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Cytomegalovirus and Epstein-Barr Virus Infection in Pediatric Liver Transplants

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Abstract

Cytomegalovirus and Epstein-Barr virus infections remain one of the main concerns in the postoperative care of children with a liver transplantation. Most of the pediatric liver transplant recipients are seronegative for cytomegalovirus and/or Epstein-Barr virus at transplantation and this places them at marked risk for the development of cytomegalovirus/Epstein-Barr virus-related diseases. In immunosuppressed patients, cytomegalovirus presents a wide range of direct and indirect effects and cytomegalovirus infection is an independent risk factor for graft loss and death. However, the availability of effective therapies, sensitive assays for diagnosis and surveillance, together with the development of effective prevention strategies have dramatically decreased the impact of cytomegalovirus infection and disease on the outcome of pediatric liver transplant recipients and cytomegalovirus infection is no longer a significant cause of morbidity or mortality in these patients.

In contrast, Epstein-Barr virus infection still represents a major cause of complications after transplantation due to its well-documented capacity to induce the development of lymphoproliferative disorders. Identifying those transplanted children at an actual risk of posttransplant lymphoproliferative disease development and defining effective and safe preventive strategies remain a challenge.

Here, we describe the clinical consequences of these viral infections and their impact on the outcome of children with a liver transplantation and review the different promoted strategies for prevention and treatment. **(Trends in Transplant. 2009;3:152-64)**

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Key words

Cytomegalovirus. Epstein-Barr virus. Prophylaxis. Preemptive treatment. Children. Liver transplantation.

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Introduction

The current one-year survival rate for children with a liver transplant is around 90%[1]. Among the factors that have contributed to these results are the improvements in diagnosis and management of infections. Transplanted children are especially vulnerable to viral infections and very particularly to certain herpes viruses: cytomegalovirus (CMV) and Epstein-Barr virus (EBV). Since most children are quite young when they are transplanted (around 50% are under two years of age) they are at a much higher risk for primary CMV and/or EBV infection in the immediate posttransplant period than adult patients.

Currently, despite the lack of standardized guidelines for prevention and treatment of CMV and EBV infections, most centers have significantly reduced the impact of these viruses on the outcomes of children with a liver transplant, but not to the same degree. Thus, while CMV infection or disease is no longer a significant cause of morbidity or mortality, EBV infection, due to its oncogenic capacity, still represents a clinical challenge in pediatric transplant programmes.

The root of this clinical difference lies in the different behavior under immunosuppression of both viruses. Thus, while CMV disease is clearly related to the lytic replication of the virus, EBV-related disease (including lymphoproliferative diseases) in transplanted patients is more dependent on the uncontrolled expansion of EBV-transformed B-cells and less on lytic viral replication, and the currently available antivirals are only effective against lytic-driven replication.

This unequal success in preventing CMV and EBV infection results in a much higher infection rate for EBV than CMV (in our recent experience 92 and 8%, respectively, in the first year after transplantation). Thus, CMV and EBV coinfection is currently a rare condition in children with liver transplant.

In the past, CMV infection has been associated with a reactivation of other herpes viruses, such as EBV[2,3], and CMV infection was reported to be one of the risk factors for post-transplant lymphoproliferative disease (PTLD) development in EBV-infected adult liver transplant recipients[4]. Unfortunately, published data regarding the role of CMV infection in the development of PTLD in pediatric liver transplant recipients are lacking.

Cytomegalovirus infection

Cytomegalovirus is a major cause of morbidity in patients with a solid organ transplant and CMV infection is an independent risk factor for graft loss and death. Direct effects of CMV disease are associated with high CMV viremia and include both CMV syndrome and invasive disease. In contrast, the indirect effects are consequences of a viral immunomodulatory effect on the recipient's immune system. Through this mechanism, CMV has been implicated in acute and ductopenic chronic liver rejection, a higher predisposition to opportunistic infections, and development of lymphoproliferative disease in EBV-infected patients or in hepatitis C infection recurrence after liver transplantation[5].

Most pediatric solid organ transplant recipients are CMV seronegative at transplantation (62% in our recent experience), so in most cases the CMV infection is due to a primary infection, newly acquired via the organ from a CMV-seropositive donor, blood products, or social contacts. Less frequently, it can be a reactivation of a latent virus acquired before transplantation or reinfection with a new strain.

