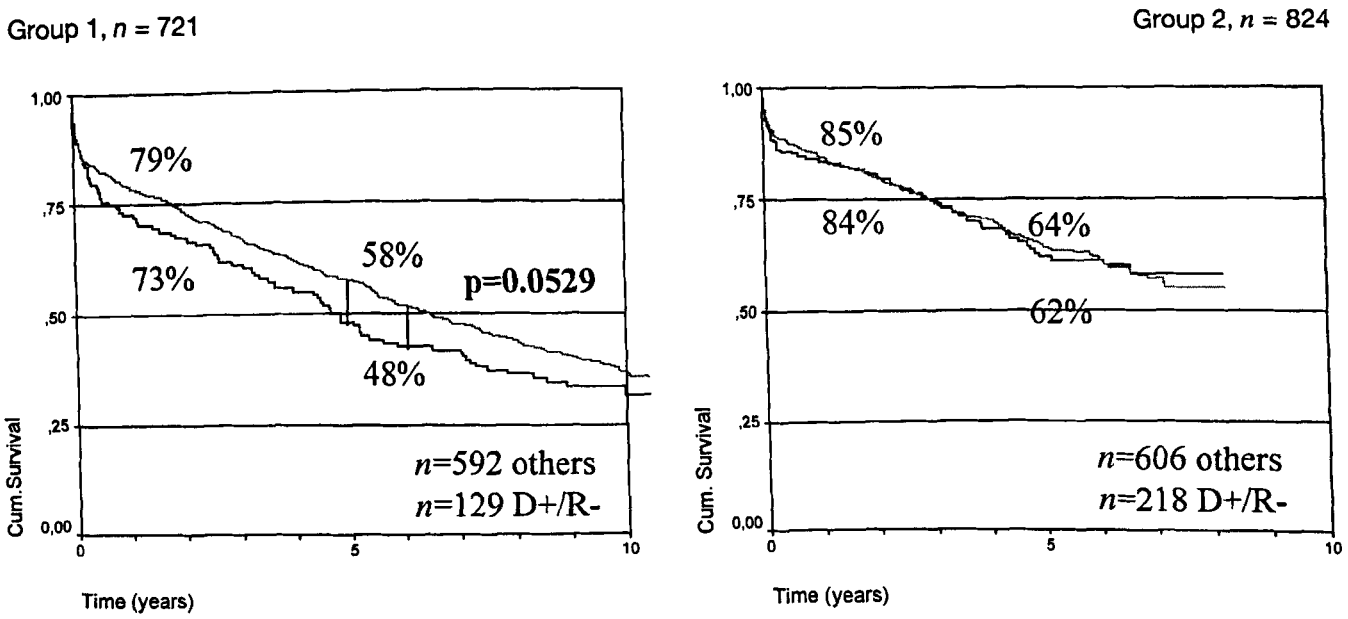

Fig. 1 Graft survival of CMV D + /R- compared with other CMV constellations

Graft survival

Comparison of graft survival in group 1 with univariate Kaplan-Maier survival tables in respect of CMV D + / R- ($n = 129$) versus the other CMV constellations (D- and D + /R + , $n = 592$) shows a reduced graft survival for CMV D + /R- of 72.9%, 61.2%, 48.1%, and 41.9% for posttransplant years 1, 3, 5, and 7, as against 79.1%, 67.4%, 57.6%, and 47.4% for the other constellations (log-rank at year 6: $P = 0.0529$). Comparison of graft survival in group 2 in terms of CMV D + /R- ($n = 218$) versus others ($n = 606$) shows equal graft survival rates for CMV D + /R- (84.5%, 74.8%, 64.2%, 57.1% for years 1, 3, 5 and 7) and the others (83.9%, 75.3%, 62.2%, 58.4%) (log-rank at year 6: $P = 0.8527$; see Fig. 1).

