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Title:

Post-transplant lymphoproliferative disorders after liver transplantation: A retrospective cohort study including 1,954 transplants

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Prognostic factors; performance status; monomorphic PTLD; living-donor liver transplantation; calcineurin inhibitors; Epstein-Barr virus

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Abbreviations:

ALF, acute liver failure; ANOVA, analysis of variance; BA, biliary atresia; BM, bone marrow; CD, cluster of differentiation; CI, confidence interval; CIT, cold ischemic time; CNS, central nervous system; CT, computed tomography; CMV, cytomegalovirus; CNI, calcineurin inhibitors; DDLT, deceased-donor liver transplantation; DLBCL, diffuse large B-cell lymphoma; EBER, Epstein-Barr virus-encoded RNA; EBV, Epstein-Barr virus; ECOG-PS, Eastern Cooperative Oncology Group performance status; FFH, florid follicular hyperplasia; FK, tacrolimus; GRWR, graft/recipient body-weight ratio; GVHD, graft-versus-host disease; HBV, hepatitis-B virus; HCV, hepatitis-C virus; HR, Hazard Ratio; IM, infectious mononucleosis; IPI, international prognostic index; IQR, interquartile range; IS, immunosuppression; KT, kidney transplantation; LDH, lactate dehydrogenase; LDLT, living-donor liver transplantation; LN, lymph node; LT, liver transplantation; LT-PTLD, Post-transplant lymphoproliferative disorder after liver transplantation; MELD, Model for End-stage Liver Disease; NA, not applicable; OS, overall survival; PCR, polymerase chain reaction; PELD, Pediatric End-stage Liver Disease; PET, positron emission tomography; PFS, progression-free survival; PH, plasmacytic hyperplasia; PS, performance status; PSC, primary sclerosing cholangitis; PTLD, Post-transplant lymphoproliferative disorder; RECIST, Response Evaluation Criteria in Solid Tumors; ROC, receiver operating characteristic, sIL-2R, soluble interleukin-2 receptor; SOT, solid organ transplantation; WIT, warm ischemic time

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Abstract

Post-transplant lymphoproliferative disorders (PTLDs) are life-threatening neoplasms after organ transplantation. Due to their rarity and multiple grades of malignancy, the incidence, outcomes, and clinicopathological features affecting patient survival after liver transplantation (LT) remain unclear. We reviewed our 1,954 LTs in 1,849 recipients (1990–2020), including 886 pediatric (<18 years) and 963 adult recipients. The following clinicopathological factors were studied: age, gender, liver etiologies, malignancy grades, Epstein-Barr virus status, performance status (PS), Ann Arbor stage, international prognostic index, and histopathological diagnosis. Of 1,849 recipients, 79 PTLD lesions (4.3%) were identified in 70 patients (3.8%). After excluding incidentally-found three autopsy cases, 67 (45 pediatric [5.1%] and 22 adult [2.3%]) patients were finally enrolled. Comorbid PTLDs significantly worsened recipient survival compared with non-complicated cases (P <0.001). The 3-/5-/10-year overall survival rates after PTLD diagnosis were 74%/66%/58%, respectively. Notably, the incidence of LT-PTLDs was significantly higher (P <0.001) with earlier onset (P =0.002) in children, while patient survival was significantly worse in adults (P =0.002). Univariate and multivariate analyses identified the following three prognostic factors: age at PTLD diagnosis ≥ 18 years (HR: 11.2, 95%CI: 2.63–47.4, P =0.001), PS ≥ 2 at diagnosis (HR: 6.77 [1.56–29.3], P =0.01), and monomorphic type (HR: 6.78 [1.40–32.9], P =0.02). A prognostic index, "LT-PTLD Score", consisting of these three factors effectively stratified patient survival and progression-free survival (P =0.003 and <0.001, respectively). In conclusion, comorbid PTLDs significantly worsened patient survival after LT. Age ≥ 18 years and PS ≥ 2 at PTLD diagnosis, and monomorphic type are independent prognostic factors, and LT-PTLD Score consisting of these three factors may allow to distinguish high-risk cases and to guide adequate interventions.

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1. Introduction

Post-transplant lymphoproliferative disorders (PTLDs) are one of the most common malignancies after solid organ transplantation (SOT)[1-5] and remain life-threatening with 5-year overall survival (OS) rates ranging 40%–70%.[6] These high mortalities and morbidities have highlighted the need to clarify the prognostic factors in PTLDs.

However, PTLDs have two difficult burdens to be investigated: rarity and heterogeneity. PTLDs develop only in transplant recipients, and their incidence has been reported to be 1.0–5.5% after liver transplantation (LT); 0.8–2.5% after kidney transplantation (KT); 0.5–5.0% for pancreas transplantation; 2.0–8.0% for heart transplantation; 3.0–10.0% for lung transplantation; and ≤ 20% for multiorgan/intestinal transplantation in adults.[2, 7, 8] Since the numbers of PTLDs in each center are limited, previous studies were mostly conducted using relatively large post-KT recipients or heterogeneous cohorts including various SOTs.[6, 9-14] PTLDs inherently have a wide range of clinicopathological characteristics, from indolent lymphoproliferation requiring only immunosuppressant modifications, to malignant lymphomas that need chemotherapies.[2] Moreover, their characteristics are reportedly different between adults and children.[2, 15] Collectively, there is no widely accepted consensus on PTLDs so far, especially after LT (LT-PTLDs).

This study thus aimed to clarify the clinicopathological features of LT-PTLDs; to identify the prognostic/risk factors therein; and to compare their characteristics between pediatric and adult LT-PTLDs, by reviewing our relatively large cohort of almost 2,000 LTs.

2. Patients and Methods

2.1. Patients

We performed a total of 1,954 LTs in 1,849 recipients at our single center between June 1990 and March 2020, including 937 pediatric (< 18 years) and 1,017 adult (≥ 18 years) LTs in

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886 children and 963 adult recipients, respectively. A total of 1,874 out of 1,954 LTs were living-donor LTs (LDLTs), while 80 were deceased-donor LTs (DDLTs). Of 1,849 recipients, 79 PTLD lesions (4.3%) were identified in 70 patients (3.8%). All lesions developed after LDLT. Of these, nine metachronous lesions in same patients and incidentally found three autopsy cases were excluded to identify prognostic factors in LT-PTLDs. Thus, 67 (45 children [5.1%] and 22 adults [2.3%]) patients were finally enrolled (Fig. S1). Written informed consent was obtained from each patient or his/her parents. This study was approved by the Ethics Committee of Kyoto University (R1473) and was conducted in accordance with the institutional guidelines as well as the ethical guidelines mandated by the Declaration of Helsinki (2013).

2.2. Peri-transplant Management

The selection criteria for donors and recipients, perioperative management, surgical procedures, and immunosuppression regimens are detailed elsewhere.[16-21] Briefly, the lower limit of graft/recipient body-weight ratio (GRWR) in adult-to-adult LDLTs are as follows: ≥ 0.8% until November 2007, ≥ 0.7% from December 2007 until March 2009, and ≥ 0.6% from April 2009.[20, 21] For biliary reconstruction, choledocho-choledochostomy was our priority in adult LT. Modulations of portal-venous pressure, such as splenectomy,[20] was performed to keep 15 mmHg or less at the end of surgery, if needed.[21] Recipients were postoperatively managed in intensive/high care units during the first several days. Blood cell counts, biochemical and coagulation examinations, and Doppler ultrasonography were performed daily until stabilized. Tacrolimus or cyclosporine, and steroid has long been used since 1990. In the early period (1990–2005), azathioprine or OKT3 was given for acute rejection. Cyclophosphamide was added in recipients undergoing ABO-incompatible LDLTs. In the late period (2006–2020), however, these three drugs were all discontinued. The combination of tacrolimus, mycophenolate mofetil, and

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steroid became the standard regimen in the last 15 years. Recently, everolimus was added to reduce the trough level of tacrolimus, if necessary. In ABO blood-type incompatible or donor-specific antibody positive cases, recipients were preoperatively treated with anti-CD20 monoclonal antibody (rituximab: 375 mg/m^2) and plasma exchange to prevent antibody-mediated rejection.[22] Acute cellular and antibody-mediated rejections were diagnosed according to the Banff criteria.[23, 24]

For pediatric LDLTs, briefly, the upper limit of GRWR was 4.0%. If the estimated GRWR exceeded 4.0%, a reduced, hyper-reduced, or S2-monosegment graft was selected.[25] For biliary reconstruction, choledocho-jejunostomy was mostly adopted because many patients underwent Kasai's operation for biliary atresia (BA). A standard immunosuppression protocol consisting of tacrolimus and steroids was used. In ABO-incompatible cases, recipients were pretreated with rituximab (≥ 2 years-old) or considered individually (1–2 years). Exchange transfusion or plasma exchange was performed as needed.