The most influential risk factor for post-transplant CMV disease is the lack of preexisting specific CMV immunity in the CMV-seronegative recipients. Risk is further increased when a graft is received from seropositive donors (CMV D^+/R). Other possible combinations, such as D^-/R^+ and D^+/R^+, are considered to be routine-risk or even low-risk as occurs in the case of D^-/R^-.

The use of grafts from a seropositive adult donor, whether a split or living-donor transplantation, is increasing in most pediatric liver transplant programs as a result of the scarcity of pediatric donors. This situation means that more and more liver transplanted children are a high-risk population for CMV infection.

Other predisposing factors are intense immunosuppression, or the occurrence of graft rejection or coinfection with other viruses such as human herpes virus-6 or hepatitis C virus. Once CMV infection is acquired, the risk for CMV disease appears to be directly related with the CMV viral load[6].

Before routine prophylaxis against CMV was implemented, primary symptomatic infection developed in 40% of all children with a liver transplant, with onset frequently occurring within the first three months after transplantation. The resulting mortality rates were as high as 20%[7].

However, the availability of effective antiviral therapies, sensitive assays for infection diagnosis and surveillance, together with the development of effective prevention strategies have dramatically decreased the impact of CMV infection and disease on the outcome of pediatric solid organ transplant recipients. Nowadays, most pediatric liver transplant programs present overall incidence rates of CMV disease of less than 20% and CMV disease is an extremely rare cause of death in this population.

As a result of prophylaxis, an increase in the incidence of late-onset CMV disease, related to impairment in the recovery of CMV-specific T-cell responses, has been reported. Thus, it developed in around 25% of the CMV D^+/R^- adult liver transplant recipients who received prophylaxis for three months[8,9]. In our experience, 30% of the seronegative pediatric transplant recipients who received a three-month antiviral prophylaxis became CMV-infected after a median of six months after transplantation, but only 25% of them presented symptoms (mostly mild leucopoenia or thrombocytopenia), and the symptoms resolved in all cases after a one-month treatment with valganciclovir (un-published data).

Currently, the most commonly used assays for infection diagnosis and surveillance are CMV DNA detection by PCR in whole blood and pp65 CMV-antigenemia determination in blood leucocytes. Both techniques have been demonstrated to be sensitive markers, but it seems that most centers prefer PCR over antigenemia. The few studies performed in pediatric liver recipients have supported the usefulness of PCR in early detection of CMV, but an optimal cutoff value for starting antiviral treatment has not yet been established[10].

Treatment

The current treatment of choice for CMV disease in children with a liver transplant consists of intravenous ganciclovir at a dose of 5 mg/kg twice-a-day for 3-4 weeks until two consecutive weekly PCR or antigenemia negative results. Another potential option for treatment of CMV disease, valganciclovir, has been proven effective in adults, but no published studies have yet confirmed its efficacy in children[11].

Cytomegalovirus resistance to ganciclovir, commonly due to mutations in the UL97 and

UL54 CMV genes, is rising in adult patients. However, it has not been reported as a frequent complication in children.

In cases of severe CMV disease, particularly with pulmonary involvement, treatment with hyperimmune CMV immunoglobulin (CMVIg) is indicated in addition to iv ganciclovir. If feasible, the degree of immunosuppression can also be decreased, but there are no standardized guidelines.

Prevention of cytomegalovirus disease

Because of the potential serious consequences of CMV infection, prevention has become the cornerstone of infection management after transplantation. However, defining a standard of care for preventing CMV disease remains controversial and undefined. There are two major strategies: universal prophylaxis and preemptive treatment. The former consists in the administration of an antiviral agent for a long period of time to all transplanted children irrespective of the individual risk for CMV disease, with the objective of avoiding both CMV infection and disease in a period of time when recipients are under intense immunosuppression. Prophylaxis suppresses viremia, thereby preventing direct, and maybe indirect, effects of CMV, and it is also likely to prevent other herpes virus infections such as EBV. Limitations of this approach include the potential toxicity of antivirals, the risk of resistance development, and the possible development of a late-onset CMV disease.

