Risk-adapted Treatment for Severe B-Lineage Posttransplant Lymphoproliferative Disease After Solid Organ Transplantation in Children

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Background. Optimal management of posttransplant lymphoproliferative disease (PTLD) remains to be defined due to heterogeneity of this condition and lack of predictors of the outcome. Here we report our experience with pediatric PTLD nonresponsive to immunosuppression (IS) withdrawal, managed after stratification into high and low risk according to the presenting features.

Methods. This is a single-center retrospective review of prospectively enrolled patients. From 2001 to 2011, 17 children were diagnosed with severe B-lineage, CD20+, PTLD after a median of 37 months (range, 5–93) from liver (12), heart (4), or multorgan (1) transplantation. Treatment was tailored on 2 risk groups: (1) standard-risk (SR) patients received IS reduction and rituximab; (2) high-risk (HR) patients received IS discontinuation, rituximab and polychemotherapy. Results. The cumulative incidence of rejection at 1 and 5 years after the diagnosis of PTLD was 35% (95% confidence interval [95% CI], 18-69%) and 53% (33-85%), respectively, whereas the disease-free survival at 1 and 5 years was 94% (95% CI, 65-99%) and 75% (45-90%), respectively. Three children died, PTLD-free, from different transplant-related complications: primary nonfunction after retransplantation (liver), cytomegalovirus disease 21 months after PTLD treatment (liver), graft dysfunction 25 months after PTLD (heart). Conclusions. Severe B-lineage PTLD after solid organ transplantation may be classified as SR or HR and treated accordingly with a tailored protocol obtaining a satisfactory long-term outcome. This approach accomplishes the control of lymphoproliferation in severe forms as well as the minimization of toxicity in milder PTLDs.

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Over the past 2 decades posttransplant lymphoproliferative disease (PTLD) has emerged as an important cause of morbidity and mortality after pediatric solid organ transplantation. In fact the term PTLD comprises of a wide spectrum of atypical lymphoid proliferations, ranging from benign lymphoid hyperplasia to malignant lymphomas. In most cases, PTLD is of B-cell origin (only 10% of cases being of T-cell immunophenotype), and Epstein Barr virus (EBV)–driven. The incidence depends on the type of transplanted organ and intensity of immunosuppression (IS), varying from 5% to 20% in different series.

Early PTLD, occurring within 12 months from transplantation, usually shows a histological pattern of early lesions, according to World Health Organization classification and is characterized by slowly progressing, localized disease, polyclonal B-cell growth, EBV-DNA integration and a good response to modulation of IS. On the contrary, late PTLD, occurring more than 1 year after transplantation, frequently presents as disseminated, often monoclonal-monomorphic disease, sometimes EBV-negative, with an aggressive clinical course and absence of response to the sole reduction of IS.

Unfavorable prognostic factors include multorgan (>2 sites), central nervous system (CNS) or bone marrow involvement, organ dysfunction, late onset, EBV negativity, poor performance status (PS), T-cell origin, and elevated lactate dehydrogenase (LDH). Monoclonality is another unfavorable prognostic factor, although even polyclonal polymorphic disease can behave as a frank lymphoma.

Therapeutic options include surgical resection, radiation, immunotherapy and chemotherapy, and recently guidelines for treatment of PTLD in adults have been published. The monoclonal anti-CD20 antibody (rituximab), characterized by a remarkable safety profile and high specificity, is...
generally recommended for PTLD of B-cell lineage.\textsuperscript{29} However, when used as a single agent in the treatment of severe PTLD, rituximab may offer an inadequate response, frequently incomplete, and of short duration\textsuperscript{30,31}; thus surgery or radiotherapy in localized disease, and chemotherapy for diffuse or progressive PTLD, should be combined to improve long-term outcome.\textsuperscript{32-39} A major concern related to the use of conventional chemotherapy is the relevant morbidity and mortality (up to 50%), secondary to severe myelosuppression and infectious complications.\textsuperscript{24,35} In an attempt to reduce cytotoxic side effects, various low-intensity chemotherapy combinations have been used, but this approach may be associated with unsatisfactory long-term results.\textsuperscript{40,41}

The multifaceted presentation of PTLD makes it difficult to discriminate mild from severe cases, with both the risks to overreact on cases that can be managed without polychemotherapy, or undertreat more aggressive cases.

The aim of this study was to evaluate the efficacy of a tailored therapeutic approach in the treatment of pediatric severe PTLDs, defining a clear-cut between forms of different severity.

**PATIENTS AND METHODS**

From October 2001 to January 2011, 492 paediatric transplants (150 heart, 332 liver, 10 intestinal/multivisceral) were carried out at our institution. During the same period, a novel approach to pediatric PTLD has been introduced.

