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CASE REPORT

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Various initial presentations of Epstein-Barr virus infection-associated post-transplant lymphoproliferative disorder in pediatric liver transplantation recipients: Case series and literature review

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Abstract

PTLD is a rare but potentially life-threatening condition, which shows a higher prevalence in children than in adults. From 129 children who underwent LT, we reported 5 cases with biopsy-proven PTLD at a single teaching hospital. Four patients had shared clinical presentations including fever, lymphadenopathy, and splenomegaly. They were noted to be given a prolonged course of IS due to the management of comorbid complications such as acute cellular rejection or severe food allergy or eosinophilic gastrointestinal disease. The other one patient presented with upper gastrointestinal bleeding from gastric mass during an early post-transplantation period. Notably, hypoalbuminemia was noted in all reported patients. Similar to previous studies, both EBV serology mismatch between the donor and recipient with high EBV viral load were noted in all except one case, whose EBV serology was unknown before LT. At least one episode of CMV reactivation was also observed in 3 of 5 patients prior to the PTLD diagnosis. The histopathology revealed 1 of 5 early PTLD, 1 of 5 polymorphic PTLD, and 3 of 5 monomorphic PTLD. The treatment included IS withdrawal, chemotherapy, and/or rituximab. One patient died of multiorgan dysfunction, one remains in complete remission, and three patients are either still on treatment or await response evaluation. Even though most of our reported PTLD cases had shared manifestations with fever, lymphadenopathy, splenomegaly, EBV serology mismatch, and high EBV viral load, various initial presentations such as respiratory symptoms, hypoalbuminemia, and prolonged use of IS from other causes such as significant food allergy were noted.

KEYWORDS

anemia, food allergy, hypoalbuminemia, immunosuppression, lymphoma

Abbreviations: BA, biliary atresia; CMV, Cytomegalovirus; D/R, donor/recipient serology status before liver transplantation; EBER-ISH, Epstein-Barr encoding region in situ hybridization; EBV, Epstein-Barr virus; IS, immunosuppression; LN, lymph node; LT, liver transplantation; MMF, mycophenolate mofetil; NA, not applicable; NHL, non-Hodgkin lymphoma; Pred, prednisolone; PTLD, post-transplant lymphoproliferative disorder; TAC, tacrolimus.

1 | INTRODUCTION

LT is currently accepted as a curative treatment in patients with end-stage liver disease which requires a highly experienced multidisciplinary team. In children, almost all patients require lifelong IS resulting in a higher risk for various opportunistic infections, such as EBV, CMV, and others. Regular long-term follow-up is important to detect these infection-related complications after LT. Post-transplant lymphoproliferative disorder (PTLD) is considered a rare but potentially life-threatening condition after LT that is a group of lymphoproliferative disorders ranging from benign lymphoid hyperplasia to high-grade malignant lymphoma.[1,2] EBV infection has been reported to have a strong association with PTLD, especially in active EBV infection. Most patients with PTLD have B-cell lymphocyte abnormalities from EBV infection.[4,5] Potential risk factors include donor-recipient EBV serological mismatch, and prolonged IS.[6,7] PTLD usually occurs during the first year after transplantation; however, the manifestation of PTLD can abruptly and aggressively present at any time after LT.[8,9]

Patients with PTLD demonstrated with a wide spectrum of clinical signs and symptoms. The disease can lead to liver graft dysfunction and graft loss.[9] The treatment of PTLD sometimes requires chemotherapy that potentially leads to more serious complications with high morbidity and mortality.[5] Herein, we reported 5 pediatric cases of PTLD with regard to clinical presentation and outcome at a tertiary care hospital that performed LT in 129 recipients between March 2001 and December 2017. The diagnosis was based on tissue histopathology according to the WHO classification of PTLD.[2,3] The pathologic evaluation of immunophenotyping and EBER-ISH was also applied in diagnosis, classification, and evaluation.

Clinical presentations of the reported cases are shown in Table 1. All patients underwent living donor LT due to end-stage liver disease. Four of them had cirrhosis due to biliary atresia and had LT before the age of 2 years. Our IS protocol included tacrolimus with corticosteroid as induction therapy and maintenance with tacrolimus, MMF, and low dose prednisolone for approximately 6 months after LT. We aimed for the tacrolimus level to be around 8-12 ng/mL at one month after LT and weaned to a level of 3-5 ng/mL after one year.