Preemptive treatment relies on strict surveillance to detect the appearance of CMV infection and, upon detection, antiviral therapy is started to prevent a progression to symptomatic CMV disease. It requires longitudinal and frequent monitoring of CMV infection and its success depends on the sensitivity and specificity of the viral marker to predict CMV disease. Again, little has been published regarding the pediatric population. A possible drawback of preemptive therapy is that it may not be entirely effective in preventing indirect CMV effects since low-grade viral replication may not be detected and therefore not treated.

Both strategies have demonstrated high efficacy in preventing CMV disease in adult recipients after liver transplantation. However, a recent survey of 58 different liver transplant programs in North America indicated that most of the centers prefer prophylaxis instead of preemptive treatment for high- and routine-risk patients[12].

There are no large controlled pediatric studies with statistically reliable data that can support any superiority of one of these strategies over the other. Only one trial is available to compare prophylaxis and preemptive treatment in children. In this recently published study, a group of 21 pediatric patients with a liver transplant were randomized to receive prophylaxis (ganciclovir for 30 days) followed by preemptive treatment (ganciclovir on reaching a threshold of 100,000 DNA copies/ml whole blood) or preemptive treatment alone. No case of CMV disease was diagnosed in either arm and the CMV-infection rates were similar (70% in the prophylaxis arm versus 81% in the preemptive alone treated group), but there was a significant increase in the median total number of days of ganciclovir in the prophylaxis plus preemptive treatment group. On the basis of their results, the authors recommended preemptive therapy over prophylaxis[13].

However, beyond this limited experience, the current strategies used in pediatric transplant programs are mainly based on the

results of trials in adults[14,15]. Some of these studies have shown that preemptive treatment may not be effective in certain CMV D^+/R^- adults with a liver transplant since viral replication can be so active and rapid that it may produce symptomatic disease prior to detection, even with a weekly monitoring scheme. In one study, nearly 25% of CMV D^+/R^- patients who developed CMV disease could not be detected early by PCR[16]. This finding is highly relevant in transplanted children since a high proportion of them are CMV-seronegative at transplantation. Indeed, prophylaxis is currently recommended in all D^+/R^- recipients by the American Society of Transplantation[17].

An additional important benefit of CMV prophylaxis in children is that of preventing other herpes-group virus infections with a potentially severe impact on outcome such as Epstein-Barr virus or human herpes virus-6.

Multiple prophylaxis strategies with different combinations of antiviral drugs and doses have been used by the different transplantation programs over the years. Currently, the most extended prophylaxis regime consists in a short course of iv ganciclovir followed by long-term therapy with oral antivirals. However, a study in liver transplanted children treated with either two weeks of iv ganciclovir followed by 50 weeks of high-dose oral acyclovir or two weeks of iv ganciclovir alone did not find an added beneficial effect of long-term prophylaxis on the incidence of CMV-disease[18].

Nevertheless, the availability of new oral antivirals, such as ganciclovir or, more recently, valganciclovir, with a superior capacity for inhibiting CMV replication than acyclovir, combined with the good results obtained from trials on adult recipients[19], have prompted the inclusion of these drugs in prophylactic protocols in children. The poor bioavailability of oral ganciclovir (under 10%) and the lack of a liquid formulation have limited its use in children. On the contrary, valganciclovir, a valine ester of ganciclovir, has demonstrated a tenfold increase in intestinal absorption, resulting in blood levels comparable to iv ganciclovir. Besides, a liquid presentation has recently been approved, providing a chance to treat small children.

There is still limited experience with the use of oral valganciclovir in children. A prospective multicentre trial enrolled 63 pediatric solid organ transplant recipients at high risk for CMV infection. All of them received treatment with valganciclovir up to 100 days posttransplantation with a 26-week follow-up. The incidence of CMV infection was 11%, mostly after ceasing valganciclovir, and there were no cases of CMV disease. Another important point was that the dosing algorithm used to adjust the body surface and renal function provided a ganciclovir exposure similar to that considered safe and effective in adult transplant recipients. The most frequent valganciclovir-related adverse effects were diarrhea (10%) and neutropenia (5%)[20].