We performed a retrospective search in the pediatric transplant and pathology database of our center to find patients with a diagnosis of PTLD in the study period. The pertinent data were retrospectively collected from the patients’ clinical records and 60 patients with a diagnosis of PTLD were identified. Forty-three patients presented with a benign form of PTLD (localized early lesions/polymorphic PTLD), responded to IS modulation and were excluded from this study. The remaining 17 were classified as severe forms of PTLD according to the criteria reported below and were treated with an antilymphoma program. Our institutional review board approves data collection and evaluation for clinical studies, provided the final data are anonymized.

The PTLD diagnosis and classification was based on histological, immunophenotypical, and molecular analysis of nodal and extranodal lesions, obtained by open biopsy or core needle aspiration, according to the official recommendations.\textsuperscript{42} Conventional staging for pediatric non-Hodgkin lymphoma (NHL)\textsuperscript{43} included bone marrow biopsy and computed tomography scan or MRI of the chest, abdomen, and pelvis. To assess clonality, rearrangements of heavy-chain immunoglobulin or T-cell receptor genes were analyzed by polymerase chain reaction. Performance status was defined as good if less than 2, poor if 2 or greater, according to the Eastern Cooperative Oncology Group.\textsuperscript{44} We analyzed our patients according to the age-adjusted International Prognostic Index (aaIPI) (Table 1),\textsuperscript{17,18,25,34,46,47} as well as to the presence of at least one of the adverse prognostic factors identified for adult PTLD (involvement of more than one organ; involvement of the bone marrow; organ dysfunction; poor PS; monomorphic histology; benign PTLD not responding to 3 weeks of IS withdrawal).\textsuperscript{17,18,25,34,46,47}

High-risk (HR) PTLD was established in presence of at least 2 of the following features:

**TABLE 1. Clinical features at diagnosis of 17 patients with PTLD**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: (range), mo</td>
<td>51 (9-206)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
</tr>
<tr>
<td>Type of transplant</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>4</td>
</tr>
<tr>
<td>Liver</td>
<td>12</td>
</tr>
<tr>
<td>Multigran</td>
<td>1</td>
</tr>
<tr>
<td>Time from transplant to PTLD, mo</td>
<td></td>
</tr>
<tr>
<td>≤ 12</td>
<td>5</td>
</tr>
<tr>
<td>13-24</td>
<td>3</td>
</tr>
<tr>
<td>25-59</td>
<td>6</td>
</tr>
<tr>
<td>≥ 60</td>
<td>3</td>
</tr>
<tr>
<td>Immunosuppression (blood level, ng/mL)</td>
<td></td>
</tr>
<tr>
<td>Cyclosporin-A</td>
<td>4</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>13</td>
</tr>
<tr>
<td>Principal localization</td>
<td></td>
</tr>
<tr>
<td>Abdomen</td>
<td>8</td>
</tr>
<tr>
<td>Lymph node</td>
<td>2</td>
</tr>
<tr>
<td>Spleen and/or liver</td>
<td>3</td>
</tr>
<tr>
<td>Lung</td>
<td>2</td>
</tr>
<tr>
<td>Esophagus</td>
<td>1</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>1</td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
</tr>
<tr>
<td>Early lesions</td>
<td>1</td>
</tr>
<tr>
<td>Polymorphic CD20+ B-lineage PTLD</td>
<td>6</td>
</tr>
<tr>
<td>Burkitt or Burkitt-like lymphoma</td>
<td>5</td>
</tr>
<tr>
<td>Diffuse large B-cell lymphoma</td>
<td>5</td>
</tr>
<tr>
<td>Clonality</td>
<td></td>
</tr>
<tr>
<td>Polyclonal</td>
<td>6</td>
</tr>
<tr>
<td>Mononclonal</td>
<td>11</td>
</tr>
<tr>
<td>EBV detection</td>
<td></td>
</tr>
<tr>
<td>Genome on biopsy only</td>
<td>4</td>
</tr>
<tr>
<td>PB viral load &gt;500 Ge/10^5 MN only</td>
<td>3</td>
</tr>
<tr>
<td>EBV Genome on biopsy and PB viral load &gt;500 Ge/10^5 MN</td>
<td>10</td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
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<tr>
<td>III</td>
<td>12</td>
</tr>
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<td>IV</td>
<td>1</td>
</tr>
<tr>
<td>Bulky presentation</td>
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<tr>
<td>ECOG performance status score</td>
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<td>&lt; 2</td>
<td>6</td>
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<tr>
<td>≥ 2</td>
<td>11</td>
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<tr>
<td>LDH (IU/L)</td>
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<td>500-1000</td>
<td>9</td>
</tr>
<tr>
<td>&gt;1000</td>
<td>6</td>
</tr>
<tr>
<td>aa-IPI</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>2-3</td>
<td>10</td>
</tr>
</tbody>
</table>

PB viral load, EBV viral load in the peripheral blood detected by real-time PCR analysis; Ge/10^5 MN, copies/10^5 mononucleated cells; EBV genome, EBV LMP-1 (EBV antigens latent membrane protein type 1) in the neoplastic tissue. ECOG, Eastern Cooperative Oncology Group; aa-IPI, age-adjusted International Prognostic Index.

