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Acyclovir plus CMV immunoglobulin prophylaxis and early therapy with ganciclovir are effective and safe in CMV high-risk renal transplant pediatric recipients

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Abstract Cytomegalovirus (CMV) infection is still a major cause of morbidity in high-risk renal transplant recipients. In the present report, we have reviewed our records of renal transplant pediatric recipients (RTPR; mean age 14.1 ± 4.9 years) since 1991, when we started a policy of CMV prophylaxis constituting high-dose oral acyclovir plus CMV hyperimmune immunoglobulins (Hlg) followed by early i.v. ganciclovir therapy in high-risk patients (i.e., CMV donor + / recipient −). Four patients received a kidney from a living relative (LR), 2 patients had one previous transplant, and 1 had a combined liver – kidney transplant. Thirty-three patients who were negative for CMV antibodies (ab) before transplantation received a kidney from CMV ab positive donors. The immunosuppressive regimen included cyclosporine A and steroids, with the addition of azathioprine in the 4 patients who received an LR kidney. Serial assessments for CMV antigenemia (pp 65) were routinely performed for 6 months after transplantation to define CMV infection. Among the 33 CMV seronegative recipients (R −) who received the graft from a CMV seropositive donor (D +), 18 (54.5%) experienced CMV infection, whereas among the 28 CMV R +, who received a graft from a CMV D +, 11 (39.3%) experienced CMV infection. With regard to CMV – related symptoms, only 2 patients suffered from a CMV syndrome (fever and leukopenia in 1 patient, fever and arthralgia in the other). In no case did the spectrum of CMV disease occur; only minor symptoms were present in 7 of the remaining CMV-infected patients (fever in 6 and leukopenia in 1). Rejection episodes and renal function did not differ between CMV-infected and non-CMV-infected patients. Our experiences support the use of prophylactic acyclovir plus CMV HIg followed by early therapy with i.v. ganciclovir to combat the risk of increased morbidity in high risk RTPR.

Key words Kidney transplant · Cytomegalovirus infection · CMV prophylaxis · CMV therapy · Pediatric recipients

Introduction

Despite the fact that more selective immunosuppressive drugs and antiviral agents active against cytomegalovirus (CMV) have recently become available, CMV infection is still a major cause of morbidity following solid organ transplantation. A total of 30–50% of all renal transplant recipients experience infections caused by CMV; in particular, the frequency of infection among seronegative recipients of kidneys from CMV seropositive donors is between 60 and 88%, with symptomatic disease occurring in approximately 60% of these [1–4].

Table 1 Details of serological and clinical data regarding cytomegalovirus (CMV) infection in 79 renal transplant pediatric recipients

Patient group	Donor	Recipient	Patient number and (%)	CMV infection	CMV syndrome[a]	CMV minor symptoms[a]
I	–	–	6 (7.6%)	0		
II	+	–	33 (41.8%)	18 (54.5%)	2 (11.1%)	5 (27.7%)
III	–	+	12 (15.2%)	1 (8.3%)		
IV	+	+	28 (35.4%)	11 (39.3%)		2 (18.1%)

[a] Patient number and percentage within CMV-infected patients belonging to each group

In this regard, pediatric patients are at higher risk of infection because of their lower rate of CMV seropositivity. Furthermore, primary CMV infection is often clinically more severe than reactivation or superinfection, thus increasing the possibility of severe CMV disease. In addition, CMV-positive donor kidneys seem to negatively influence graft and patient survival rates in certain categories of patients [5].

For the above reasons and in an effort to prevent serious clinical CMV disease in high-risk recipients, immunological and pharmacological strategies have been developed. As it is not feasible to only transplant organs from seronegative donors to seronegative recipients, various approaches have been adopted: (1) passive antibody protection with polyspecific Ig or CMV hyperimmune immunoglobulins (HIg), either alone or in association with other antiviral agent, (2) active immunization with a live attenuated vaccine, or (3) antiviral agents such as high-dose oral acyclovir or pre-emptive ganciclovir [6–17]. Finally, the early diagnosis of CMV infection remains an additional important goal, with a view to starting antiviral therapies as soon as possible [18–21].

In the present report, we have reviewed our records of renal transplant recipients since 1991, when we started a policy of CMV-infection prophylaxis constituting high-dose oral acyclovir plus CMV HIg followed by early i.v. ganciclovir therapy in the presence of infection in high-risk CMV-seronegative patients.

Materials and methods

The records of 79 renal transplant pediatric recipients who received a kidney between July 1991 and August 1996, were reviewed. All patients were followed up for a minimum of 12 months. There were 45 males and 34 females with a mean age at the time of kidney transplantation of 14.1 ± 4.9 years (range 2.5–20). Four patients received a kidney from a living relative, 2 patients had had one previous transplant, and 1 patient had a combined liver-kidney transplantation. Patients were subdivided into four groups according to the CMV infection antibody status of the donors and recipients and the details of the CMV serological data and CMV infection are listed in Table 1. The high-risk recipient group, i.e., CMV-seronegative recipients receiving a CMV-positive graft, consisted of 33 patients (group 2).

