

The value of immunoprophylaxis for cytomegalovirus infection with intravenous immunoglobulin in pediatric liver transplant recipients receiving a low-dose immunosuppressive regimen

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Abstract: The incidence of CMV infection following pediatric Ltx is particularly high, which can be attributed to the increased number of patients at high risk for primary infection (donor CMV+, recipient CMV–). Current approaches to cope with this complication producing post-operative morbidity include prophylactic or preemptive ganciclovir therapy. As the risk for symptomatic CMV infection is directly correlated with the intensity of immunosuppression, the aim of our study was to assess the value of IVIG in order to protect children receiving low-dose immunosuppression from CMV disease. Twenty-eight consecutive children (median age 62.2 months) at high risk prospectively received three infusions of IVIG on days four, 14, and 28 post-transplant and were monitored for six months post-Ltx. Immunosuppression consisted of cyclosporine (initial trough levels 170–200 µg/L) and prednisolone (starting dose 15 mg/m²) as well as basiliximab induction therapy. Patient survival was 100% and graft survival was 92.9%. Two subjects developed laboratory findings of CMV infection (8%) and one child suffered from tissue invasive CMV disease (4%). Three patients were excluded from the study because of protocol violations. We conclude that there was a low incidence of CMV disease among a prospective cohort receiving low-dose immunosuppression and a standard IVIG product.

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CMV infection plays a major role producing morbidity and mortality following pediatric Ltx with an incidence of up to 40% in the previous decade and a reported mortality of up to 19% (1). During the first three months following Ltx, the incidence of CMV infection is highest, especially in the first six wk (2). Recently, improvements in diagnostic testing, immunosup-

pression, and therapy of CMV disease have led to a significant reduction in post-operative CMV related morbidity and mortality.

Among CMV prevention strategies in children, there are the following approaches: ganciclovir alone (as prophylaxis or preemptive therapy), ganciclovir plus immunoglobulin and immunoglobulin alone (3), with the latter being the least frequently employed approach. However, the appropriate duration of ganciclovir therapy and the role of IVIG in preventing CMV disease remain equivocal in pediatric Ltx recipients (4).

Abbreviations: CMV, cytomegalovirus; EBV, Epstein–Barr virus; IVIG, intravenous immunoglobulin; Ltx, liver transplantation; PFIC, progressive familial intrahepatic cholestasis; PTLD, post-transplant lymphoproliferative disease.

Immunoglobulin products are produced from pooled human plasma using the Cohn-Oncley process. They contain mostly IgG (>95%), and only traces of IgA and IgM (5). Adverse events are rare and include chills, headaches, fever, urticaria, arterial hypotension, and rheumatoid complaints. Serious adverse events, such as anaphylactic shock, occur in isolated cases.

CMV infection can be divided into direct and indirect effects, particularly in high-risk CMV-negative transplant recipients (R-) receiving an organ from a CMV-positive donor (D+). As a direct consequence of viral replication and tissue invasion, CMV may lead to fever, hepatitis, nephritis, pneumonitis, gastroenteritis, and retinitis. In contrast, there is strong evidence that indirect effects of CMV increase rates of acute and chronic graft rejection, bacterial and fungal superinfections, and EBV-associated PTLD (6).

It has been well established that CD4+ and CD8+ cytotoxic T cells are important in the control of CMV infection in solid-organ transplant recipients. Therefore, the risk of CMV correlates with the intensity of immunosuppression as has been shown by Sester et al. (7) demonstrating that calcineurin inhibitors decrease CMV-specific T-cell reactivity in a dose-dependent manner.

This study was based on the hypothesis that a low-dose immunosuppression (with respect to calcineurin inhibitors and steroids) enables safe and effective CMV prophylaxis with IVIG in pediatric liver transplant recipients.

Patients and methods

Patients

Twenty-eight children underwent Ltx included in this trial. Inclusion criteria were *de novo* Ltx and risk for CMV (donor CMV+, recipient CMV-). Exclusion criteria were fulminant hepatic failure of unknown origin, CMV acute or chronic liver failure, and re-transplantation.

We prospectively monitored the clinical, laboratory, and microbiological course of consecutive children with at risk for CMV infection in the first six months following Ltx. Transplants were performed between September 2005 and April 2008. In that period, a total of 71 pediatric Ltx have been performed in our center. All parents of the children gave written informed consent to participate in the study.

Study design

This was a prospective, non-controlled study. We used IVIG prophylactically coupled with monitoring to perform pre-emptive ganciclovir therapy.