2.3. Diagnosis of PTLDs

PTLDs were all histologically diagnosed by expert pathologists with excisional biopsies just except for a case with needle biopsy. PTLDs were classified according to the WHO classification revised in 2017.[26] After confirming histopathological diagnosis, patients underwent staging work-up, including whole-body computed tomography (CT), bone marrow biopsy/aspiration, [18F]-fluorodeoxyglucose positron emission tomography (PET)/CT, and cerebrospinal fluid test to check central nervous system (CNS) invasion. Serological tests for Epstein-Barr virus (EBV) and cytomegalovirus (CMV) infection were conducted preoperatively. Tumor EBV-positivity was determined by *in situ* hybridization assays for EBV-encoded RNA

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(EBER).[27, 28] Serological status was confirmed by polymerase chain reaction (PCR) for the quantification of EBV-viral load.[27, 28]

2.4. PTLD-like lesions

In this study, we defined "PTLD-like lesions" as any post-transplant lymphoproliferative lesions that showed clinical manifestations but did not fulfill the PTLD criteria according to the WHO classification.[26] The followings were included as PTLD-like lesions: indolent small B-cell lymphomas, including follicular lymphoma or marginal zone lymphoma,[29] EBV-negative reactive lymphadenopathy, EBV-positive mucocutaneous ulcer,[30] hairy cell leukemia,[31, 32] and EBV-associated pleural effusion/ascites without malignant cells (Table-S1). Although excluded from a strict definition of PTLDs,[26] the clinical presentations of PTLD-like lesions were similar to those of PTLDs, and patients with these lesions often required immunosuppressant modifications or chemotherapies that could affect patient prognosis.

2.5. EBV Monitoring

EBV status in both donors and recipients was determined serologically before LT. In pediatric recipients, EBV-PCR was used to be measured every month for the first 6 months regardless of EBV-seropositivity. Over the last decade, EBV-PCR has been performed every week before discharge after transplant, followed by biweekly-to-monthly after discharge for the first 6 months, to detect not only EBV primary infection in naive recipients but also EBV reactivation in seropositive patients as early as possible. If the result remains negative, intervals between EBV-PCRs will be prolonged. High-risk (D+R-: EBV-naive recipients with EBV-infected donor livers) recipients are more carefully followed by checking clinical symptoms (lymphadenopathy,

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fever, or hepatitis, *etc*.) and EBV-PCR. In adults, EBV-PCR is performed when recipients have some symptoms of EBV infection or unexplained fever.

2.6. Monitoring and Treatment of PTLDs

Recipients are usually followed up once a month for the first 6 months, every 2 months from 6 to 12 months, and every 3 months thereafter if the postoperative course is uneventful. Patients with high EBV-viral load, unexplained fever, or lymphadenopathy undergo thorough examinations.

In patients with LT-PTLDs, we first considered to modify immunosuppressants, i.e., cessation, reduction, or switching of calcineurin inhibitors (CNI). In pediatric patients, we conducted the 1st-line chemotherapy according to the recommendation from the Japanese Pediatric Leukemia/Lymphoma Study Group[33] since 2007, in combination with rituximab. For non-responders to the 1st-line, we used more intensified 2nd-line chemotherapy, incorporating cisplatin (DECAL: dexamethasone, etoposide, cisplatin, cytarabine, L-asparaginase) or high-dose cytarabine[33]. In adults, R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) has long been the mainstay, and dose-adjusted (DA)-EPOCH-R (etoposide, vincristine, doxorubicin, cyclophosphamide, prednisolone, and rituximab) has recently been applied in high-risk PTLDs. Surgical resections were performed for localized, perforated, and obstructed intestinal lesions. Radiation therapy was indicated in patients not eligible for chemotherapies, non-responders to chemotherapies, or those with CNS involvement. Restaging CT scans were performed timely, and the therapeutic effects were assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines.[34]

2.7. Variables

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The preoperative clinical variables included recipient/donor age at LT, gender, underlying liver etiologies, malignancy grades, ABO blood-type compatibility, Pediatric End-stage Liver Disease (PELD) or Model for End-stage Liver Disease (MELD) scores, and pre-transplant EBV/CMV serological status.

The operative variables included graft-type (left- or right-lobe), GRWR, operation time, intraoperative blood loss, cold ischemic time (CIT), and warm ischemic time (WIT). As listed in Table-1, PTLDs-related variables are as follows: Recipient age at PTLD diagnosis, serological and histopathological positivity of EBV, Eastern Cooperative Oncology Group performance status (ECOG-PS),[35] Ann Arbor Stage,[36] tumor size, presence/absence of extranodal lesions, soluble interleukin-2 receptor (sIL-2R), international prognostic index (IPI),[37] intervals between LT and PTLD diagnosis, histological classifications, *etc*.

2.8. Statistical Analysis

Data are expressed as median with interquartile ranges (IQRs) for continuous variables and counts for categorical variables. Comparisons of continuous variables and categorical variables were performed using Mann-Whitney U-tests or chi-square tests, as appropriate. In cases with multiple LTs, the intervals from the first transplant to PTLD diagnosis were adopted to account for the duration of immunosuppressants exposure. Prognostic factors for LT-PTLDs were analyzed using univariate and multivariate Cox regression analyses. Overall patient survival (OS) and progression-free survival (PFS) were counted from the date of PTLD diagnosis to the patient's death or the last follow-up (OS) and to death or disease relapse/progression (PFS), respectively. These survivals were estimated by the Kaplan-Meier method, followed by log-rank tests. All analyses were two-sided, and $P < 0.05$ was considered statistically significant. Variables with $P <$

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0.10 in the univariable analysis were included in the multivariable analysis. All statistical analyses were performed using JMP Pro14 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Incidence, Timing, and Treatments of LT-PTLDs

Overall, the incidence of pediatric PTLDs were significantly higher than in adults (n =45 [5.1%] *vs.* n =22 [2.3%], $P < 0.001$). The intervals between LT and PTLDs diagnosis varied widely, from 19 days to 24.5 years (median 23 months [IQR: 5–53]); however, PTLD onsets were significantly earlier in children than in adults (14 [4–32] *vs.* 57 [25–111] months, $P = 0.002$). Notably, almost half of pediatric PTLDs (49%) developed within a year after LT (Fig. 1-A). Two pediatric recipients (age < 18 years at LT) developed PTLDs in adulthood (≥ 18 years); therefore, a total of 43 pediatric and 24 adult PTLDs were identified. The most common treatment was immunosuppressant modifications (n = 27 [40%]) followed by chemotherapies (n = 23 [34%]).

3.2. Patient Characteristics and Clinicopathological Variables

Patient characteristics, perioperative variables, and PTLD-related variables are summarized in Table-1. The most common etiology was BA in 38 (57%), followed by hepatitis-C virus (HCV) in 8 (12%) cases. Regarding blood-type combinations, 11 cases (16%) were ABO-incompatible, and the remaining 56 (84%) were identical or compatible. Pre-transplant serological statuses for EBV and CMV were positive in 39 (58%) and 37 (55%) patients, respectively. The median age at PTLD diagnosis was 6.1 years (range: 19 days–24.7 years). EBV-PCR (blood) and histopathological EBV statuses were positive in 31 (46%) and 35 (52%), respectively. ECOG-PS was 0–1 in 48 (72%) and 2–4 in 19 (28%) patients. Ann Arbor Stage was 1–2 in 44 (65%) and 3–4 in 22 (33%). Extranodal lesions were found in 28 (42%) patients. LDH

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and sIL-2R at PTLD diagnosis were 372 U/L (IQR: 258–524) and 2,480 U/mL (IQR: 1,605–4,225), respectively. IPI was 0–2 in 44 (65%) and 3–4 in 13 (19%). Histopathologically, monomorphic type was the most common (n = 24 [36%]), followed by PTLDs-like lesions (n = 17 [25%]) and infectious mononucleosis type (n = 14 [21%]). Monomorphic type included diffuse large B-cell lymphoma (DLBCL, n = 13), Burkitt lymphoma (n = 7), and T-cell neoplasms (n = 2).