ECOG indicates Eastern Cooperative Oncology Group; aa-IPI, age-adjusted International Prognostic Index.
– stage III or IV;
– monomorphic histology;
– poor PS;
– LDH ≥ 2 times the upper normal level for age (or ≥1000 IU/L);

The remainders were classified as standard-risk (SR) PTLD.

**Treatment According to Stratification**

The treatment was tailored on the 2 risk groups described above.

In SR patients, initial therapy included rituximab and IS reduction associated with surgical resection in case of fully resectable masses.

The HR patients received rituximab and polychemotherapy after IS discontinuation. Polychemotherapy included blocks of fludarabine, cyclophosphamide, doxorubicin, and rituximab (FCD-R), and reduced intensity Berlin-Frankfurt-Münster (BFM) blocks for a maximum of 6 blocks. The number of blocks given depended on disease stage, resectability and serum LDH, according to BFM strategy for B-NHL; Rituximab (375 mg/m²/dose intravenously) was given for a total of 4 doses at the beginning of each of the first 4 blocks. The CNS prophylaxis with intrathecal methotrexate, methylprednisolone and cytarabine (ITT) was given from the third block onward.

**Response Assessment**

Response to treatment was assessed by ultrasound examination after each cytotoxic cycle, and computed tomography scan or MRI after the first 2 cycles of chemotherapy and/or at end of treatment. Definition of response was based on Cheson’s criteria. Complete remission (CR) and partial remission were defined, respectively, as absence of the disease or reduction by 50% or more in the number and/or in the size of measurable masses. Absence of response or disease progression was defined, respectively, as a reduction by less than 50% or an increase in lesion size. Relapse was defined as reappearance of PTLD after obtaining CR.

**Statistical Analysis**

The cumulative incidence of rejection was measured from the time of PTLD diagnosis to the date of graft rejection or death or last follow-up in a competing risk setting. Death was considered a competing event. The disease-free survival was calculated from the date of CR to date of relapse or death in CR, whatever the cause, or last follow-up. The overall survival (OS) was calculated from the date of diagnosis to the date of death, whatever the cause, or last follow-up.

Survival probabilities were calculated using the life table method, and survival curves were estimated by the method of Kaplan-Meier. The binomial exact 95% confidence interval (95% CI) was calculated for percentages.

Statistical analysis was conducted using the STATA/SE 8.2 (Stata Corporation, 4905 Lakeway Drive, Collage Station, TX) and R (version 3.1.2) software packages.

**RESULTS**

Sixty patients with a diagnosis of PTLD were found in our pediatric transplant database. Seventeen patients (7 male and 10 female), of a median age of 51 months (range, 9-206), were categorized as severe PTLD and represent the core group of this study. Eleven patients had the criteria for severe PTLD at presentation, whereas 6 were initially defined as benign, but then did not respond to IS weaning. These latter patients were moved to the severe PTLD group and they were classified as standard risk PTLD. Figure 1 shows the chart
representation of the patients’ selection. Among them, 6 were classified as SR, whereas 11 were classified as HR. The clinical history and outcome of 4 cases (3 with diffuse large B-cell lymphoma and 1 with disseminated polymorphic B-PTLD) have been already reported.38 Features at onset of the disease are shown in Table 1. Median time from transplantation to PTLD occurrence was 37 months (range, 5-93), with 5 cases having an early onset (within 12 months from transplantation). All patients were under IS with a calcineurin inhibitor (CNI), and namely, Tacrolimus (n = 13) or Cyclosporin (n = 4) with a median level of 5.5 ng/mL (range, 3-14.4) and 117.5 ng/mL (30-151), respectively. Fifty percent of the patients had experienced acute graft rejection in the early postoperative period. According to the World Health Organization classification, the distribution of PTLD was as follows: (a) early lesions (n = 1); (b) polymorphic (n = 6); (c) CD20+ B-cell (NHL) (n = 10), including Burkitt/Burkitt-like lymphoma (BL) (n = 5) and diffuse large B-cell lymphoma (DLBCL) (n = 5). One patient presenting with a histology compatible with early lesions PTLD was included into HR severe PTLD group because the disease was characterized by a multisystemic involvement with organ dysfunction and poor PS. Stage of disease was I, II, III, and IV in 2, 2, 12, and 1 cases, respectively. Eleven children had a poor PS. The LDH serum concentration greater than 500 IU/L was observed in 15 cases (>2 normal value in 6 of them). Ten of 17 patients proved to be high risk according to aalIPI (aalIPI = 2-3) as well as our stratification adopted for severe PTLD; among the remaining patients, 1 lymphoma patient with a low aalIPI score was also treated with our immune-chemotherapy program. Thirty cases were associated with serological evidence of high EBV replication (median, 13191; range, 536-50000 Ge/105 MN) and/or histologically proven EBV positivity (EBER) in 14 of them. Initially, all patients underwent a reduction or discontinuation of CNIs. Additional treatment was given according to the institutional program based on stratification described above (Table 2).