In contrast to antivirals, the role for immunoglobulin preparations used alone or in combination with antiviral drugs to prevent CMV infection is much less defined. Thus, a recent review of all the published trials, which included a large number of solid organ transplant recipients, showed that immunoglobulin did not reduce the risk of CMV disease compared with either no treatment or placebo, and that the combination of CMVIg with antivirals (acyclovir or ganciclovir) had no additional benefits in preventing CMV disease or all-cause mortality compared to the use of antivirals alone[21]. The few trials conducted in children have not shown a significant benefit of immunoglobulins in preventing CMV disease[22,23].

Hybrid strategies represent a promising alternative, combining the advantages of prophylaxis and preemptive treatment. In this context, a recently published retrospective study described the experience with a hybrid prevention strategy in 119 pediatric liver transplant recipients (84 CMV-seronegative), combining a minimum of 14 postoperative days iv ganciclovir followed by CMV viremia monitoring, biweekly for the first three months, monthly for the rest of the first year, and every three months thereafter. Children with detectable CMV DNA restarted iv ganciclovir until they became negative or symptoms ceased in CMV-disease cases. After a median follow-up of 2.3 years, the overall incidence for CMV infection was 34.4% (58% for the D^+/R^- subgroup) with 9.8% for the disease[24].

In conclusion, since most pediatric liver recipients are seronegative for CMV and EBV pretransplantation and so are at high risk for both viral diseases, it seems reasonable to consider prophylaxis the best strategy. Unsettled points, such as the best antiviral regimen and its duration, need to be defined. No synergy between immunoglobulins and antivirals has been demonstrated in CMV prophylaxis. Preemptive strategies seem to be a promising and cost-effective approach, but demand extremely careful infection surveillance, which would probably limit their use in children (number of venipunctures) and, more importantly, there are doubts related to their efficacy in D^+/R^- recipients. Well-designed hybrid strategies may represent a valuable alternative to the currently extended use of prophylaxis.

Finally, another important consideration is that contrary to adult patients, children are at high risk for other herpes virus (i.e. EBV) related disease, so any decision we take in CMV management can have an impact, positive or negative, on these other infections.

Epstein-Barr virus infection

Epstein-Barr virus infection remains one of the main concerns in the postoperative care of children with a liver transplant because of the well-documented capacity of this virus to induce PTLD. This term defines a wide spectrum of diseases, from reactive polyclonal hyperplasia to diffuse lymphoma, characterized by an uncontrolled proliferation of EBV-transformed lymphocytes (B-cells in most cases). The currently reported incidence of PTLD ranges from 5-15% and about 80% of PTLD cases have been reported to develop in the first two years after transplantation. Posttransplant lymphoproliferative disease has an overall mortality rate that has decreased from as high as 60% in old series to around 10-20% in the more recent ones[25-28]. It has been recognized as an independent risk factor for graft loss and death in a large series of liver transplanted children and it is the most frequent tumor in this population (around 50% of all tumors)[29].

Other potentially important consequences of EBV infection are those derived from treating it, such as the risk for graft rejection secondary to the reduction of the immunosuppression treatment.

Briefly, the imbalance between viral infection and the deteriorated EBV-specific T-cell-based host surveillance caused by inappropriate immunosuppression is the main causal mechanism for PTLD. As a result of it, EBV-infected cells are deficiently controlled by the immune response and eventually may proliferate and result in PTLD[30].

Several factors have been associated with an increase in the incidence of PTLD; the most decisive is primary EBV infection. Indeed, severe EBV infection and PTLD occur

10-20 times more frequently in patients who were seronegative at transplantation. Other reported risk factors are intense immunosuppression or CMV disease.

The current standard for diagnosis and monitoring of EBV infection in transplanted children is measurement of EBV DNA in whole blood by real time polymerase chain reaction (RT-PCR).

A majority (60-80%) of pediatric liver recipients is EBV seronegative at transplantation, and more than 75% of them develop a primary infection in the first six months after transplantation. In the case of EBV-seropositive recipients, only 20-30% of them become reinfected. Most of these primary infections or reinfections are asymptomatic and only around 20% present with a great diversity of symptoms, ranging from the unspecific (fever, weight loss, anorexia) to a more specific infectious mononucleosis syndrome or a frank PTLD.