3.3. Histopathological types and time to PTLDs

The median follow-up period was 12.3 (range 0.2–29.2) years. Notably, non-monomorphic PTLDs developed significantly earlier than monomorphic types ($P < 0.001$, Fig. 1-C). Furthermore, EBV-positive PTLD lesions developed significantly earlier than those without ($P < 0.001$, Fig. 1-D), while there was no significant association between serological EBV-positivity and the timing of PTLD onset ($P = 0.55$, Fig. 1-E). These trends were observed in both pediatric and adult patients (Fig. S2).

3.4. Historical Transition of LT-PTLDs Incidence and tacrolimus trough level

Then we compared the incidence of LT-PTLDs between the early (1990–2005) and the late period (2006–2020). Although overall patient survival with LT-PTLDs was not significantly different between the two periods in both children and adults (data not shown), the incidence of pediatric LT-PTLDs decreased in the late period ($P = 0.06$, Fig. 2-A and -B). As a possible reason for this, the trough level of tacrolimus in pediatric patients with LT-PTLDs was significantly higher in the early period than that in the late ($P < 0.001$, Fig. 2-C), whereas no significant differences were observed in adults between the two eras (Fig. 3).

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3.5. Patient Survival and Cause of Death

As shown in Fig. 4A–C, comorbid PTLDs in the whole, pediatric, and adult cohorts significantly worsened OS after LT compared with non-complicated cases ($P < 0.001$, < 0.001, and $= 0.005$, respectively). The 3-/5-/10-year OS rates in the whole, pediatric, and adult cohorts after PTLD diagnosis were 74/66/58%, 81/79/71%, and 61/38/28%, respectively (Fig. 4-D and -E). Although the incidence of LT-PTLDs was significantly lower in adults ($P < 0.001$), patient survival was significantly worse in adults than in pediatric patients ($P = 0.002$, Fig. 4-E and -F).

Overall, 30 patients (44.8%) died in the present series. Tumor progression was the leading cause of death (14 patients [47%]), followed by graft failure (7 patients [23.3%]), sepsis (2 patients [6.7%]), and cerebral hemorrhage (1 patients [3.3%]). (Table-S3).

3.6. Prognostic Factors in LT-PTLDs

PTLD-related mortality included deaths from tumor progression and treatment toxicities only. Univariate Cox regression analysis revealed that age at PTLD diagnosis ≥ 18 years (Hazard Ratio [HR]: 5.69, 95% confidence interval [CI]: 1.70–19.0, $P = 0.005$), primary disease: non-BA (HR: 3.60, 95%CI: 1.17–11.1, $P = 0.03$), PS ≥ 2 at PTLD diagnosis (HR: 3.21, 95%CI: 1.12–9.17, $P = 0.03$), presence of extranodal lesions at diagnosis (HR: 5.00, 95%CI: 1.53–16.3, $P = 0.008$), and monomorphic PTLDs (HR: 6.43, 95%CI: 1.98–20.8, $P = 0.002$) were significant prognostic factors in LT-PTLDs.

To eliminate confounding bias, "primary disease: non-BA" was excluded because it had a strong correlation with "age at PTLD diagnosis". As summarized in Table-2, age at PTLD diagnosis ≥18 years (HR: 11.2, 95%CI: 2.63–47.4; $P = 0.001$); PS ≥ 2 at PTLD diagnosis (HR: 6.77, 95%CI: 1.56–29.3; $P = 0.01$); and monomorphic type (HR: 6.78, 95%CI: 1.40–32.9; $P = 0.02$), were identified as independent prognostic factors in LT-PTLDs.

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3.7. Stratification of Patient Prognosis by LT-PTLD Score

We introduce "LT-PTLD Score" consisting of the three prognostic factors identified by the multivariate analysis, i.e., A: age at PTLD diagnosis > 18 years; B: PS ≥ 2; and C: monomorphic PTLDs. According to the hazard ratio of each factor (A: 11.2, B: 6.77, and C: 6.78), we have constructed a prognostic scoring with a weighting of 2 points for A and 1 point for B and C. Patients with LT-PTLDs were classified into 0–4 points, by which the patient prognosis was effectively stratified (Fig. 5-A). As seen, LT-PTLD patients with point 0 showed 100% survival. When 0, 1–2, and 3–4 points are regarded as low, intermediate, and high risk for PTLD-related deaths, respectively (Fig. 5-B), the prognostic score significantly stratified the patient survival with LT-PTLDs-related mortality (Fig. 5-C), overall patient survival (Fig. 5-D), and progression-free survival (Fig. 5-E). As shown in Fig. 5-C, LT-PTLDs-related mortality significantly worsened as the prognostic score increased ($P = 0.003$ in 0 *vs* 1–2; $P = 0.04$ in 1–2 *vs* 3–4; and $P < 0.001$ in 0 *vs* 3–4). These results demonstrated that LT-PTLD Score allows more precise estimation of patient prognosis with LT-PTLDs.

3.8. Subgroup Analyses: Children vs. Adults

Pediatric (age at PTLD diagnosis < 18 years, $n = 43$) and adult (≥ 18 years, $n = 24$) patients were then separately analyzed. Patient characteristics, perioperative and PTLD-related variables are summarized in Table-1. Similarly, PTLD-associated mortality included deaths from tumor progression or treatment toxicities only. Univariate analysis demonstrated that PS ≥ 2 at PTLD diagnosis (HR: 9.64, 95%CI: 1.07–86.8, $P = 0.04$) and monomorphic type (HR: 13.7 [1.51–124.2], $P = 0.02$) were significant prognostic factors in pediatric PTLDs. Though statistically not

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significant, similar results were also obtained in adults, as follows: PS ≥ 2 at diagnosis (HR: 3.69 [0.89–15.4], $P = 0.07$) and monomorphic type (HR: 4.71 [0.87–25.5], $P = 0.07$).

4. Discussion

Due to high morbidity and mortality, PTLDs have been investigated in various SOT; however, their rarity only in transplant recipients, as well as the heterogeneity, from non-destructive to destructive PTLDs,[26] have hampered detailed assessments of these critical complications. In the present study, using a relatively large cohort of 1,954 LTs in 1,849 patients, we found that the overall incidence of LT-PTLDs was 3.8%. Of these, the incidence in pediatric recipients (< 18 years at LT) was 5.1%, more than double the incidence of 2.3% in adults (≥ 18 years). Moreover, almost half of pediatric LT-PTLDs developed within a year after transplant (Fig. S3), and the intervals between LT and PTLD occurrence were significantly shorter in pediatric than in adult recipients. These results may imply that LT-PTLDs are more critical in pediatric rather than in adult recipients; however, the prognosis of adult LT-PTLDs was significantly worse than that of pediatric cases. Notably, the 3-, 5-, and 10-year OS rates after PTLD diagnosis were 81%, 79%, and 71% in children, whereas those in adults were as low as 61%, 38%, and 28%, respectively. These different oncological behaviors between children and adults may be attributable to the proportion of monomorphic PTLDs, which tended to occur more often in adults than in children (50% *vs.* 28%, $P = 0.07$). In other words, pediatric recipients are more likely to develop non-monomorphic PTLDs early after LT, which may have had a positive impact on patient survival.

As CNI is a well-known risk for PTLD development[2], we investigated the relationship between the incidence of PTLDs and the tacrolimus concentration. Of note, the incidence of pediatric LT-PTLDs decreased in the late period (2006–2020). As a possible reason for this, the

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tacrolimus trough in pediatric patients with LT-PTLDs was significantly higher in the early period than that in the late. These results may suggest that the high concentration of tacrolimus may, at least in part, be involved in the development of pediatric LT-PTLDs[38].

In this study, we identified the following three prognostic factors in LT-PTLDs: age ≥ 18 years at PTLD diagnosis, PS ≥ 2 at PTLD diagnosis, and monomorphic PTLDs. LT-PTLD Score consisting of these three factors significantly stratified patient survival and progression-free survival in LT-PTLDs. To date, the following prognostic factors have been reported for PTLDs after KT or various SOTs (Table-4): PS ≥ 2 or 3;[9-12, 14] monomorphic PTLDs;[6, 10] age ≥ 16,[14] > 55[6] or 60[11] years; LDH elevation;[6, 11, 12] hypoalbuminemia;[13] manifested B symptoms;[12] number of sites involved;[9] and involvement of transplanted organs,[10] CNS,[13] or bone marrow.[13] Taken together with the current results, age ≥ 18 years and PS ≥ 2 at PTLD diagnosis and monomorphic PTLDs may be universal prognostic factors for patient prognosis, regardless of transplanted organs.