The EBV and CMV viral load were monitored every 2 weeks in the first 3 months then every 4 weeks during 3-12 months, and every 2-3 months during 12-24 months post-LT. The serum EBV PCR viral load was measured by Abbott RealTime assay with the limit of detection of 210 copies/mL. IS was decreased if EBV viral load was higher than 800-1000 copies/mL, and investigations for PTLD were performed if the patients had suggestive clinical presentation. All IS were discontinued when the diagnosis of PTLD was made. Clinical presentation and course of the five cases are explained in the following:

2 | CASE 1

A 2-year-old girl with cirrhosis of unknown etiology presented with prolonged fever, anemia, enlargement of multiple lymph nodes, and splenomegaly 14 months after LT. She initially had an EBV viral load of 372 copies/mL since 6 months after LT and rose up to 19 550 copies/mL which did not fully respond to a reduction of tacrolimus. Finally, the diagnosis of polymorphic PTLD was made by tissue biopsy from an enlarged submandibular lymph node. She received four weekly doses of rituximab and achieved complete remission. She has been well after a reintroduction of tacrolimus.

3 | CASE 2

A 4-year-old boy with biliary atresia and a previous history of one episode of CMV reactivation and two episodes of recurrent herpetic gingivostomatitis after LT developed fever and non-productive cough for 2 weeks at 36 months post-LT. Pneumonia was diagnosed that did not improve after a one-week course of oral antibiotics and further complicated with persistent right upper and middle lung atelectasis. Bronchoscopy revealed external compression at the right bronchus suggestive of a mass lesion. CT scan showed multiple enlarged matted lymph nodes at the bilateral cervical and thoracic level with a lobulated lesion at the torcular herophili, later confirmed by an MRI brain likely to be a PTLD lesion. Burkitt lymphoma (ie, monomorphic PTLD) was diagnosed by tissue from liver biopsy. He was treated with rituximab and chemotherapy for 4 months. He had clinical improvement, and the latest CT scan revealed decreased size of lymph nodes, hypoechoic lesions in the liver, spleen, and both kidneys.

4 | CASE 3

A 3-year-old girl with biliary atresia had multiple postoperative complications including portal vein thrombosis, an episode of acute cellular rejection, multiple food allergy, three episodes of CMV reactivation, and multiple episodes of elevated EBV viral load. She also had persistent elevated liver enzymes despite high dose IS. Her EBV viral load ranged between 400 and 16 000 copies/mL during the follow-up without clinical symptoms. She presented with fever and mucous bloody diarrhea for 2 weeks. Cervical lymphadenopathy and splenomegaly were noted with elevated transaminases. Stool tests were negative for infection. The chronically elevated EBV viral load led to an imaging and subsequent cervical lymph node biopsy which confirmed NK/T-cell lymphoma (ie, monomorphic PTLD, CD-20 negative). She was treated with chemotherapy without the need for rituximab. After a month of treatment, she remained well but the latest CT scan four months after treatment revealed the increased size of mediastinal and intraabdominal lymph nodes. The EBV viral load remained undetectable, and she was free of IS. The evaluation was ongoing.

5 | CASE 4

A 3-year-old girl with biliary atresia, and also later diagnosed with biopsy-proven eosinophilic gastrointestinal disorder with allergic rhinitis after LT, and worsening snoring with stridor at rest was

TABLE 1 Features of five patients with post-transplant lymphoproliferative disorder

No	Age of LT (mo)	Indication for LT	Time at diagnosis (mo after LT)	EBV (D/R)	EBV viral load at diagnosis (copies/ml)	TAC level at diagnosis (ng/ml)	Other IS (mg/kg/day)	Steroid accumulation dose (mg/kg)	CMV reactivation (mo after LT) &CMV (D/R)	Associated findings	Pathology	Treatment	Outcome
1	12	Cirrhosis with unknown cause	14	+/-	107 768	7	Pred (0.28)	182	NA	Fever, anemia	**Cervical LN**	Discontinue IS	**Complete recovery**
									+/+	Lymphadenopathy (cervical, mediastinal, intraabdominal)	Polymorphic PTLD		3-year follow-up
							MMF (27.8)			Splenomegaly	CD20+	Rituximab	No recurrence
										Hypoalbuminemia	EBER-ISH +		
2	11	BA	36	+/ equivocal	192 090	4.9	NA	71	3	Fever	**Liver tissue**	Discontinue IS	**Finished therapy**
									+/+	Lymphadenopathy (mediastinal up to 8 cm, cervical)	Burkitt lymphoma (monomorphic PTLD)		
										Right lung atelectasis from lymph node compression	CD20+ Bcl-6+		
										Lymphoma in the liver, spleen, kidney and brain	CD10+ Ki67+	Rituximab	2-mo follow-up
										Hypoalbuminemia	EBER-ISH + C-myc+	Chemotherapy (NHL: Burkitt lymphoma, high-risk regimen with cyclophosphamide, vincristine, intrathecal methotrexate)	Evaluation of cure remained pending