After either asymptomatic infection or the resolution of clinically symptomatic infection or PTLD, some children maintain persistently elevated EBV viral loads for a long time. Among these are a group of high viral load carriers, defined as those that maintain high viremia levels for more than six months and who have been reported to represent 18-41% of the whole population. In this group, two studies respectively reported PTLD rates of 3 and 25% for liver transplant recipients and up to 45% in another study on a group of heart transplanted children. In contrast, these studies did not find cases of PTLD among children whose viremia was either a low positive or negative[31-33].

At the present time, it is not entirely known what the clinical significance of this chronic high-viremia carriage is, but the reported higher incidence of PTLD in this subpopulation converts these patients into the main target for a strict surveillance of PTLD and preemptive treatment. Identifying the ones who are at a real risk of PTLD among these high-load EBV carriers remains a challenge.

Management of Epstein-Barr virus infection in children with a liver transplant

Due to the poor outcome associated with PTLD once it is diagnosed, efforts have been directed to develop preventive strategies. Thus, the goals of management are reducing the incidence of EBV infection, or at least minimizing its consequences, and above all preventing PTLD development while maintaining the graft rejection-free.

The best strategy for PTLD prevention has not yet been established, mainly because its low incidence makes statistically strong trials (randomized or cohort studies) prohibitive in terms of time and cost. Thus, many of the available studies have relied on historical control groups of patients at single centers or include only a small number of patients.

Different sequential approaches, such as pretransplant and posttransplant prophylaxis and preemptive treatment, are considered.

Pretransplant prophylaxis

At this time there is not an option for pretransplant prophylaxis in EBV-seronegative recipients since a vaccine is not yet available. Nevertheless, there are some trials in course (phase I/II) with vaccines based on different lytic (gp350) or latent (EBNA2, EBNA-3C) viral proteins[34,35].

Posttransplant prophylaxis

No published study to date has demonstrated a significant positive influence in preventing EBV infection or PTLD in association with any specific protocol for primary immunosuppression[36,37].

On the contrary, the effect of the overall intensity of immunosuppression on PTLD rates has been demonstrated. Thus, a low-dose immunosuppressive protocol with lower than usual target cyclosporine or tacrolimus blood levels resulted in a significant reduction in the incidence of PTLD[38]. However, it may present excessive risk for rejection beyond its potential benefits. Not every transplanted child has the same risk of PTLD; thus an individual evaluation of the risk/benefit relation before applying low-dose immunosuppression makes sense. On the other hand, it has been also demonstrated that intensive immunosuppression, such as the use of cytotoxic antibodies like OKT3, significantly predisposes to PTLD[39].

Antiviral prophylaxis

The theoretical objective of prophylaxis is that of preventing, or at least reducing, the transmission of replicative viruses from the transplanted organ or blood products to the recipient's B-cells and thus preventing their expansion. However, since we know that most of the recipients will become infected in the first months after transplantation, a more realistic approach would probably be to just modify or delay that primary EBV infection during the first months after liver transplantation when immunosuppression is heaviest.

Antivirals are currently used in many centers as simultaneous prophylaxis for CMV and EBV infection. However, the studies designed to assess the effectiveness of antivirals on EBV-infection prophylaxis have had variable results.

The only published randomized trial that compared the efficacy of a sequential prophylaxis consisting of two weeks of iv ganciclovir followed by 50 weeks of oral acyclovir with two weeks of iv ganciclovir alone in liver transplanted children found similar symptomatic EBV-disease rates in both groups (33% for the combined treatment and 21% for the group treated only with ganciclovir; p = NS), concluding that a long prophylaxis regimen did not represent any advantage in preventing EBV disease[40].

However, several nonrandomized trials suggest that antiviral prophylaxis may play a role in preventing EBV disease or PTLD[41-43]. In a case-control study in a large cohort of children and adults with a kidney transplant, a significant decrease in the incidence of early PTLD (< 1 year after transplant) was found in those patients that had been treated with either ganciclovir or acyclovir compared to those who had not received antiviral prophylaxis, with an up to 82% reduction in the risk of PTLD, depending on the antiviral agent. However, a protective effect for late-onset PTLD was not demonstrated.