Consistent with a previous report,[2] we demonstrated that EBV-positive PTLDs developed significantly earlier than EBV-negative ones ($P < 0.001$). In contrast, several reports have shown that EBV-naive recipients are at the highest risk to develop PTLDs,[39-41] and that primary EBV infection after SOT in EBV-naive recipients is a risk factor for early PTLDs.[42] In this study, however, pre-transplant EBV serologies were all positive in adults (100%), and even in the pediatric cohorts, 65.5% (19/29 cases) were EBV-positive preoperatively. Notably, no significant difference was found between EBV-seropositive and -negative patients in the timing of overall LT-PTLD onset. Although EBV-positive patients tended to develop LT-PTLDs earlier than EBV-naive cases in children, this difference did not reach statistical significance ($P = 0.06$, Fig. S2-E). It remains unclear whether these results are characteristic in LT-PTLDs; however, given

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that more than 95% of adults worldwide are infected with EBV,[43] further large-scale studies are needed, especially focusing on pediatric LT-PTLDs.

As for monitoring interventions for early detection of PTLDs, we have focused on sIL-2R. We examined sIL-2R in 25 LT-PTLD patients at diagnosis, in which 22 patients (88%) showed an increase in serum sIL-2R. Notably, monomorphic PTLDs showed higher sIL-2R than non-monomorphic types ($P = 0.09$, Table-S3). When the cutoff value of 1,800 U/mL, calculated from the receiver operating characteristic (ROC) curve, is indicated, serum sIL-2R is significantly higher in monomorphic PTLDs than in the others ($P = 0.02$, Table-S3). Although EBV-PCR was used for monitoring the PTLDs onset[44], early detection of monomorphic PTLDs seems difficult because EBV-PCR viral load was significantly lower in monomorphic PTLDs than in the others ($P = 0.03$, Table-S3). These results suggest that monomorphic PTLDs are less associated with EBV infection than non-monomorphic types. Taken together, satisfying both high sIL-2R and low viral load by EBV-PCR may suggest the presence of monomorphic PTLDs. Although further large-scale studies are needed, the combination of these two parameters may be useful for early detection of monomorphic PTLDs that require intensive treatments including chemotherapies.

The current study has several limitations. First, this is a retrospective, single-center study, which could not avoid potential selection bias. A multicenter study with a larger cohort is required to validate our findings. Second, we included PTLD-like lesions in the current analysis, although they are excluded from PTLDs in the standard definition.[26] However, their characteristics and required treatments are not different from those of PTLDs. Since PTLD-like lesions accounted for as much as 25% of the current cohorts, we consider that they should be recognized more widely as important forms of PTLDs. Third, there were missing values in some variables, including pre-transplant EBV/CMV serologies, EBV-PCR, or sIL-2R. Despite their significance in PTLD pathogenesis, they were not always measured, especially in the earlier era.

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In conclusion, LT-PTLDs occurred in 3.8% overall, 5.1% in pediatric, and 2.3% in adult LT recipients, which significantly worsened patient survival. Age ≥ 18 years and PS ≥ 2 at PTLD diagnosis, and monomorphic PTLDs were identified as independent prognostic factors for patient survival with LT-PTLDs. LT-PTLD Score consisting of these three factors effectively stratified patient survival and progression-free survival. Although required to be validated in an independent cohort, LT-PTLD Score may allow to distinguish high-risk cases of LT-PTLDs and to provide a potential guide for adequate interventions.

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Figure Legends

Figure 1. Time between LT and PTLD diagnosis

A. Annual incidence of LT-PTLDs

Annual incidence of LT-PTLDs was counted in the overall, pediatric (< 18 years at LT), and adult cohorts (≥ 18 years), separately (n = 67, 45, 22, respectively). While LT-PTLDs occurred at any time from early to late post-LT, it is noteworthy that almost half (49%) of pediatric LT-PTLDs developed within the first year after transplants.

B. Cumulative incidence of LT-PTLDs: Pediatric *vs.* Adult recipients

Pediatric LT-PTLDs developed significantly earlier than adult cases (P = 0.002 by a log-rank test). The shaded areas show 95% confidence intervals (CI) hereafter, unless otherwise indicated.

C. Cumulative incidence of LT-PTLDs: Monomorphic *vs.* Others

Monomorphic PTLDs developed significantly later than other types of LT-PTLDs (P < 0.001).

D. Cumulative incidence of LT-PTLDs: EBER+ *vs.* EBER-

LT-PTLDs with positive EBER developed significantly earlier than those without (P < 0.001).

E. Cumulative incidence of LT-PTLDs: EBV-seropositive *vs.* -naive

The curves for both cumulative incidence rates almost matched with each other, indicating no significant difference regarding the timing of PTLDs occurrence between EBV-seropositive and -naive recipients (P = 0.55).

Abbreviations: EBER, *Epstein-Barr* virus-encoded RNA; EBV, *Epstein-Barr* virus; LT, liver transplantation; PTLDs, post-transplant lymphoproliferative disorders.

Figure 2. Historical Transition of the Incidence Rate of Pediatric LT-PTLDs

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A. Annual incidence rate of monomorphic and non-monomorphic LT-PTLDs in children (< 18 years), given as the number of LT-PTLDs occurrence/number of LT per year, was investigated in the early (1990–2005) and the late period (2006–2020).

B. Cumulative incidence of pediatric LT-PTLDs: Early *vs.* Late period

The incidence of pediatric LT-PTLDs tended to decrease in the late than those in the early period ($P = 0.06$ by a log-rank test).

C. Historical Transition of tacrolimus trough level: Early *vs.* Late period

The trough level of tacrolimus in pediatric patients with LT-PTLDs was significantly higher in the early period than that in the late ($P < 0.001$ by a two-way ANOVA).

Abbreviations: ANOVA, analysis of variance; FK, tacrolimus; LT, liver transplantation; PTLDs, post-transplant lymphoproliferative disorders.

Figure 3. Historical Transition of the Incidence Rate of Adult LT-PTLDs

A. Annual incidence rate of monomorphic and non-monomorphic LT-PTLDs in adults (≥ 18 years), given as the number of LT-PTLDs occurrence/number of LT per year, was investigated in the early (1990–2005) and the late period (2006–2020).

B. Cumulative incidence of adult LT-PTLDs: Early *vs.* Late period

In contrast to pediatric LT-PTLDs, the incidence of adult LT-PTLDs showed no significant differences in adults between the two eras ($P = 0.18$ by a log-rank test).

C. Historical Transition of tacrolimus trough level: Early *vs.* Late period

The trough level of tacrolimus in adult patients with LT-PTLDs was not different between the two eras ($P = 0.10$ by a two-way ANOVA).

Abbreviations: ANOVA, analysis of variance; FK, tacrolimus; LT, liver transplantation; PTLDs, post-transplant lymphoproliferative disorders.

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Figure 4. Patient Survival after LT and PTLD diagnosis

The recipients who died within three months were excluded to eliminate the influence of early mortality from other causes, i.e. severe infections, refractory rejections, or intracranial bleeding in Figure 2-A, -B, and -C. The shaded areas show 95% CI.

A. Overall recipient survival: PTLDs *vs.* non-PTLDs

Comorbid LT-PTLDs significantly worsened recipient survival compared with those without ($P < 0.001$ by a log-rank test).

B. Pediatric recipient survival: PTLDs *vs.* non-PTLDs

Similarly, comorbid LT-PTLDs in the pediatric cohort (< 18 years) significantly worsened recipient survival compared with those without ($P < 0.001$).

C. Adult recipient survival: PTLDs *vs.* non-PTLDs

In the adult cohort, comorbid PTLDs significantly worsened recipient survival compared with those without ($P = 0.005$).

D. Overall patient survival in LT-PTLDs

The overall 3-, 5-, and 10-year patient survivals after LT-PTLD diagnosis were 74%, 66%, and 58%, respectively.

E. Overall patient survival in LT-PTLDs: pediatric *vs.* adult cases

The 3-, 5-, and 10-year pediatric patient survivals after LT-PTLD diagnosis were 81%, 79%, and 71%, respectively, while those of adults were 61%, 38%, and 28%, respectively. Pediatric PTLDs showed significantly better patient survival than adult PTLDs ($P = 0.005$).

F. Patient survival in LT-PTLDs: pediatric *vs.* adult cases

Patient deaths not related to PTLD were treated as "censored". LT-PTLDs-associated mortality was significantly lower in pediatric PTLDs than in adult ($P = 0.002$).

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Abbreviations: LT, liver transplantation; PTLDs, post-transplant lymphoproliferative disorders.