IVIG product

IVIG (0.4–0.5 g/kg body weight; Gamunex® 10%, Talecris GmbH, Frankfurt, Germany) was administered at

an infusion rate of 0.1 mL/kg/min on days four, 14, and 28 post-Ltx. The CMV IgG antibody concentration of the IVIG product was between 1:18 000 and 1:>40 000 (according to product information from the company).

Immunosuppression

All children received cyclosporine A (initial trough levels between 170 and 200 µg/L) and prednisolone (starting dose 15 mg/m²) as primary immunosuppression. Furthermore, all children received two doses of basiliximab (Simulect®; Novartis Pharma GmbH, Basel, Switzerland) on days one and four post-transplant. The corticosteroids were continuously tapered.

Acute graft rejection

Clinically suspected acute graft rejection was confirmed by percutaneous liver biopsy and histologic grading according to the Banff criteria (8) and was treated with a three- to five-day course of intravenous methylprednisolone bolus therapy (10 mg/kg/day).

CMV status and antiviral treatment

Serum anti-CMV IgM and IgG antibody titers, pp65, and CMV PCR (in blood and urine; Roche Pharma AG, Grenzach-Wyhlen, Germany) were quantified weekly during the first month following Ltx and thereafter on a monthly basis up to six months following Ltx. All tests were performed in the same laboratory. CMV infection and disease were defined according to established criteria (9). All cases of proven CMV disease were treated with intravenous ganciclovir (10 mg/kg/day, divided into two doses) for at least four wk. Pre-emptive therapy with ganciclovir was given to children who were positive for pp65 and additionally positive for CMV DNA in the blood by PCR.

Results

Patient characteristics

The patient characteristics are shown in Table 1. There were 14 male and 14 female children, the median age of the study cohort patients was 62.2 months (range 3–189 months). A total of three patients had to be excluded from the study because of protocol violation. Two patients accidentally received another IVIG product (patients 7 and 8), and one patient did not receive the second dose of IVIG (patient 26). None of the three patients developed CMV infection or disease.

Patient and graft survival

Patient survival was 100% (six-month follow-up), graft survival was 92.9%. Two patients received a second transplant because of primary graft non-function on days three and five post-transplant, respectively.

Table 1. Patient characteristics and results of CMV diagnostics and infections in 28 pediatric patients following liver transplantation and receiving low-dose immunosuppression

Patient no.	Age at Ltx (months)	Diagnosis leading to Ltx	Graft source	Graft rejection	CMV pp65 and PCR positive	CMV disease
1	58	Crigger Najjar	Split liver	No	+	–
2	8	Biliary atresia	Split liver	Yes	–	–
3	3	Biliary atresia	Split liver	No	–	–
4	4	Biliary atresia	Living donation	No	–	–
5	9	Biliary atresia	Split liver	No	–	–
6	33	Alagille's syndrome	Split liver	Yes	–	–
7	13	Hepatoblastoma	Split liver	Yes	–	–
8	7	Mitochondrial disease	Split liver	No	–	–
9	172	Unknown	Red. sized liver	No	+	–
10	11	Biliary atresia	Living donation	No	–	–
11	9	Biliary atresia	Split liver	No	–	–
12	88	Biliary atresia	Living donation	No	–	–
13	57	Fulm. hepatic failure (Enterovirus)	Split liver	Yes	–	–
14	131	Biliary atresia	Split liver	Yes	–	–
15	9	Biliary atresia	Living donation	No	+	On day 35 (gastroenteritis)
16	100	PFIC	Living donation	No	–	–
17	39	Cryptic liver cirrhosis	Split liver	No	–	–
18	84	Alagille's syndrome	Split liver	Yes	–	–
19	14	Biliary atresia	Split liver	No	–	–
20	8	Biliary atresia	Split liver	No	–	–
21	84	Alagille's syndrome	Red. sized liver	No	–	–
22	174	PFIC	Living donation	Yes	–	–
23	16	Biliary atresia	Split liver	No	–	–
24	135	Urea cycle defect	Whole liver	No	–	–
25	68	Cryptic liver cirrhosis	Split liver	No	–	–
26	146	Urea cycle defect	Red. sized liver	Yes	–	–
27	73	Unknown cirrhosis	Split liver	No	–	–
28	189	PFIC	Living donation	No	–	–

All children were at high risk for CMV (D+R–) and received IVIG prophylactically on days four, 14, and 28 post-Ltx. Patients 7, 8, and 26 were excluded from the study because of protocol violations.

CMV infection and CMV disease

There were two cases of positive pp65 and CMV PCR (days 23 and 28 post-Ltx), and one case of CMV disease (day 35 post-Ltx). The patient with CMV disease developed gastroenteritis and CMV was cultured from the stools.