Patient survival

Comparison of patient survival of group 1 with univariate Kaplan-Maier survival tables in terms of CMV D + /R- versus the other CMV constellations ($n = 592$) shows lower patient survival for CMV D + /R-: 93.0%, 89.9%, 83.0%, and 81.4% for posttransplant years 1, 3, 5 and 7, as opposed to 96.6%, 90.7%, 84.8%, and 79.4% for the others (log-rank at 18 months: $P = 0.0496$). Comparing patient survival in group 2 in terms of CMV D + /R- versus others shows equality of patient survival rates for CMV D + /R- (96.8%, 91.8%, 85.3%, and 83.4% for years 1, 3, 5 and 7) and the other patients (97.0%, 91.9%, 87.7%, and 80.9%; log-rank at 18 months; $P = 0.8567$).

Results in patients with diagnosis of CMV infection

When comparing transplant cases in group 1 in terms of patients with a diagnosis of CMV after transplant versus CMV-free patients in the same group, we found most of the covariables were comparable, with the exception that the CMV patients had more acute rejection episodes (ARE 1.02 + 0.99, $P = 0.009$). In group 2 covariables were comparable with the exception that the patients with CMV diagnosis were older (47.3 + 11.8 years, $P = 0.012$), received grafts from older donors (45.9 + 15.2 years, $P = 0.000$) and also had more ARE (1.12 + 0.65). Comparison of graft survival in group 1 with univariate Kaplan-Maier survival tables with respect to patients with a diagnosis of CMV after transplant ($n = 59$) versus CMV-free patients ($n = 662$) shows a lower graft survival rate for patients with a diagnosis of CMV, of 69.5%, 54.2%, 44.1%, and 37.3% for posttransplant years 1, 3, 5, and 7, as against 78.9%, 67.4%, 57.0%, and 47.4% for the CMV-free patients (log-rank at year 6: $P = 0.0294$). Comparison of graft survival in group 2 in terms of patients with the diagnosis of CMV posttransplant ($n = 195$) versus CMV-free patients ($n = 629$) shows comparable graft survival rates for patients with CMV diagnosis (82.6%, 75.5%, 58.4%, and 50.5% for years 1, 3, 5 and 7) and for CMV-free patients (84.9%, 74.8%, 64.6%, and 58.5%, log-rank at year 6: $P = 0.4900$). Comparing patient survival in group 1 with univariate Kaplan-Maier survival tables in terms of patients with a diagnosis of CMV after transplant versus CMV-free patients shows a reduced graft survival for

Table 3 Cox proportional hazards regression of 5-year graft survival

Group 1	$n = 721$		Group 2	$n = 824$	
Variable	P	Relative risk	Variable	P	Relative risk
CMV D+/R−	0.0483	1.32 (1.0–1.7)	reKTx	0.0007	1.59 (1.2–2.1)
reKTx	0.103	1.44 (1.1–1.9)	ARE (0, 1, 2, 3)	0.0012	1.22 (1.1–1.4)
ARE (0, 1, 2, 3)	0.0001	1.28 (1.1–1.4)	Old Donor	0.0089	1.43 (1.1–1.9)
Old donor	0.0000	1.92 (1.4–2.6)	MM DR	0.0311	1.32 (1.0–1.7)
Immunized	0.0045	1.66 (1.2–2.4)	DGF	0.0026	1.48 (1.2–1.9)
Not in the equation:		Diagnosis of CMV, CIT, MM DR, DGF	Not in the equation:		CMVD+/R−, diagnosis of CMV, immunized, CIT

Table 4 Cox proportional hazards regression of 18-month patient survival

Group 1	$n = 721$		Group 2	$n = 824$	
Variable	P	Relative risk	Variable	P	Relative risk
Diagnosis of CMV	0.0371	2.55 (1.1–6.2)	Old recipient > 50 y	0.0021	2.97 (1.5–5.9)
Old recipient > 50 years	0.0096	2.36 (1.2–4.5)			
Not in the equation:		CMV D+/R−, ReKTx, ARE, old donor, immunized, CIT, MM DR, DGF	Not in the equation:		CMV D+/R−, diagnosis of CMV, ReKTx, ARE, old donor, immunized, CIT, MM DR, DGF

patients with a CMV diagnosis: 89.8%, 83.5%, 79.7%, and 74.6% for posttransplant years 1, 3, 5, and 7, as opposed to 96.5%, 91.2%, 85.1%, and 80.2% in the CMV-free patients (log-rank at year 1: $P = 0.0106$). Comparing patient survival in group 2 in the same way shows idenzical patient survival rates for patients with the diagnosis of CMV (98.0%, 94.3%, 85.9%, and 79.3% for years 1, 3, 5 and 7) or without the diagnosis of CMV (97.0%, 91.2%, 86.8%, and 81.6%, log-rank at year 1: $P = 0.3574$).