Figure 5. Significant Stratification of Patient Prognosis by LT-PTLD Score

Patient survival and progression-free survival proportions were analyzed according to "LT-PTLD Score" consisting of the following 3 independent prognostic factors: age ≥ 18 years (2 points) and performance status ≥ 2 (1 point) at LT-PTLD diagnosis, and monomorphic PTLDs (1 point). Patient deaths not related to PTLD were treated as "censored" in A and C.

A. Patients with LT-PTLDs were classified into 0–4 points by LT-PTLD Score, which effectively stratified the patient prognosis. As seen, LT-PTLD patients with point 0 showed 100% survival. The higher the LT-PTLD score, the worse the patient survival. ($P < 0.001$ by a log-rank test).

B. A flow-chart illustrating the prognostic scoring system, by which 0, 1–2, and 3–4 points are regarded as low, intermediate, and high risk for PTLD-related deaths, respectively,

C. LT-PTLD Score significantly stratified the patient prognosis. As clearly seen, LT-PTLDs-related mortality significantly worsened as the score increased ($P = 0.003$ in 0 *vs* 1–2; $P = 0.04$ in 1–2 *vs* 3–4; and $P < 0.001$ in 0 *vs* 3–4).

D and **E.** Similarly, overall survival including other causes of death ($P = 0.02$ in 0 *vs* 1–2, $P = 0.17$ in 1–2 *vs* 3–4, $P < 0.001$ in 0 *vs* 3–4, $P = 0.003$ in 0 *vs* 1–4, D) and progression-free survival ($P = 0.003$ in 0 *vs* 1–2, $P = 0.37$ in 1–2 *vs* 3–4, $P < 0.001$ in 0 *vs* 3–4, $P < 0.001$ in 0 *vs* 1–4, E) were both significantly and effectively stratified by LT-PTLD Score.

Taken all these together, these results demonstrated that LT-PTLD Score allows more precise estimation of patient prognosis with LT-PTLDs.

Abbreviations: Mono., monomorphic type; Non-mono., non-monomorphic type; PS, performance status; PTLDs, post-transplant lymphoproliferative disorders.

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Supplemental Figure 1. Flow-chart showing patient inclusion and exclusion

Abbreviations: DDLT, deceased-donor liver transplantation; LT, liver transplantation; LDLT, living-donor liver transplantation; Re-LT, liver re-transplantation

Supplemental Figure 2. Cumulative incidence of LT-PTLDs by Children and Adults

A and **B. Cumulative incidence of LT-PTLDs: Monomorphic type *vs.* Others**

Non-monomorphic PTLDs developed significantly earlier than the monomorphic type in both children (A) and adults (B) ($P = 0.04$ and $P = 0.007$ by log-rank tests, respectively).

C and **D. Cumulative incidence of LT-PTLDs: EBER+ *vs.* EBER-**

In children, LT-PTLDs with positive EBER developed significantly earlier than those without ($P = 0.04$). Though statistically not significant ($P = 0.40$) presumably due to the small number of cases, a similar trend was also observed in the adult cohort.

E and **F. Cumulative incidence of LT-PTLDs: EBV-seropositive *vs.* EBV-naive**

Although it did not reach a statistically significant difference ($P = 0.06$), EBV-seropositive children tended to develop LT-PTLDs earlier than in EBV-naive patients (E). In adult patients, Pre-transplant EBV serostatus was all positive (F).

Abbreviations: EBER, *Epstein-Barr* virus-encoded RNA; EBV, *Epstein-Barr* virus; LT, liver transplantation; PTLDs, post-transplant lymphoproliferative disorders.

Supplemental Figure 3. Time between LT and PTLD diagnosis by children and adults

Annual incidence of monomorphic and non-monomorphic LT-PTLDs were counted in both the pediatric (A, < 18 years at LT) and adult (B, ≥ 18 years) cohorts, separately ($n = 45$ and 22, respectively).

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A. Notably, 25 out of 33 non-monomorphic LT-PTLDs (75.8%) occurred within the first two years after pediatric LT, while such trend was not observed in the monomorphic type.

B. In contrast, LT-PTLDs occurred at any time from early to late post-LT in adults, regardless of morphological classifications (monomorphic or others).

Abbreviations: LT, liver transplantation; PTLDs, post-transplant lymphoproliferative disorders

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Table 1: Patient Characteristics, Perioperative Variables, and PTLD-related Variables

Characteristics	All (*n* = 67)	Pediatric (*n* = 43)	Adult (*n* = 24)	P-value
	No. or Median (IQR)			
Male/ Female	31/ 36	19/ 24	12/ 12	0.65
Age at LT (years)	2.1 (0.9–45.2)	1.2 (0.6–2.0)	54.8 (31.5–61.1)	**< 0.001**
Age at PTLDs (years)	6.1 (1.9–53.4)	2.8 (1.6−4.2)	60.0 (38.6−66.3)	**< 0.001**
BA/ Metabolic/ ALF/ HCV/ Others	38/ 5/ 6/ 8/ 10	35/ 3/ 2/ 0/ 3	3/ 2/ 4/ 8/ 7	**< 0.001**
Malignant/ Benign liver etiology	7/ 60	1/ 42	6/ 18	**0.004**
ABO incompatible/ not	11/ 56	9/ 34	2/ 18	0.26
EBV serology: +/ - at LT	39/ 10	19/ 10	20/ 0	**< 0.001**
CMV serology: +/ - at LT	37/ 11	20/ 9	17/ 2	0.09
EBV-PCR: +/-	31/ 9	25/ 1	6/ 8	**< 0.001**
EBER: +/-	35/ 24	30/ 9	5/ 15	**< 0.001**
PS: 0/ 1/ 2/ 3/ 4	2/ 46/ 13/ 4/ 2	0/ 28/ 10/ 4/ 1	2/ 18/ 3/ 0/ 1	0.06
Ann Arbor stage: I/ II/ III/ IV	35/ 9/ 10/ 12	22/ 4/ 8/ 8	13/ 5/ 2/ 4	0.44
Bulky tumor (>5cm)/ not	7/ 58	3/ 38	4/ 20	0.25

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Extranodal lesion ≥ 1/ not	28/ 39	13/ 30	15/ 9	**0.01**
LDH at PTLD (U/L)	372 (258–524)	375 (262-525)	346 (244-523)	0.70
sIL-2R at PTLD (U/mL)	2480 (1605–4225)	2660 (1433-4390)	2420 (1710-4150)	0.96
IPI: 0/ 1/ 2/ 3/ 4/ 5	7/ 21/ 16/ 8/ 5	5/ 13/ 9/ 5/ 2	2/ 8/ 7/ 3/ 3	0.85
Tacrolimus/ Cyclosporine	57/ 2	36/ 0	21/ 2	**0.049**
Monomorphic (DLBCL/ Burkitt)/ Polymorphic/ Non-destructive (PH/ IM/ FFH)/ PTLD-like	24 (13/ 7)/ 5/ 21 (5/ 14/ 2)/ 17	12 (6/ 4)/ 2/ 20 (5/ 13/ 2)/ 9	12 (7/ 3)/ 3/ 1 (0/ 1/ 0)/ 8	**0.005**
Chemotherapy/ not	23/ 39	11/ 28	12/ 11	0.16

The data are presented as median (interquartile range) for continuous variables and number for categorical variables. P-values < 0.05 is highlighted in bold.

Abbreviations: ALF, acute liver failure; BA, biliary atresia; CIT, cold ischemic time; CMV, cytomegalovirus; CNI, calcineurin inhibitor; DLBCL, diffuse large B-cell lymphoma; EBER, EBV-encoded RNA; EBV, Epstein-Barr virus; FFH, florid follicular hyperplasia; Follow-up, close follow-up/observation; GRWR, graft/recipient body-weight ratio; HCV, hepatitis-C virus; IM, infectious mononucleosis; IPI, international prognostic index; IS, immunosuppression; LDH, lactate dehydrogenase; LT, liver transplantation; MELD, Model for End-stage Liver Disease; NA, not applicable; OS, overall survival; PCR, polymerase chain reaction; PELD, Pediatric End-stage Liver Disease; PFS, progression-free survival; PH, plasmacytic hyperplasia; PS, performance status; PSC, primary sclerosing cholangitis; PTLD, post-transplant lymphoproliferative disorder; sIL-2R, soluble interleukin-2 receptor; WIT, warm ischemic time; +, positive; -, negative.