(*Continues*)

TABLE 1 (Continued)

No	Age of LT (mo)	Indication for LT	Time at diagnosis (mo after LT)	EBV (D/R)	EBV viral load at diagnosis (copies/ml)	TAC level at diagnosis (ng/ml)	Other IS (mg/kg/day)	Steroid accumulation dose (mg/kg)	CMV reactivation (mo after LT) &CMV (D/R)	Associated findings	Pathology	Treatment	Outcome
3	17	BA	23	+/-	78 012	1.9	Pred (0.42)	253	2, 4, 8 +/+	Fever Lymphadenopathy (cervical, mediastinal, intraabdominal) Splenomegaly Hypoalbuminemia	**Cervical LN** Monomorphic PTLD (T/NK cell) CD3+ CD8+ Ki67+ CD56+ CD20 - GranzymeB+ EBER-ISH +	Discontinue IS Chemotherapy (NHL: anaplastic large cell lymphoma, low risk regimen)	**Ongoing therapy**
4	14	BA	21	+/-	8969	3.0	NA (stopped 3 mo before diagnosis)	121	NA +/+	Fever, anemia Lymphadenopathy (cervical) Splenomegaly Adenotonsillar hypertrophy Hypoalbuminemia	**Tonsil + adenoid** Florid follicular hyperplasia (early PTLD) CD20+ EBER-ISH +	Discontinue IS	**Improved** 5-mo follow-up No recurrence
5	209	BA	3	+/NA	2689	NA	Pred (0.33) Cyclosporin (2.78)	109	1 +/+	Upper gastrointestinal bleeding	**Gastric mass** Monomorphic PTLD CD20+ EBER-ISH +	NA	Expired (8 days after diagnosis PTLD)

sent to an otolaryngologist for evaluation. She had donor-recipient serology mismatch of EBV and later had fluctuating EBV viral load of 500-50 000 copies/mL. Tonsillectomy and adenoidectomy with submental lymph node biopsy showed an early lesion of PTLD. Tacrolimus was discontinued, and the oncologist decided to continue monitoring without initiating chemotherapy or rituximab; she has remained well.

6 | CASE 5

A 17-year-old female with biliary atresia underwent LT due to uncontrolled variceal bleeding. Her postoperative complications were bleeding from mesenteric artery, acute cellular rejection, CMV reactivation, and poor graft function without an identifiable cause. Three months after LT, she had an episode of upper gastrointestinal bleeding. Esophagogastroduodenoscopy showed 3 gastric polypoid masses with ulceration on top. Biopsy revealed monomorphic PTLD. She later deteriorated with pulmonary edema, multiple organ failure, and diastolic dysfunction requiring multiple inotropic agents and died before starting the treatment for PTLD.

7 | DISCUSSION

7.1 | Incidence, risk factors, and onset of PTLD

PTLD is a well-known complication that can develop after solid organ transplantation. The pathophysiology is not completely understood but it may relate to EBV infection, EBV-driven proliferation, and/or impaired T cells to control EBV-induced B-cell proliferation.[11] PTLD is more common in pediatric LT recipients than in adults with the prevalence of >10% in some reports.[6] These young children may not contract EBV infection during the first two years of life (ie, before the time of LT) while receiving an organ from a donor with positive EBV serology (donor IgG positive/recipient IgG negative status).

The incidence of PTLD also varies with the type of transplanted organ. LT recipients are less likely to develop PTLD as compared to intestinal (19%),[11] lung (8%), or heart (3%) transplant recipients,[12] but more likely than the kidney transplant recipients (<1%).[13] A clear explanation on the differences in incidence is yet to be defined but may relate to the function of immune cells in each transplanted organ.

At our center, we noted an incidence of 3.9% (5 cases of 129 transplanted patients). PTLD typically occurs within the first year after LT (ie, early onset PTLD) during the highest degree of IS.[4,8,13,14] However, at our center, we noted that only one patient was diagnosed within the first year after LT. We hypothesized that the occurrence of PTLD may also relate to the degree of IS. Case Nos. 1 and 3, who developed PTLD after a year post-LT, received a combination of tacrolimus and other IS at the time of PTLD diagnosis either due to a previous episode of recent acute cellular rejection or diagnosed multiple food allergy, and case No. 4 had just recently discontinued prednisolone within 3 months (Table 1).