Cox proportional hazards regression

Graft Survival

The risk of losing graft function within 5 years after the transplant was analyzed using Cox proportional hazards regression in both groups. The model for group 1 determines that the constellation of CMV D + /R− represents a significant risk factor for earlier graft loss ($P = 0.0483$) and estimated the relative risk at 1.32 (95% confidence interval 1.0015–1.7372), whereas in group 2 the influence of CMV D + /R− constellation was found not to be significant ($P = 0.6061$); see Table 3.

Patient survival

When analyzing the risk of patient's death within 18 months after renal transplantation by means of Cox proportional hazards regression we found that in both the age of the recipient was the most important risk factor (group 1 $P = 0.0096$, rRisk 2.36; group 2 $P = 0.0021$, rRisk 2.97), whereas in group 2 patients age was the only significant risk factor found in the Cox proportional hazards regression equation the model determined in group 1 that patients with a postoperative diagnosis of CMV have a significantly ($P = 0.0371$) higher risk (2.55-fold) of dying within 18 months than those without a CMV diagnosis (see Table 4).

Discussion

Nearly all covariables havechanged in a significant way from the late 1980s (group 1) to the 1990s (group 2). Therefore, the two groups cannot be compared directly. In group 2 we found more mismatches on HLA-DR, lower levels of pretransplant immunization, older donors, shorter cold ischemia times, other preservation liquids in use, less delayed graft function, fewer acute rejection episodes and at a frequency of 8.7% the use of another primary immunosuppressant, i.e. tacrolismus (Tables 1, 2). Especially important for this analysis is that far more sensitive diagnostic tools were available for CMV in the time of group 2 (CMV-IgM and Clo-

nab). Therefore, it is not correct to compare patients at risk of CMV in group 1 with those in group 2. The analysis can only be done in group 1 and group 2 separately.

Conclusion

In a retrospective study like this over a long period of time (14 years), in which a lot of medical procedures and options have changed, it is difficult to form a conclusion on the effect of a single therapeutic procedure, such as the introduction of ganciclovir therapy. Of course, the considerable improvements in diagnosis of CMV infections and the opportunity of interfering in the CMV disease process at a very early stage are the most important advantage today. We have demonstrated, however, that a strictly applied anti-CMV regimen, use of anti-CMV hyperimmunglobulins for prophylaxis, monitoring CMV posttransplant and use of ganciclovir in anti-CMV therapy even preemptively can avoid disadvantages concerning long-term transplant kidney graft function completely. The procedure eliminated the risk of CMV infections in kidney-transplanted patients, resulting in allograft and patient outcome identical to those in patients free of CMV risk. In addition, it has been shown that before ganciclovir was available there was a significant risk of the patient's death when a CMV infection was diagnosed. This risk is also completely abolished by the regimen described. Probably the fairest way to demonstrate the advatages of CMV monitoring and consistently applied therapies using ganciclovir in this study is to pay attention to the results seen in the CMV D + /R patients. In CMV D + /R– patients in group 1, who were treated without ganciclovir, not only the long-term graft survival observed was significantly reduced, but also the death rate within 18 month after transplantation was increased. Under the described anti-CMV regimen, these risks of CMV D + /R– are no longer seen. The question of how far these results are caused by the use of anti-CMV hyperimmunoglobulins in prophylaxis cannot be answered in this study, because all patients in groups 1 and 2 received the same prophylaxis regimen. However, these results are achieved on the basis of the use of anti-CMV hyperimmunoglobulins in anti-CMV prophylaxis. Ganciclovir is a very effective drug that has increased the safety of immunosuppressive therapies enormously. Ganciclovir makes a substantial contribution to the safety of immunosuppressive therapies and is thus an indispensable part of modern immunosuppression. A substantial part of the progress in organ transplantation today is due to ganciclovir.