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Table 2: Univariate and Multivariate Analyses of Clinical Factors Affecting Patient Survival with LT-PTLDs

	Univariate Analysis			Multivariate Analysis		
	HR	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
Male sex	0.84	0.29–2.42	0.75			
Age at PTLDs ≥ 18 years	5.69	1.70–19.0	**0.005**	11.2	2.63–47.4	**0.001**
Primary disease: non-BA	3.60	1.17–11.1	**0.03**			
ABO incompatible	0.37	0.05‑2.82	0.38			
EBV-naive	NA*	NA*	NA*			
Positive EBV-PCR	0.72	0.14–3.76	0.70			
Positive EBER	0.62	0.18–2.17	0.46			
Performance Status ≥ 2	3.21	1.12‑9.17	**0.03**	6.77	1.56–29.3	**0.01**
Ann Arbor stage ≥ III	0.88	0.27‑2.86	0.83			
Bulky tumor ≥ 5cm	2.11	0.46‑9.80	0.34			
Extranodal lesion ≥ 1	5.00	1.53–16.3	**0.008**	0.38	0.07–2.20	0.28
LDH elevation	3.69	0.48‑28.7	0.21			

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IPI ≥ 3	1.36	0.36–5.16	0.65			
Time to PTLDs ≥ 1 year	1.23	0.41–3.69	0.72			
Monomorphic PTLDs	6.43	1.98‑20.8	**0.002**	6.78	1.40–32.9	**0.02**

CI indicates confidence interval. HR, hazard ratio. P-values < 0.05 is highlighted in bold.

Abbreviations: BA, biliary atresia; EBER, EBV-encoded RNA; EBV, Epstein-Barr virus; IPI, international prognostic index; LDH, lactate dehydrogenase; LT, liver transplantation; NA, not applicable; PCR, polymerase chain reaction; PTLDs, post-transplant lymphoproliferative disorders.

NA*: not applicable because no patients died due to PTLDs in either or both groups of the analyzed variable.

Table 3: Univariate Analyses of Clinical Factors Affecting Pediatric and Adult Patient Survival with LT-PTLDs

	Pediatric (*n* =43)			**Adult (*n* =24)**		
	HR	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
Male sex	1.67	0.28–10.0	0.57	0.43	0.10–1.75	0.24
Primary disease: non-BA	NA*	NA*	NA*	NA*	NA*	NA*
ABO incompatible	0.95	0.11‑8.47	0.96	NA*	NA*	NA*
PELD score	1.04	0.90–1.18	0.54	NA*	NA*	NA*
MELD score	NA*	NA*	NA*	1.02	0.94‑1.10	0.63

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EBV-naive	NA*	NA*	NA*	NA*	NA*	NA*
Positive EBV-PCR	NA*	NA*	NA*	2.71	0.36–20.6	0.34
Positive EBER	NA*	NA*	NA*	0.93	0.16–5.40	0.94
Performance Status ≥ 2	9.64	1.07-86.8	**0.04**	3.69	0.89-15.4	0.07
Ann Arbor stage ≥ III	1.90	0.27-13.5	0.52	0.64	0.13-3.08	0.57
Bulky tumor ≥ 5cm	5.94	0.54-65.5	0.15	0.82	0.01-6.66	0.85
Extranodal lesion ≥ 1	4.46	0.73–27.3	0.11	4.05	0.79–20.8	0.09
LDH elevation	NA*	NA*	NA*	3.17	0.38–26.1	0.28
IPI ≥ 3	NA*	NA*	NA*	1.45	0.34–6.14	0.61
Time to PTLDs ≥ 1 year	0.68	0.11–4.14	0.68	1.14	0.23–5.76	0.87
Monomorphic PTLDs	13.7	1.51-124.2	**0.02**	4.71	0.87-25.5	0.07

CI indicates confidence interval. HR, hazard ratio. P-values < 0.05 is highlighted in bold.

Abbreviations: BA, biliary atresia; EBER, EBV-encoded RNA; EBV, Epstein-Barr virus; LDH, lactate dehydrogenase; IPI, international prognostic index; LT, liver transplantation; MELD, Model for End-stage Liver Disease; NA, not applicable; PCR, polymerase chain reaction; PELD, Pediatric End-stage Liver Disease; PTLDs, post-transplant lymphoproliferative disorders.

NA*: not applicable because no patients died due to PTLDs in either or both groups of the analyzed variable.

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Table 4: Previously Reported Prognostic Factors in PTLDs

Year	Journal	First Author	Transplanted organs and patient numbers	Prognostic factors
2001	JCO	Leblond V.	Kidney: 35, Heart: 19, Lung: 5, Liver: 3	PS ≥ 2, number of involved sites
2005	JCO	Ghobrial IM.	Kidney: 36, Kidney & Pancreas: 7, Pancreas: 5, Liver: 35, Lung: 4, Heart: 15, Multiorgan/ Other: 5	PS ≥ 3, monomorphic PTLDs, graft organ involvement
2007	Ann Hematol.	Choquet S.	Heart: 16, Kidney: 22, Lung: 11, Kidney & Pancreas/ Heart & Lung: 11	Age > 60 years, PS ≥ 2, elevated LDH
2008	Br J Haematol.	Hourigan MJ.	Kidney: 42	PS ≥ 2, elevated LDH, B symptoms
2010	JCO	Evens AM.	Kidney: 37, Kidney & Pancreas: 9, Pancreas: 4, Liver: 17, Heart: 8, Lung: 5	CNS involvement, BM involvement, hypoalbuminemia
2013	JCO	Caillard S.	Kidney: 500	Age > 55 years; serum creatinine > 133 μmol/L; elevated LDH; disseminated PTLDs; CNS involvement; serous membrane invasion; T-cell PTLDs; monomorphic PTLDs
2015	Br J	Montanari F.	Heart: 63, Kidney: 32, liver: 22, Other: 10	Age ≥ 16 years, PS ≥ 2, CD20 negative

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	Haematol.			

Abbreviations: Ann Hematol., Annals of Hematology; BM, bone marrow; Br J Haematol., British Journal of Haematology; CD, cluster of differentiation; CNS, central nervous system; JCO, Journal of Clinical Oncology; LDH, lactate dehydrogenase; PS, performance status; PTLDs, post-transplant lymphoproliferative disorders.