Acute graft rejection

The incidence of acute graft rejection was 28.6% (eight of 28 patients). All rejection episodes were graded as mild to moderate in histology. No patient had more than one acute rejection episode.

Discussion

In adult solid organ transplantation, the strategy for CMV prevention is mainly based on antiviral therapy with ganciclovir, which has to be administered intravenously every 12 h for a period of four to 12 wk (10). However, previous studies demonstrated the benefit to using CMV IVIG

prophylactically in adult liver transplant recipients (11). Alternatively, the oral prodrug of ganciclovir, valganciclovir, can be used. In pediatric Ltx recipients, IVIG provides a feasible alternative to ganciclovir for at least three reasons: to avoid the side effects of ganciclovir in the young population, to avoid the need for a higher frequency/number of venapunctures with the often limited venous access in children, and to account for the relatively low level of immunosuppression post-Ltx. The latter being due to the fact that Ltx children have the lowest need for immunosuppression as compared with other solid organ transplantation recipients. Therefore, we conducted the present prospective study in order to test our hypothesis that a regular IVIG product is capable of effectively preventing CMV disease in pediatric Ltx recipients at highest risk (D + R–).

Despite published guidelines for the prevention of CMV disease in transplant recipients in different organ transplant groups (12, 13), there is still no consensus regarding the optimal

prophylactic therapy in Ltx children with different immunosuppressive regimens. A recently published meta-analysis suggests that the use of CMV hyperimmune IVIG can lead to some benefits in transplant recipients with improved survival, reduced CMV disease, and CMV-associated deaths (14).

In this study, we demonstrated that it is possible to experience low rates of CMV infection in children at risk post-Ltx effectively in the setting where a standard IVIG product is used. The latter is much less expensive compared with a CMV hyperimmune IVIG. Despite the design of our study, we speculate that the low rates of CMV infection were due at least in part to the use of standard IVIG. We acknowledge that a definitive cause and effect relation cannot be established using this design. However, the results cannot be transferred to children receiving other immunosuppressive protocols such as tacrolimus-based immunosuppression or a triple therapy consisting of a calcineurin inhibitor, steroids and azathioprine or mycophenolate mofetil as the current practice in other centers.

In this study, there was one patient presenting with CMV viral symptoms and beginning tissue invasive disease (gastroenteritis) despite prophylaxis with IVIG. Two others developed positive pp65 assays and positive CMV PCR. All three patients were treated successfully with ganciclovir therapy without further complications. Therefore, the overall incidence of CMV infection was 12% (three of 25), which was low compared with children with a risk constellation (D+/R-) following Ltx receiving higher immunosuppression.

An additional risk factor in high-risk children represents the steroid bolus therapy with acute graft rejection, as has been documented in the literature (15, 16). In this study, no patient with acute graft rejection (n = 8) and consecutive methylprednisolone bolus therapy developed CMV infection.

Before 2000, we used more intensive immunosuppression with higher initial cyclosporine A trough levels (180–250 µg/L) and higher steroid doses (beginning with 60 mg/m²). In children at highest risk (D+, R-), we administered CMV hyperimmunoglobulin and had an incidence of CMV infection of 20% (nine of 45 patients) (data not shown). It is important to note that because of the different immunosuppressive protocols data from this study cannot be compared directly with historical data.

In recently published study in pediatric kidney transplant recipients receiving pre-emptive ganciclovir and CMV hyperimmune globulin prophylaxis in high risk patients, the incidence of CMV

infection was remarkably higher (72%), and 9.6% of the patients developed tissue invasive CMV disease (17). Differences with our cohort can be clearly explained by the significantly more intensive immunosuppression following kidney transplantation.

In contrast, we used a standard IVIG product which was much less expensive than hyperimmunoglobulin. Furthermore, additional hospital stays were significantly reduced by avoiding intravenous ganciclovir therapy in our IVIG approach.

This study is limited by its design and the relatively small patient number. Additionally, the follow-up of six months does not recognize late CMV infection which may occur. However, the very good results of IVIG in children post-Ltx receiving low-dose immunosuppression to avoid CMV infection justifies the continued use of this protocol, but the initiation of a randomized controlled trial should be discussed. Furthermore, it seems to be rationale to perform a future study using oral valganciclovir to prevent and treat CMV infection, as data in children are limited.

In conclusion, we have demonstrated a low incidence of CMV disease among a prospective cohort receiving low-dose immunosuppression and a standard IVIG product. However, prophylactic IVIG administration may not be effective in children following other solid organ transplantations or in the case of more intensive immunosuppression. In the future, the role of pre-emptive therapy with oral valganciclovir should be evaluated, for example, in comparison to IVIG prophylaxis.

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