SR Patients (n = 6)
The IS levels detected at diagnosis were reduced weekly by 50% in liver and 25% in heart or multiorgan recipients; shortly thereafter rituximab was administered, as single agent (n = 5) or in combination with surgery (in 1 resectable tumor). Four patients obtained CR with the first course of rituximab. Four additional doses of rituximab, at 3 weeks interval, were also given to 1 patient (n = 4) to obtain CR. In 2 patients (1 liver and 1 heart), who presented a partial response, a mechanistic target of rapamycin inhibitor was added to low dosage of Tacrolimus.

Patient 2, with a PTLD localized to the liver, developed liver acute cellular rejection 7 days after the second dose of rituximab; tacrolimus at full dosage plus intravenous pulse steroids were administered with limited benefit. Three months later, the patient underwent a second liver transplantation for chronic rejection and portal vein thrombosis after the reduction of IS, but died of graft primary nonfunction.

No relevant toxicity after the PTLD treatment was observed. A graft (2 liver, 1 bowel) cellular rejection was observed in 3 more cases, respectively, 5, 24 and 25 months from the diagnosis of PTLD, and was successfully treated by intensified IS.

HR Patients (n = 11)
The HR patients underwent a rapid discontinuation of IS through immediate IS withdrawal; thereafter, rituximab was given in combination with chemotherapy as described below. Induction treatment for 3 cases with DLBCL and 1 case of disseminated CD20+ polymorphic-PTLD consisted of 2 or 3 blocks of FCD-R, followed by reduced intensity BFM blocks for a total of 6 blocks. The remaining 7 patients (5 BL, 1 DLBCL and 1 diffuse early lesions PTLD) received reduced intensity BFM blocks only (Tab. 2). Rituximab was given for a total of 4 doses at the beginning of each of the first 4 blocks (the last 2 doses were administered after chemotherapy in the patient treated with 2 blocks only). The CNS prophylaxis with TIT was given from the first block in the first patient with BL (pt 7) but a subsequent severe CNS toxicity was observed; thereafter, in the following patients, to reduce CNS toxicity, TIT was administered from the third block onward. Complete remission was achieved after the first, the second, and the third block in 3, 7, and 1 cases, respectively.

Toxicity in HR
Hematological toxicity was the most common side effect observed in HR group. After FCD-R blocks, however, cytopenia was mild and/or of short duration (grade 4 neutropenia for 3-7 days; only grade 2 thrombocytopenia). A profound and prolonged myelotoxicity was associated instead with BFM-like blocks with grade 4 neutropenia and thrombocytopenia after each block in 9 patients. The interval between BFM-like blocks (at reduced intensity) was always longer than planned, with a median of 20 days rest (range, 15-41). Two children experienced a bacterial or fungal pneumonia, promptly controlled by antimicrobial therapy. Only the patient who received TIT from the beginning (patient 7, with stage IV BL) experienced relevant nonhematological toxicity with a transient acute renal failure and neurological impairment. No patients, including heart transplant recipients, presented cardiac toxicity (maximal cumulative doses of anthracyclines: 140 mg/m²).

Graft Rejection in HR
Antirejection therapy was restarted cautiously. In the first 2 liver recipients, IS was started only when clinical and histological signs of graft rejection were documented, respectively, at 4 and 9 months after discontinuation of chemotherapy. In the remaining 7 liver recipients, tacrolimus-based IS was restarted within 3 months after the end of chemotherapy, and only in one of them liver rejection occurred, 8 months later. In the 2 heart transplant recipients who stopped IS, cyclosporin was initially restored at very low dosage (plasma trough levels under 50 ng/mL) 1 month after ending chemotherapy, when CD3 + CD4+ T-lymphocyte count was greater than 150/mm³. Both of them experienced an acute heart rejection at 3 months from chemotherapy. In all cases, however, heart rejection was successfully treated by intensifying IS.

Survival
The overall follow up period of survivors is a median of 52 months (range, 31-140), whereas the median follow-up of all patients is 44 months (range, 4-8-140).