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Figure 1

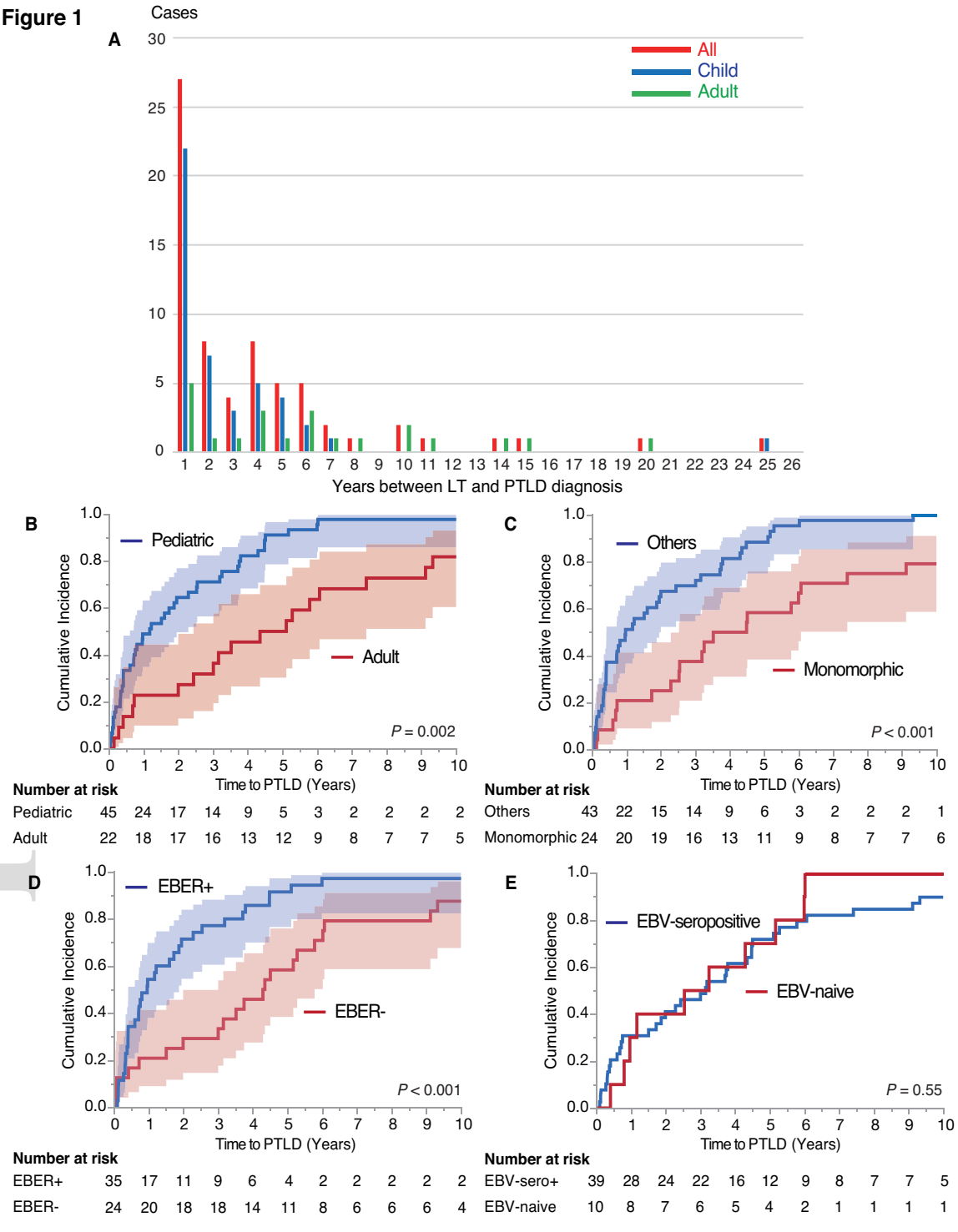

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Figure 2

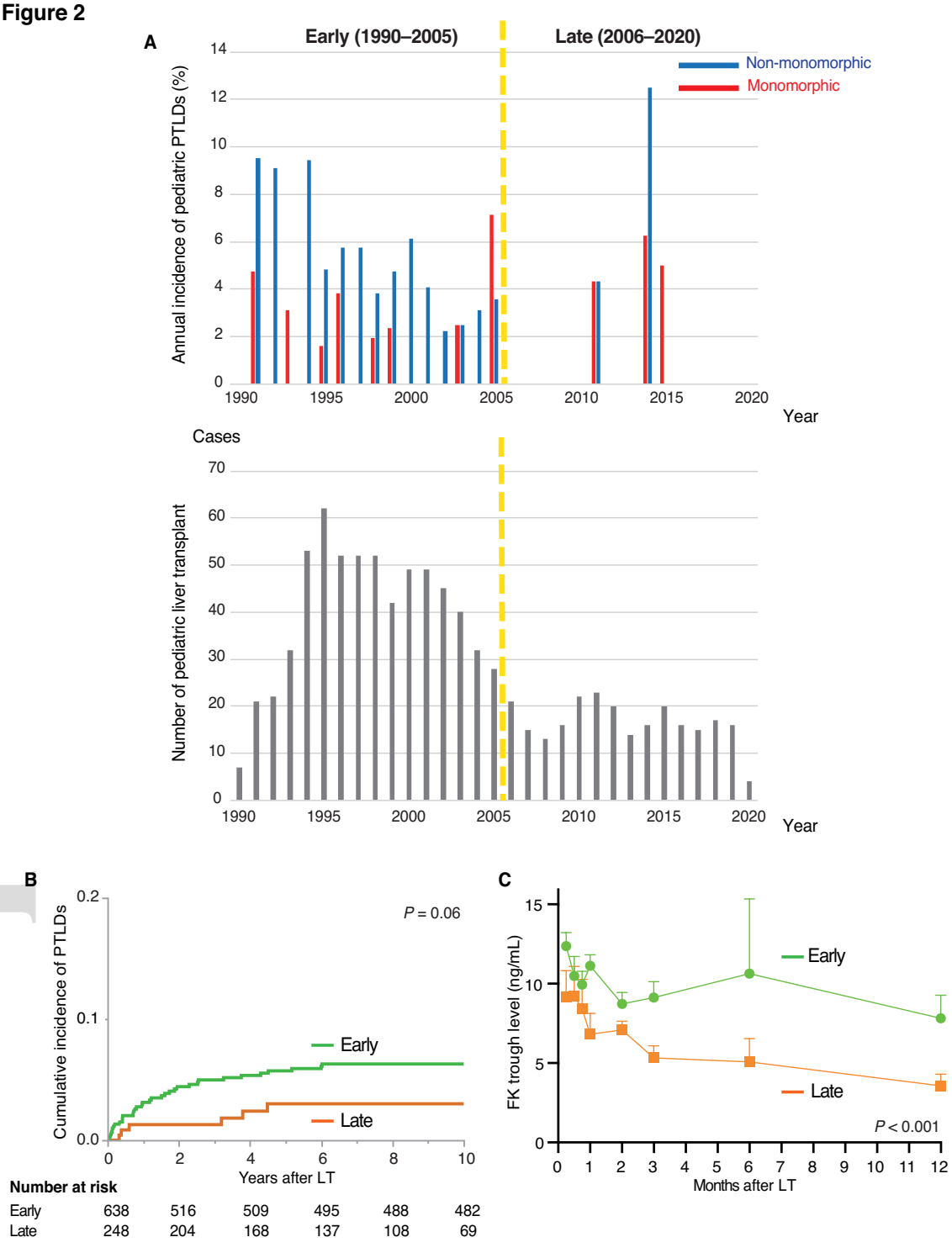

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Figure 3

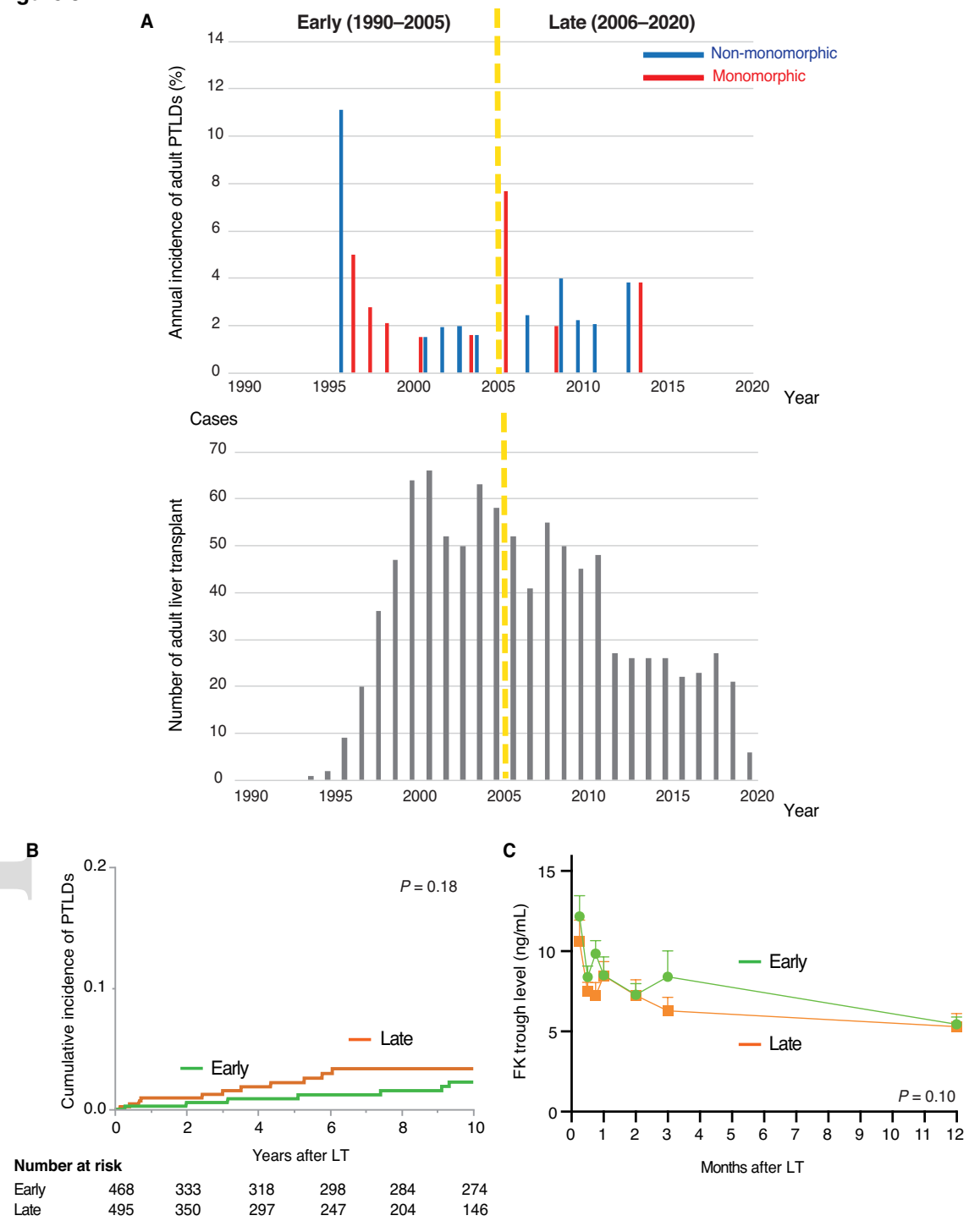