7.1.1 | Epstein-Barr virus

Up to 95% of pediatric PTLD have EBV infection or reactivation, especially in cases with EBV serology mismatch (donor IgG positive/recipient IgG negative).[4,6,10,15,16] Most patients in our series also had EBV serology mismatch which is common in children. Prolonged high viral load of EBV (ie, chronic high load carrier) is an important risk factor for PTLD. Several studies showed that viral load >4000 copies/mL and sustained high viral load more than 6 months (as in Case Nos. 3 and 4) are considered a risk for developing PTLD,[18] even if the IS was weaned.

Clinical signs and symptoms of PTLD are non-specific, so the diagnosis of PTLD requires a high index of suspicion. Surveillance EBV viral load remains crucial in post-LT children in this regard. Patients may benefit from an early IS reduction and treatment especially in recipients with EBV serology mismatch.[6,9,16] Adult studies suggest monitoring viral load for EBV mismatched recipients every 2-4 weeks in the first 3 months, monthly until 6 months, and every 3 months till one year after transplantation.[6] However, the optimal time of routine monitoring in high-risk pediatric recipients is under debate.

7.2 | Immunosuppressive agents

The IS in patients after solid organ transplantation has the beneficial mechanism to inhibit cell-mediated immune response. However, agents that directly inhibit T-cell activation such as anti-thymocyte globulin and calcineurin inhibitor are associated with PTLD.[19] T-cell depleting agents have a strong association with PTLD because of the lack of control of B-cell proliferation. We hypothesized that calcineurin inhibitor, which is frequently used in our LT children, blocks the production of cytokines such as interleukin-2 and inhibits T-cell activation and proliferation. Therefore, it may lead to opportunistic viral infection such as EBV. Our center mainly used calcineurin inhibitor for both during the induction and maintenance phases.

Furthermore, four of our PTLD patients had recently used corticosteroid for various reasons. The cumulative steroid dose ranged from 71 to 253 mg/kg of prednisolone (mean 147 mg/kg); only case No. 2 received <100 mg/kg. Thus, monitoring for PTLD may be beneficial in patients who have used high accumulative dose of corticosteroids. Mechanistically, corticosteroids inhibit T-cell function, cytokines, and macrophage function. It also lowers circulating CD4 T cells and inhibits T-cell activation.[19]

7.3 | Associated CMV infection

PTLD has been linked to CMV infection that is also at risk with high level of IS,[20] although the effect is not as strong as for EBV. Three cases (Case Nos. 2, 3, and 5) had at least one episode of CMV reactivation. More studies are needed to define an association between CMV infection and PTLD.

7.4 | Clinical manifestations

All except one patient in our series had fever, lymphadenopathy, and splenomegaly as presenting symptoms, similar to previous studies.[4,17,21] The central nervous system is infrequently involved (as in case No. 2).[7] One patient had pulmonary involvement leading to obstructive atelectasis. Interestingly, we found that all of our reported patients had hypoalbuminemia. Factors that may cause low serum albumin were the following: (i) loss of albumin from the gastrointestinal tract (ie, potential protein-losing enteropathy), which is potentially caused by food allergy or even gastrointestinal PTLD itself. Previous study of PTLD in heart and lung transplanted recipients showed that hypoalbuminemia may not only be associated with the diagnosis of PTLD but also be related with a poor prognosis[11]; and (ii) systemic inflammation and response to malignancy.

7.5 | Treatment and outcome

There is a lack of consensus in treating PTLD in children undergoing LT. The most accepted initial management is to reduce or stop IS for reconstitution of the immune response. Balancing treatment of EBV-associated PTLD and graft rejection while adjusting IS has always been challenging. We discontinued all IS immediately after biopsy-proven PTLD was noted. Furthermore, different treatment options (eg, chemotherapy, anti CD-20 monoclonal antibody such as rituximab, surgery, radiotherapy) have a crucial role in the management of PTLD, and the approach depends on the histologic subtype of PTLD. The transplanted patients also remain at risk for PTLD as long as they remain on IS. After the treatment of PTLD, one patient remains in complete remission, while one patient with an early onset PTLD died within 1 week after upper gastrointestinal bleeding from the gastric PTLD lesion. Three patients are either still on treatment or await response evaluation.

8 | CONCLUSION

Most of the five reported children with PTLD had well-known shared manifestations of EBV serology mismatch, fever, lymphadenopathy, and splenomegaly. However, clinicians should be made aware of various initial presentations such as respiratory symptoms, hypoalbuminemia, and history of prolonged IS use.

CONFLICT OF INTEREST

The authors have no conflicts of interest to disclose.

AUTHORS' CONTRIBUTION

LS: prepared the first draft of the manuscript and revised the manuscript; PT: provided study concept and critically revised the manuscript; CL, UA, SP, SH ST, and PP: critically reviewed the manuscript. All authors approved the final draft of the manuscript.

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