Cumulative incidence of rejection at 1 and 5 year after diagnosis of PTLD was respectively 35% (95% CI, 18%-69%)
## TABLE 2.
Clinical features, treatment and outcome of patients with aggressive PTLD

<table>
<thead>
<tr>
<th>Pt</th>
<th>sex/age at diagnosis, mo</th>
<th>Organ transplanted</th>
<th>Histology</th>
<th>Stage</th>
<th>Sites</th>
<th>LDH</th>
<th>PS</th>
<th>Risk</th>
<th>Therapy (Type/No. cycles)</th>
<th>Major complications</th>
<th>Response</th>
<th>Graft rejection/moons from PTLD diagnosis</th>
<th>Outcome/FU, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M/9</td>
<td>Liver</td>
<td>Monoclonal CD20 + DLBCL, EBV-LMP1+</td>
<td>IIB</td>
<td>Jejunum/ Mesenteric-LNs</td>
<td>730</td>
<td>&lt;2</td>
<td>SR</td>
<td>CNI weaning surgery</td>
<td>—</td>
<td>CR</td>
<td>yes/25</td>
<td>AW/140+</td>
</tr>
<tr>
<td>2</td>
<td>F/37</td>
<td>Liver</td>
<td>Polyclonal Polymorphic CD20 + PTLD, EBV-LMP1+</td>
<td>I</td>
<td>Liver</td>
<td>700</td>
<td>&lt;2</td>
<td>SR</td>
<td>CNI weaning</td>
<td>Raised transaminases/ Pneumocystis J. pneumonia</td>
<td>CR</td>
<td>Yes/0.5</td>
<td>AW/69+</td>
</tr>
<tr>
<td>3</td>
<td>M/14</td>
<td>Liver</td>
<td>Polyclonal Polymorphic CD20 + PTLD, EBV-LMP1+</td>
<td>I</td>
<td>Esophagus</td>
<td>543</td>
<td>&lt;2</td>
<td>SR</td>
<td>CNI weaning Plus m-TORi</td>
<td>—</td>
<td>CR</td>
<td>Yes/24</td>
<td>AW/60+</td>
</tr>
<tr>
<td>4</td>
<td>F/83</td>
<td>Heart</td>
<td>Monoclonal polymorphic CD20 + PTLD, EBV-LMP1+</td>
<td>III</td>
<td>Liver/lung</td>
<td>500</td>
<td>&lt;2</td>
<td>SR</td>
<td>CNI weaning</td>
<td>—</td>
<td>CR</td>
<td>No</td>
<td>AW/59+</td>
</tr>
<tr>
<td>5</td>
<td>F/18</td>
<td>Heart</td>
<td>Polyclonal Polymorphic CD20 + PTLD, EBV-LMP1+</td>
<td>IIB</td>
<td>Small bowel</td>
<td>658</td>
<td>&lt;2</td>
<td>SR</td>
<td>CNI weaning</td>
<td>—</td>
<td>CR</td>
<td>No</td>
<td>AW/35+</td>
</tr>
<tr>
<td>6</td>
<td>F/44</td>
<td>Multiorgan</td>
<td>Polyclonal Polymorphic CD20 + PTLD, EBV-LMP1+</td>
<td>IIB</td>
<td>Small bowel/Celiac-LNs</td>
<td>694</td>
<td>&lt;2</td>
<td>SR</td>
<td>CNI weaning</td>
<td>Sepsis</td>
<td>CR</td>
<td>Yes/5</td>
<td>AW/34+</td>
</tr>
<tr>
<td>7</td>
<td>M/32</td>
<td>Liver</td>
<td>Monoclonal Burkitt LNH, EBV-LMP1+</td>
<td>IV</td>
<td>Liver, Bone Marrow</td>
<td>3250</td>
<td>&gt;2</td>
<td>HR</td>
<td>Stop CNI prephase</td>
<td>Acute renal/neurologic impairment</td>
<td>CR</td>
<td>Yes/8</td>
<td>AW/121+</td>
</tr>
<tr>
<td>8</td>
<td>F/51</td>
<td>Liver</td>
<td>Monoclonal CD20 + DLBCL, EBV-LMP1+</td>
<td>III</td>
<td>Abdomen (bulky), kidney, peritoneal/pleural effusion</td>
<td>3596</td>
<td>&gt;2</td>
<td>HR</td>
<td>Stop CNI prephase</td>
<td>—</td>
<td>CR</td>
<td>yes/14</td>
<td>AW/136+</td>
</tr>
<tr>
<td>9</td>
<td>F/53</td>
<td>Liver</td>
<td>Monoclonal CD20 + DLBCL, EBV-LMP1+</td>
<td>IIB</td>
<td>Ileocecal-tract (bulky)</td>
<td>467</td>
<td>&gt;2</td>
<td>HR</td>
<td>Stop CNI prephase</td>
<td>—</td>
<td>CR</td>
<td>No</td>
<td>AW/99+</td>
</tr>
<tr>
<td>10</td>
<td>M/106</td>
<td>Heart</td>
<td>Monoclonal CD20 + DLBCL, EBV-LMP1+</td>
<td>IIB</td>
<td>Multiple LNs, spleen</td>
<td>663</td>
<td>&gt;2</td>
<td>HR</td>
<td>Stop CNI prephase</td>
<td>Pneumonia</td>
<td>CR</td>
<td>Yes/7</td>
<td>AW/112+</td>
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<tr>
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<td>M/70</td>
<td>Heart</td>
<td>Polyclonal polymorphic CD20 + PTLD, EBV-LMP1+</td>
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<td>Lung, pleura, paranasal-sinuses, multiple LNs, liver</td>
<td>620</td>
<td>&gt;2</td>
<td>HR</td>
<td>Stop CNI prephase</td>
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<td>CR</td>
<td>Yes/7</td>
<td>DEAD/CR25</td>
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<tr>
<td>Pt \ sex/age at diagnosis, mo</td>
<td>Organ transplanted</td>
<td>Histology</td>
<td>Stage</td>
<td>Sites</td>
<td>LDH</td>
<td>PS</td>
<td>Risk</td>
<td>Therapy (Type/No. cycles)</td>
<td>Major complications</td>
<td>Response</td>
<td>Outcome/ FU, mo</td>
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| 12 M/206                    | Liver               | Monoclonal Burkitt-like LNH EBV-LMP1+ | IIIB   | Abdomen (bulky)/ multiple LNs/Liver | 1191 | >2 | HR   | Stop CNI prephase
  - Block AA\(^a\) \(\times 2\)
  - Block BB\(^a\) \(\times 1\)
  - Block CC\(^a\) \(\times 1\) | Pneumonia          | CR       | No   | 10\(\wedge\)DEAD, CR/21 |
| 13 F/59                     | Liver               | Monoclonal Burkitt LNH EBV-LMP1+       | III    | Abdomen (Bulky) | 1088 | >2 | HR   | Stop CNI prephase
  - Block AA\(^a\) \(\times 2\)
  - Block BB\(^a\) \(\times 2\)
  - Block CC\(^a\) \(\times 2\) | —                | CR       | No   | AW/44+ |
| 14 F/16                     | Liver               | Monoclonal CD20 + DLBCL EBV-LMP1+      | IIIB   | Abdomen, Liver/Spleen | 1595 | >2 | HR   | Stop CNI prephase
  - Block AA\(^a\) \(\times 2\)
  - Block BB\(^a\) \(\times 2\)
  - Block CC\(^a\) \(\times 2\) | —                | CR       | no   | AW/44+ |
| 15 M/108                    | Liver               | Monoclonal Burkitt-like LNH EBV-LMP1+  | III    | Abdomen (Bulky) | 2825 | >2 | HR   | Stop CNI prephase
  - Block AA\(^a\) \(\times 2\)
  - Block BB\(^a\) \(\times 2\)
  - Block CC\(^a\) \(\times 2\) | —                | CR       | no   | AW/42+ |
| 16 F/60                     | Liver               | Polyclonal CD20+ Early Lesions EBV-LMP1+ | IIIB   | Multiple LNs, Liver, Small Bowel | 830  | >2 | HR   | Stop CNI prephase
  - Block A\(^b\) \(\times 1\)
  - Block B\(^b\) \(\times 1\)
  - Rituximab \((\times 4)\) | —                | II CR    | yes/9 | AW/46+ |
| 17 F/49                     | Liver               | Monoclonal Burkitt LNH EBV-LMP1+       | IIIB   | Abdomen (Bulky), Kidney, Mediastinum | 572  | >2 | HR   | Stop CNI prephase
  - block A\(^c\) \(\times 2\)
  - block B\(^c\) \(\times 2\)
  - block CC\(^c\) \(\times 1\) | —                | CR       | no   | AW/31+ |