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Figure 4

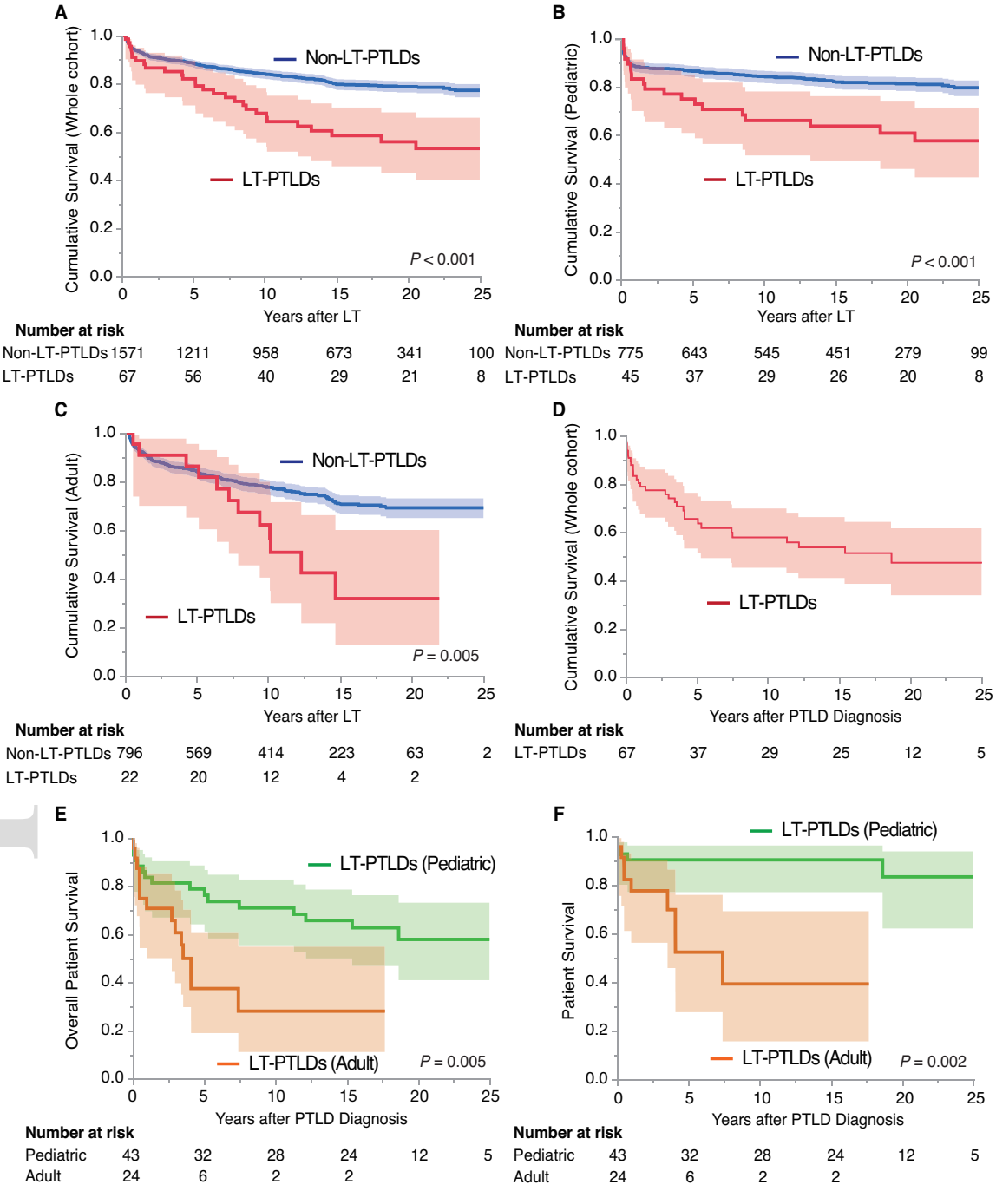

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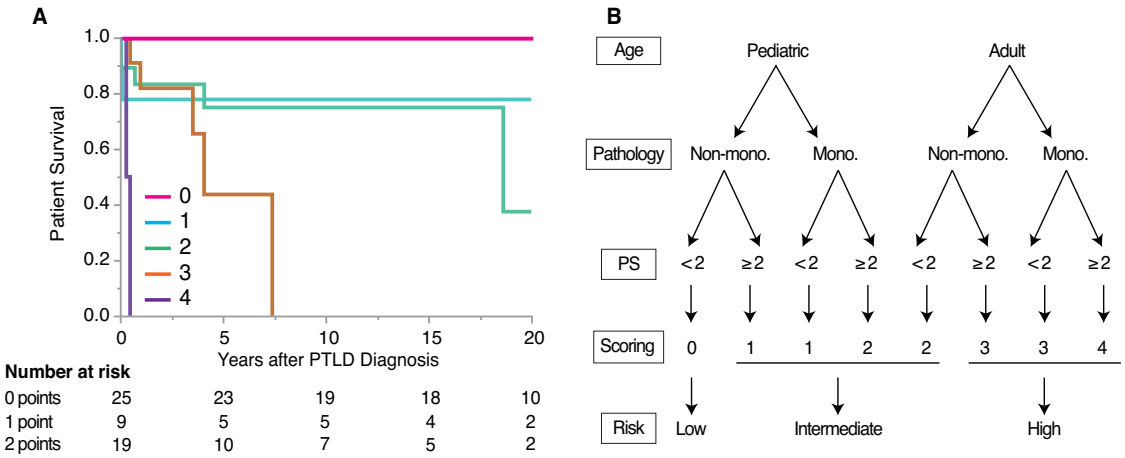
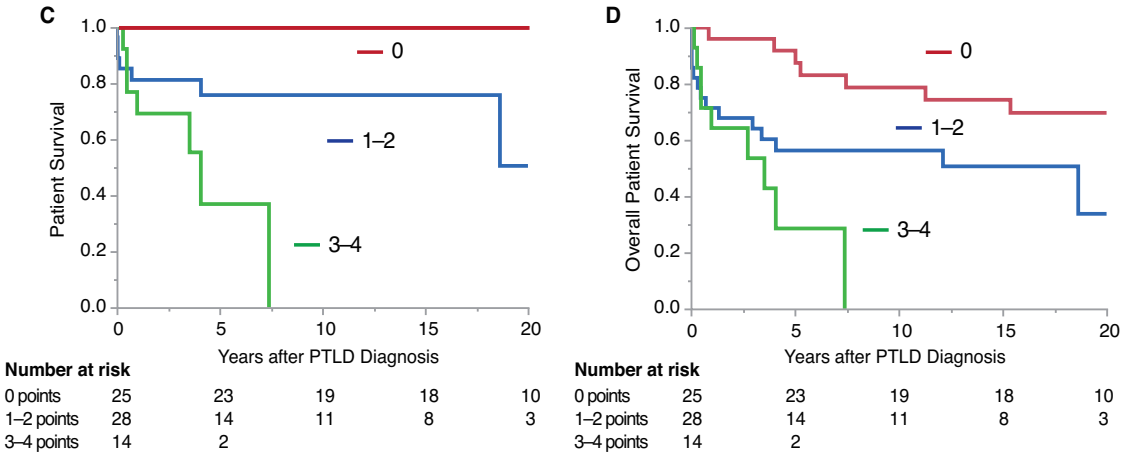
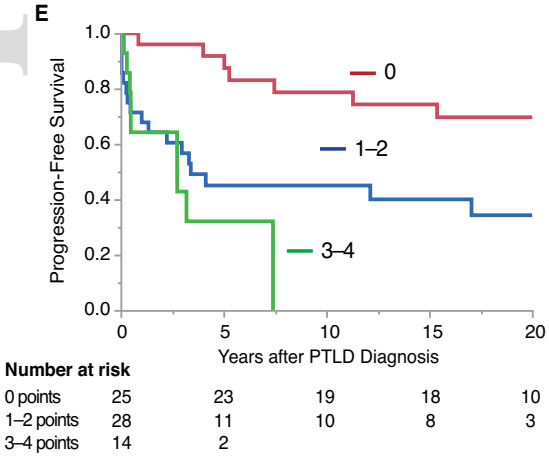
Figure 5







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