The immunosuppressive regimen included cyclosporine and steroids, with the addition of azathioprine in the 4 patients who received a living donor-related kidney. Acute rejection episodes were treated with i.v. methylprednisolone pulses; 4 patients received adjunctive therapy with antilymphocytic serum (Lymphoglobuline; Pasteur Mérieux, 2 patients) or monoclonal antibodies [Ortochlone (OKT3); Cilag, 2 patients] for steroid-resistant rejection (CMV constellation: D +/R – in 3 patients, D –/R + in 1 patient). Three patients received antilymphocytic serum for acute tubular necrosis (1 D +/R –, 2 D +/R +).

The CMV prophylactic regimen in seronegative recipients consisted of oral acyclovir (Zovirax; Wellcome, 40 mg/kg per day until January 1995 and subsequently 80 mg/kg per day) associated with CMV HIg (Cytotect; Biotest) as follows. A dose of 150 mg/kg was given on the first postoperative day, 100 mg/kg on days 15 and 30 after transplantation, and 50 mg/kg on days 45, 60, and 120. Since February 1995 dosage was modified as follows: a dose of 150 mg/kg was given on the first postoperative day and twice monthly for 2 months after transplantation, and 100 mg/kg in the 3rd and 4th month after transplant. CMV-seropositive patients received only oral acyclovir at the same dosage. During OKT3 or antilymphocytic serum therapy, prophylactic i.v. ganciclovir (Cymevene; Biotest) was administered (half dose).

In the presence of CMV infection, 10 mg/kg body weight per day i.v. ganciclovir was administered for at least 2 weeks, or until negative antigenemia was obtained. Ganciclovir and acyclovir dosages were adjusted in response to renal failure according to the manufacturers' schedules. In these patients, in the presence of CMV seroconversion, prophylactic CMV HIg were discontinued. Specific patients' informed consent was not obtained as this protocol is a part of the routine posttransplant management of high-risk patients and is in accordance with the Helsinki Declaration of 1975.

Antibody titers for CMV were determined by an enzyme-linked immunoadsorbent assay (ELISA) technique for the IgG titer and DS ELISA for the IgM titer. An immunocytological assay was used for the detection of CMV antigens (pp 65 KD) in circulating peripheral blood leukocytes (PBL). By the use of a mixture of monoclonal antibodies and a second step fluorescence antibody as a detection system, CMV lower matrix protein pp 65 was detected in cytocentrifuged PBL and the results expressed quantitatively by reporting the number of CMV antigen positive cells per 200000 PBL [17]. The presence of five or more positive cells per 200000 PBL was considered to define CMV infection and prompted ganciclovir treatment. All patients were monitored for CMV antigenemia (and CMV antibody level) for 6 months after transplantation.

CMV-related symptoms were classified as a CMV syndrome if the patients showed unexplained fever for more than 2 days accompanied by CMV infection, as described above, and one of the following symptoms: arthralgia, leukopenia ($< 3 \times 10^9$/l), or thrombocytopenia ($< 100 \times 10^9$/l). CMV disease was defined as a CMV

syndrome plus pneumonia, hepatitis, or central nervous system involvement.

Results

A total of 79 records were reviewed; among these 30 patients (38%) suffered CMV infection, while 49 (62%) did not (Table 1). Among recipients who were CMV seronegative before transplantation, none of those who received the graft from a CMV-seronegative donor (group I) developed CMV infection; of the 33 recipients who received the graft from a CMV-seropositive donor (group II), 18 (54.5%) experienced CMV infection, while 15 (45.5%) did not. Among CMV-seropositive recipients, 1 of the 12 who received a kidney from a CMV-seronegative donor developed infection (group III); of the 28 recipients who underwent kidney transplantation from a CMV-seropositive donor (group IV), 11 (39.3%) experienced CMV infection, while 17 (60.7%) did not. Of the 7 patients who were treated with adjunctive immunosuppressive therapy (antilymphocytic serum or monoclonal antibodies) for steroid-resistant rejection or acute tubular necrosis, 4 developed CMV infection (2 from group II, both treated for rejection; 1 from group III, treated for rejection; 1 from group IV, treated for acute tubular necrosis).

The median time to onset of CMV infection was 48 ± 4.1 days (range 14–105); no statistically significant difference was seen between seronegative and seropositive recipients (45.4 ± 5.8 and 58.2 ± 8.2 days, respectively). With regard to CMV-related symptoms, only 2 patients from group II (Table 1) experienced CMV syndrome (fever and leukopenia in one case, fever and arthralgia in the other). In no case did the spectrum of CMV disease occur and only minor symptoms were present in 7 of the remaining CMV-infected recipients; in particular, fever in 6 (4 patients from group II and 2 patients from group IV) and leukopenia in 1 (group II) (Table 1).