\(^a\) Death due to complication of second liver transplantation.
\(^b\) Rituximab was administered in the first 4 cycles of therapy.
\(^c\) Block AA: high dose methotrexate (HDMTX; from 1.5 to 3 g/m\(^2\)); vincristine (VCR; 1.5 mg/m\(^2\)); cytarabine (from 120 to 150 mg/m\(^2\) q 12 hours \(\times 4\)); ifosfamide (from 600 to 800 mg/m\(^2\) per day \(\times 5\)); VP-16 (from 80 to 100 mg/m\(^2\) per day \(\times 2\)); DXM (10 mg/m\(^2\) per day \(\times 5\)); Intrathecal MTX-cytarabine-methylprednisolone (TIT).
\(^d\) Block BB: HDMTX (from 1.5 to 3 g/m\(^2\)); VCR (1.5 mg/m\(^2\)); daunomycin (from 20 to 25 mg/m\(^2\) per day \(\times 2\)); cyclophosphamide (from 160 to 200 mg/m\(^2\) per day \(\times 5\)); DXM (10 mg/m\(^2\) per day \(\times 5\)); TIT.
\(^e\) Block CC: Vindesine (3 mg/m\(^2\)); ARA-C (3000 mg/m\(^2\) q 12 hours \(\times 4\)); VP-16 (100 mg/m\(^2\) q 12 hours \(\times 4\)); DXM (20 mg/m\(^2\) per day \(\times 5\)); TIT.
\(^f\) FCD-R: fludarabine (30 mg/m\(^2\) per day \(\times 3\)); cyclophosphamide (150 mg/m\(^2\)); doxorubicin (30 mg/m\(^2\)); rituximab (375 mg/m\(^2\)).
\(^g\) Death due to heart chronic rejection.
\(^h\) Death due to invasive aspergillosis.