A second option for preventing EBV disease and PTLD that has been evaluated is immunoprophylaxis using intravenous EBV-enriched antibody gamma globulin. This option is based on the observation that although cytotoxic T-cells play the main role in controlling EBV infection, some reports have documented an increased risk for PTLD in patients unable to produce antibodies against certain EBV antigens (i.e. EBV nuclear antigen; EBNA)[44]. Two randomized trials comparing the treatment with CMVIg with placebo have been published and both of them showed little benefit from immunoglobulin in preventing PTLD[23,45].

In conclusion, the available data indicate that antiviral prophylaxis does not prevent EBV infection or reinfection and a high proportion of children (60-90%) become infected in the first six months after liver transplantation. But, in the absence of randomized trials to confirm it, it seems that prophylaxis reduces the incidence of early PTLD. At any rate, it prevents a reported risk factor for EBV infection, namely CMV. However, some aspects of prophylaxis, such as the specific antiviral protocol or the prophylaxis duration, remain undefined.

Preemptive treatment

The objective of preemptive treatment is to prevent the development of PTLD once the EBV infection is detected. Considering the fact that a majority of transplanted children will acquire EBV infection, it is fundamental to identify the ones with an increased risk for PTLD and who therefore would be candidates for this type of treatment.

The current practice for EBV-infection surveillance after transplantation in most centers is longitudinal monitoring of viral load in peripheral blood with DNA amplification techniques. This practice is based on the well-documented correlation that exists between a sustained viral load increase plus high EBV DNA levels with an increased risk of PTLD, and that there is usually a variably long gap in time between EBV-viral load increases to high levels and the development PTLD[46-50].

Nowadays, RT-PCR is the standard assay for detection and quantitation of EBV viral load. Current recommendations include frequent monitoring during the first year after a transplant when the patient is at greatest risk (at two-week intervals for the first three months, then monthly until one year) and not so frequent (every 3-4 months) thereafter.

However, the main limitation of this technique is that of a poor positive-predictive value of 50-70% in contrast with its excellent negative-predictive value of 95-100%. This means that an increased viral load is not always predictive of impending PTLD. This lack of specificity has precluded the search for alternative markers that would more accurately evaluate PTLD risk among high viral load carriers. Thus, the detection of a low specific anti-EBV cellular immune response using anti-EBV T lymphocyte quantitation in peripheral blood, combined with the detection of a high EBV viral load, has significantly increased the specificity for predicting PTLD development compared with DNA measurement alone.

The chance to identify patients at risk prior to the appearance of clinical disease has led to the investigation of different preemptive approaches to prevent EBV-related complications, but up until now there is no general consensus regarding the best strategy. Basically, the treatment modalities used by most centers are immunosuppression reduction, antiviral treatment, or a combination of both.

However, the lack of randomized controlled trials that permit a comparison of the effectiveness of the different strategies, as well as the combined use of these in some published studies, makes it difficult to extract conclusions.

There is general agreement on the beneficial effects of reducing immunosuppression in these patients to restore their capacity for an EBV-specific immunologic response that would be effective against both the lytic-phase virus and the latently infected B-cells. However, the risk of developing an acute graft rejection in some high EBV-viremia carriers who are nevertheless not at real high-risk for PTLD is the major limitation of this strategy and must be considered. This

approach requires a close and very careful monitoring of immunosuppressor blood levels and liver function tests for an early diagnosis of a potential graft rejection. Timing of immunosuppression restoration is still not determined and currently it mostly depends on the appearance of the first signs of rejection. Thus, the availability of more accurate tests for monitoring the degree of immunosuppression and improving the criteria for identifying the children at an actual high risk for PTLD beyond the present-day standards (PCR) should improve the safety of this strategy in the future.

The results obtained in a group of 43 liver transplanted children (40% EBV-seronegative at transplant) with a median follow-up of 26 months support this approach. Their immunosuppression was tapered (tacrolimus trough levels 4-6 ng/ml) when high viremia levels (> 4,000 copies/µg DNA) were detected. The PTLD incidence in this group was 2.3%, significantly lower than the historical rate of 16% ($p < 0.05$) and the acute rejection incidence was very low (2.3%)[51].