Six patients (4 in group II, 2 in group IV) required two (4 patients) or three (2 patients) separate courses of ganciclovir therapy for relapsing CMV infections (i.e., recurrent rises and falls in CMV antigenemia count); the median time to recurrence of CMV infection was 14.1 ± 4.3 days (range 4–33). None of these patients manifested CMV syndrome or disease when CMV infection relapsed or they experienced the emergence of CMV strains resistant to ganciclovir. Acyclovir, CMV HIg, and ganciclovir were generally well tolerated; in addition there were no clinical adverse events that required a patient to discontinue the treatment. All CMV-infected patients seroconverted; moreover a different pattern was observed in CMV-relapsing recipients characterized by a persistent IgM titer and a later shift to IgG response. Acute rejection episodes occurred in 33.3% (10 out of 30) of the CMV-infected recipients and in 51% (25 out of 49) of the non-infected ones, but the difference did not appear statistically significant. Renal function in the 61 recipients with a follow up of at least 1 year was no different between CMV-infected and non-infected patient groups: the mean calculated creatinine clearance was 77.6 ml/min per 1.73 $m^2 \pm 17.3$ SD (range 29.8–105) in the infected patients and 73.2 ml/min per 1.73 $m^2 \pm 17.4$ SD (range 37.3–120) in the non-infected ones.

Discussion

CMV infection is still a major cause of morbidity in high-risk renal transplant recipients. CMV serological matching and type and intensity of immunosuppression play an important role in the incidence of symptomatic CMV infections [1–4]. Since the policy of transplanting only kidneys from seronegative donors to seronegative recipients is not feasible, various strategies have been developed. These strategies, which are not mutually exclusive, include the induction of a CMV immunological protection that can obtained actively, with attenuated Towne strain, or passively, through the administration of intravenous standard or hyperimmune immunoglobulin preparations. Furthermore, the administration of high-dose acyclovir or pre-emptive ganciclovir is also widely used [6–17].

As a result of successful prophylactic strategies, serious symptomatic CMV infections have now been significantly reduced at most transplant centers, but none of these strategies has proved entirely satisfactory in the management of high-risk recipients. Such prophylactic strategies have not been made redundant by the availability of specific antiviral agents active against CMV, such as ganciclovir, since, in addition to producing clinical syndromes, CMV infection may have indirect effects on the outcome of transplantation. In fact, kidney grafts from CMV-positive donors have been shown to have a lower survival rate than those from CMV-negative donors [5] and there is a relationship between CMV infection and rejection. The above observations have indicated CMV infection as an important risk factor for subsequent rejection episodes [22]. As far as the concomitant presence of acute rejection episodes, despite antiviral therapy, in a proportion of CMV-infected patients the same authors have suggested that antiviral therapy, given once the CMV infection is overt, cannot prevent rejection episodes [22]. Furthermore, recent data from Murray have shown relapsing CMV infections only in patients who received a kidney from a seropositive donor; in the same study the infection developed despite early therapy with ganciclovir, and morbidity, hospitalization, and graft losses were greater [23]. Finally, a striking concomitance of severely symptomatic active

CMV infection and Epstein-Barr virus (EBV) reactivation has been demonstrated [24], thus raising another intriguing problem regarding the possibility of the development of posttransplant lymphoproliferative disorders.

Bearing in mind the great potential morbidity related to CMV infection in high-risk renal transplant recipients, the controversial effect of acyclovir and CMV HIg prophylaxis, and the limited efficacy of pre-emptive or early therapy with ganciclovir, we decided to start the following anti-CMV-infection scheme: acyclovir plus CMV HIg as prophylaxis, in accordance with previous studies by Carrieri [25] and Uber [26], followed by early therapy with ganciclovir in the presence of infection, as determined by positive CMV antigenemia. Although the protocol reported in our study was expensive and required frequent laboratory tests, the cost was outweighed by its effectiveness. Only 18 out of 33 seronegative recipients who received a kidney from a seropositive donor experienced CMV infection and in no case did significant symptomatic infection occur and no adjunctive hospitalization was required for CMV infection. There were no graft losses due to CMV infection and no differences in the incidence of acute rejection episodes between CMV-infected and non-infected patients. In this regard, although the rejection episodes reported in our patient series were not all biopsy proven, it is tempting to speculate that the absence of a difference in the rejection incidence between CMV-infected and non-infected patients could be due to the effect of our combined anti-CMV-infection treatment.

Renal function at 1 year was no different between infected and non-infected recipients. In our experience, relapsing CMV infection did not carry a risk of increased morbidity. No patient experienced the emergence of CMV strains resistant to ganciclovir, and generally this therapeutic scheme was well tolerated. Finally, no differences in EBV concomitant infection episodes were observed (data not shown). Similar results have been achieved in marrow and liver transplant recipients through long-term administration of i.v. ganciclovir [27–29], however, this involves maintaining intravenous access for a long time and exposes the patients to the toxicity of ganciclovir. Oral ganciclovir could be an effective means of preventing CMV infection in renal transplant recipients, however, despite a few promising data [30, 31], the question requires further clinical studies. Until such time as a more economical and equally efficacious therapeutic option becomes available, we recommend the use of prophylactic acyclovir plus HIg followed by early therapy with i.v. ganciclovir to combat the risk of increased morbidity in high-risk renal transplant recipients.

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