DLBCL, diffuse large B-cell lymphoma; EBV-LMP-1, EBV antigens latent membrane protein type 1; B, B symptoms (fever, night sweats, or weight loss); bulky, any mass having a maximum diameter \(\geq 10\) cm in its largest dimension; LN, lymph node; m-TORi, mechanistic target of rapamycin inhibitors; prephase, dexamethasone (DXM; 5 mg/m\(^2\) per day \(\times 5\)) alone or in combination with cyclophosphamide (200 mg/m\(^2\) per day \(\times 2\)); FU, follow up; AW, alive well; M, male; F, female.
and 53% (95% CI, 33%-85%) (Figure 2A), whereas the disease-free survival at 1 and 5 years was, respectively, 94% (95% CI, 0.65-0.99) and 75% (95% CI, 0.45-0.90) (17 patients). DFS indicates disease-free survival.

Three children died, PTLD-free, from different transplant-related complications. One SR patient died of graft primary non function after a second liver transplantation for chronic rejection and portal vein thrombosis. Two HR patients died, PTLD-free, at 21 and 25 months from PTLD diagnosis, respectively. The first one was a 16-year-old patient who had a liver transplant because of hepatopulmonary syndrome complicating hepatoportal sclerosis; 8 years after transplant and 18 months after PTLD treatment, he developed cytomegalovirus infection unresponsive to antiviral treatment; he received third-party cytomegalovirus-specific cytotoxic lymphocytes infusion but then had cytomegalovirus pneumonia and a superimposed invasive aspergillosis from which he died 3 months after presentation (patient 12). The second one was a boy who at 2 years of age underwent heart transplantation because of viral myocarditis; 5 years later and 12 months after PTLD diagnosis, he developed a severe myocardial fibrosis of unknown origin but without any signs of acute rejection. Two years later, he died of a heart failure (autopsy was not performed) (patient 11).

One patient relapsed 37 months after ICR, obtained with the first-line immune-chemotherapy. She was successfully treated with a second course of immune-chemotherapy and was alive, PTLD-free 8 months after the recurrence of the disease.

Overall, 13 of 17 children are alive, in first CR and with normal graft function with a median follow-up of 52 months (range, 31-140). The OS at 1 and 5 years after diagnosis of PTLD is, respectively, 94% (95% CI, 0.65-0.99), and 82% (95% CI, 0.55-0.94) with a median follow-up of 44 months (4.8-140) (Figure 3).

**DISCUSSION**

Although modulation of IS is considered the gold standard treatment of early, usually polyclonal PTLDs, the optimal management of severe PTLDs remains to be defined, taking into account the pathological and clinical heterogeneity of the disease. Several therapeutic strategies have been explored, and historically, stepwise approaches have been suggested. In this context, it is crucial to identify prognostic factors to define which patients will respond to rituximab alone and which patients might benefit from low-dose or conventional chemotherapy ± rituximab.

Recently, Gross et al reported a prospective trial on the largest cohort of pediatric refractory B-lineage PTLD. This study demonstrated that the combination of rituximab to low-dose chemotherapy was safe and effective to control lymphoproliferation in a group of patients with a wide range of disease severity, including milder (ie, localized, polymorphic PTLD) and more severe forms (clinical stage III/IV, monomorphic histology or fulminant disease); the 2-year event free and OS was 71% and 83%, respectively. In another relevant prospective multicenter trial carried out in adults, Trappe et al showed that a sequential treatment with Rituximab followed by CHOP achieved a 5-year progression-free and OS of 50% and 55%, respectively. Our results, showing a 5-year disease-free and OS, respectively, of 75% and 82%, can be considered comparable to the aforementioned studies.