Antivirals, either alone or associated with tapered immunosuppression, have been considered by some centers as an alternative for PTLD-preemptive treatment. However, the potential efficacy of this strategy is more controversial since antiviral drugs (ganciclovir or valganciclovir) act by means of inhibiting the lytic-EBV replication cycle and have no effect on EBV-infected lymphocytes in the latent state of the infection. Therefore, the efficacy of antivirals in preventing PTLD in asymptomatic children with a high viral load will depend on the role, if any, that lytic viral replication plays in the development of the lymphoproliferative process, or whether this process is only a consequence of the proliferation of the latent virus-infected B-cells. At present, the concrete mechanism that triggers PTLD is not completely defined, but some data from different studies could support the intervention of viral lytic replication[52,53]. Thus, RNA transcripts of genes involved in EBV-lytic replication were identified in peripheral blood lymphocytes of liver transplanted children, all of them high-viral load carriers that ultimately developed PTLD, as if latent EBV enters a replicative cycle with B-cell cellular division.

Several non-controlled studies support the use of different antiviral-based protocols as preemptive treatment for EBV-related PTLD. Thus, in one of these studies, a combined treatment with iv ganciclovir and reduction of immunosuppression in children with liver transplantation and a rising viral load detected by prospective PCR monitoring resulted in a drop in the incidence of PTLD from a historical 10% down to 5%[54].

Another study, in this case in children with an intestinal transplant, showed a reduction in PTLD incidence from 46 to 23% after a combined preemptive treatment with iv ganciclovir and CMVIg, without modifying the immunosuppression[55].

The recently available oral valganciclovir allows long-term treatment, without a need of hospitalization, for small children. A few studies have recently been published using valganciclovir as preemptive treatment of EBV-induced PTLD in children. A retrospective study on a continued treatment (7.2 ± 3.8 months) with valganciclovir (520 mg/m^2 twice daily), without modification of immunosuppression in liver transplanted children with a chronic EBV infection (72% asymptomatic) and EBV DNA detected by qualitative PCR and a follow-up 12 months after therapy was started, reported an overall incidence of PTLD of 2.3% opposed to a historical rate of 5.1%. The treatment was well-tolerated, with no severe effects attributable to valganciclovir, and neutropenia was the most frequent adverse effect (12%)[56].

More recently, a 30-day preemptive treatment with valganciclovir in pediatric liver recipients with a PCR-detected high EBV DNA resulted in a completely negative EBV DNA in 34% of the children, a reduction of at least 50% of the value in 41% of the children, and no change in the remaining 23%. In a non-treated historical group, the results were 6, 25, and 68%, respectively (p = 0.01). Other interesting findings of this preliminary study were that EBV viral load was directly related with valganciclovir blood levels, and that most children did not achieve the recommended levels and needed an increase in their valganciclovir dose. Thus, the authors recommended monitoring valganciclovir blood levels in order to optimize treatment[57].

The current protocol in our centre for preventing PTLD after liver transplantation in children consists in a six-month antiviral prophylaxis (one month iv ganciclovir and five months valganciclovir) with EBV DNA monitoring by RT-PCR (monthly for the first three months after transplant and every three months thereafter) and restart of valganciclovir upon detection of EBV DNA, combined with a reduction in immunosuppression in those cases with detected high viral load (> 16,000 copies/ml). A group of 25 consecutive first-liver graft recipients (64% were EBV-seronegative at transplantation) were prospectively followed-up for one year. Most children (92%) became EBV-infected in the first year after transplantation, and 61% showed high EBV DNA values. Only one patient presented EBV-related symptoms and there were no cases of PTLD (non-published data).

Finally, new treatment modalities like adoptive immunotherapy with autologous EBV-specific cytotoxic T lymphocytes or anti-CD20 antibody treatment offer certain future possibilities, based on the promising results of some published trials[58-60]. However, it is reasonable to think that the technical difficulty and/or elevated cost and/or potential secondary effects of these new treatments will necessitate a marked improvement in our capacity to better identify the patients at actual high risk for PTLD.

In conclusion, reduction of immunosuppression is considered the mainstay in the management of liver transplanted children with EBV infection and a high viral load. Antivirals may also play a role in controlling EBV viremia and thus preventing PTLD and its efficacy should be confirmed in future controlled trials on large series of patients so that different treatment options can be compared.

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