In our study, the treatment was more tailored to severity of PTLD because we stratified the severe forms into 2 further risk groups to avoid overtreatment in milder as well as undertreatment in more aggressive forms. Because this is a single-center study, the population may have been selected
and managed more homogeneously. In our patients, the introduction of a risk-adapted therapy was effective in controlling lymphoproliferation. All patients rapidly achieved CR with the first-line therapy, only one of them relapsed and none died due to PTLD. The SR patients were successfully treated with minimal therapy, that is, early use of rituximab associated to IS reduction, and surgery in 1 case of resectable mass, as previously reported. Conversely, HR patients responded well to a more aggressive intervention, such as a combination of rituximab/dose-adjusted chemotherapy and rapid IS discontinuation, without major side effects. As already reported, the use of 2 FCD-R blocks as initial treatment was safe and effective in polymorphic PTLD and in non-BL. Burkitt-like lymphomas received a BFM-based program since the beginning of treatment; this strategy was successful in all cases, but in our experience, it was more toxic than FCD-R regimens, even in the context of the treatment intensity reduction described above. Neurotoxicity was seen only in the patient treated with TIT from the beginning; delaying intrathecal chemotherapy and discontinuing CNIs may have been beneficial here. Thus, our experience supports the hypothesis that the marked toxicity reported in the past might have been because of the concomitant administration of IS and chemotherapy, particularly in heart and lung transplant recipients. As reported previously, unfavorable histology is not sufficient to reliably predict the course of the disease; in fact, some cases of disseminated polyclonal early lesions and polymorphic PTLD present with a poor clinical course, similar to a monoclonal monomorphic disease. Thus, at least 2 of the 4 adverse prognostic factors listed above are needed to classify PTLD as high risk.

We suggest that in SR patients, initial therapy should include rituximab and IS reduction with surgical resection, in case of fully resectable masses. For patients achieving only a partial remission after the first course of rituximab, a second course may be given. In the case of failure to respond to rituximab-based treatment, the therapeutic strategy adopted for HR group may be considered.

For HR patients, IS should be discontinued and rituximab/polychemotherapy started promptly. Initial treatment in our opinion should be mild and could be based on FCD-R blocks. If only a BFM strategy is used, treatment intensity in the first 2 blocks should be markedly reduced, that is, using methotrexate at intermediate doses (500 mg/m² in 24 hours) with a 20% reduction of the other drugs. Thereafter, a more intensive treatment strategy may be better tolerated. Intrathecal chemotherapy may be delayed to reduce the risk of neurotoxicity associated with the concomitant administration of CNIs.

In PTLD treatment a fine balance between control of lymphoproliferation and preservation of allograft is essential. In this relatively small cohort of patients, we could not determine features predicting rejection, which remains a major concern after completing PTLD treatment. Nevertheless, in our cohort, the 2 cardiac transplant recipients who stopped IS for HR PTLD developed acute rejection soon after PTLD treatment, suggesting that early restart of full dose IS may be considered in these patients. In our series, no clinical or histological signs of graft rejection during the treatment of HR-PTLD were observed, despite the prompt discontinuation of IS, suggesting an appropriate antirejection effect of our chemotherapeutic regimen combined with rituximab.

The IS therapy was restarted with caution in HR patients to decrease the risk of PTLD reactivation, and interestingly, 5 of 11 patients experienced graft rejection. Patient 2 had a severe rejection leading to retransplantation, and this should be regarded as a complication of PTLD treatment. Optimal timing and modality of IS reinstitution after PTLD treatment remain to be established. Based on our experience, IS should be restarted as soon as the chemotherapy program is completed in heart transplants, and within 3 months in liver recipients. It may be debated however if liver recipients could be left off IS, providing strict biochemical and histological monitoring because a relevant proportion may achieve spontaneous tolerance. Although this could not be demonstrated in our cohort, it may be argued that patients who had a previous episode of acute rejection should be considered at higher risk of graft loss after PTLD. We have not considered previous rejection episodes as relevant to decide the overall management of PTLD. Mechanistic target of rapamycin inhibitors have become popular after treatment of PTLD; however, we used them only to accomplish safe reduction of CNI in 2 patients with SR PTLD because so far there is no proof that treatment with this class of immunosuppressants is beneficial either on PTLD relapse or occurrence of rejection.

In summary, our center experience suggests that it is possible to identify 2 risk groups (SR and HR) in children with severe PTLD, on the basis of extension of the disease, histology, PS, and LDH level. This stratification allowed a satisfactory survival rate, controlling lymphoproliferation in aggressive forms, but at the same time minimizing toxicity in milder PTLDs. The benefit of our approach compared to that of a nonrisk adapted strategy, though, remains to be confirmed by additional prospective studies.

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