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Risk Factors Associated with PTLD Related Mortality in Adult Multivisceral Transplant Recipients – A Single Centre Cohort Study

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Abbreviations:
ACR: Acute Cellular Rejection;
ATG: rabbit antithymocyte globulin;
CHOP: cyclophosphamide, doxorubicin hydrochloride (hydroxydaunorubicin), vincristine sulfate (Oncovin), and prednisone;
CMV: Cytomegalovirus;
CTL: cytotoxic T lymphocyte;
EBV: Ebstein-Barr Virus;
ECOG: Eastern Cooperative Oncology Group;
EFS: Event-free Survival;
IPI: International Prognostic Index;
IS: immunosuppression;
MVTx: Full Multivisceral Transplant;

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Rezumat

Factori de risc asociați cu mortalitatea TLPT în transplantul multivisceral la primitori adulți - un studiu de cohortă

Background: Tulburările limfoproliferative post transplant (TLPT) constituie un grup heterogen de afecțiuni limfoproliferative care contribuie semnificativ la mortalitatea după transplantul multivisceral (MVTx). Acest studiu îşi propune să identifice potențialii factori de risc asociați cu mortalitatea primitorilor de grefe abdominale multiviscerale care au dezvoltat TLPT.
Metode: În studiu au fost incluşi toți primitorii adulți transplantați cu grefe multiviscerale care cuprind şi intestinul, în cadrul instituției noastre între 2013-2022, şi care au dezvoltat TLPT (21 de pacienți).
Rezultate: Mortalitatea asociată cu TLPT a fost 28.6% (6/21). Un risc relativ crescut de mortalitate s-a asociat cu un stadiu de performanță ECOG=3 (p=0.005; HR 34.77; 95%CI 2.94-410.91), dacă primitorii au avut o splenectomie prealabilă (p=0.036; HR 14.36; 95%CI 1.19- 172.89), sau un retransplant cu grefă multivisceralã (p=0.039; HR 11.23; 95% CI 1.13-112.12). S-a observat o tendință semnificativă pentru creşterea riscului de mortalitate prin TLPT în cazurile cu încărcătură virală EBV crescută (p=0.008), cu interval de timp crescut de la momentul transplantului la momentul diagnosticării TLPT (p=0.008), şi la

MMVTx: Modified Multivisceral Transplant;
OS: Overall Survival;
PTLD: Post-transplant lymphoproliferative disorder;
PCR: Polymerase Chain Reaction;
SOT: Solid Organ Transplantation,
TRM: treatment-related mortality.

donatorii cu vârstă înaintată (p<0.001). Valoarea maximă a LDH înainte de inițierea terapiei pentru TLPT a fost semnificativ mai mare în grupul de pacienți decedați comparativ cu grupul de pacienți supraviețuitori (520.3 ± 422.8 IU/L vs 321.8 ± 154.4 IU/L; HR 1.00, 95%CI 1.00 to 1.01, p=0.019). Valoarea maximă a încărcăturii virale înainte de inițierea terapiei antiTLPT (Cycle Threshold (CT) cutoff = 32) s-a corelat cu riscul relativ de deces în grupul de pacienți transplantați multivisceral care au dezvoltat TLPT [(29.4 ± 3.5 CT) la supraviețuitori, comparativ cu 23 ± 4.0 CT la pacienții decedați].
Concluzii: Acesta este primul studiu care identifică factorii de risc pentru mortalitatea TLPT la primitorii adulți de transplant multivisceral. Validarea acestor rezultate în cadrul unor cohorte mai ample, în studii multicentrice şi stratificarea ulterioară a riscurilor cuantificate în conformitate cu aceşti factori de risc, au potențialul de a contribui la o supraviețuire post transplant mai îndelungată a acestor pacienți.

Cuvinte cheie: transplant multivisceral, tulburări limfoproliferative post transplant, încărcătură virală Epstein-Barr

Abstract

Background: PTLD is a heterogeneous group of lymphoproliferative diseases which can add significant mortality following multivisceral transplantation (MVTx). Our study aimed to identify potential risk factors of mortality in adult MVTx recipients who developed PTLD.
Methods: All adult recipients of intestinal-containing grafts transplanted in our institution between 2013 and 2022, and who developed PTLD, were included in the study.
Results: PTLD-associated mortality was 28.6% (6/21). Increased relative risk of mortality was associated with Stage 3 ECOG performance score (p=0.005; HR 34.77; 95%CI 2.94-410.91), if the recipients had a splenectomy (p=0.036; HR 14.36; 95%CI 1.19-172.89), or required retransplantation (p=0.039; HR 11.23; 95% CI 1.13-112.12). There was a significant trend for increased risk of PTLD mortality with higher peak EBV load (p=0.008), longer time from MVTx to PTLD diagnosis (p=0.008), and higher donor age (p<0.001). Peak LDH before treatment commencement was significantly higher in the mortality group vs the survival group (520.3 ± 422.8 IU/L vs 321.8 ± 154.4 IU/L; HR 1.00, 95%CI 1.00 to 1.01, p=0.019). Peak viral load prior to treatment initiation (Cycle Threshold (CT) cutoff = 32) correlated with the relative risk of death in MVTx patients who developed PTLD [29.4 (3.5) CTs in survivors compared to 23.0 (4.0) CTs in the mortality group].
Conclusions: This is the first study to identify risk factors for PTLD-associated mortality in an adult MVTx recipient cohort. Validation in larger multicentre studies and subsequent risk stratification according to these risk factors may contribute to better survival in this group of patients.

Key words: multivisceral Transplantation, post-transplant Lymphoproliferative disorders, Epstein-Barr viraemia

Introduction

PTLD continues to be a frequent complication (1), occurring at fivefold increased rates compared with the general population, and constitutes the leading cause of malignancy-related mortality in SOT (2). EBV has a pivotal role in the genesis of PTLD due to its ability to transform and immortalize B lymphocytes (3). Such cells have the potential for uncontrolled proliferation, which occurs particularly in the absence of cytotoxic T-cell control, such as during immunosuppression associated with organ transplantation. The ubiquity of EBV (4), associated with engraftment of multiple organs, rich with lymphatic tissue, as well as the requirement of highly immunosuppressive regimens in MVTx recipients account for the increased incidence (13-32%) (5) and unfavourable prognosis of PTLD in this population. Key to decreasing the high mortality in this cohort of patients is understanding the risk factors associated with decreased survival. The incidence of EBV+ PTLD is highest in intestinal transplant recipients and portends a more severe prognosis compared to the pediatric population. Previous studies (6), performed on a mixture of SOT recipients analysing outcomes depending on gene expression of MYC, BCL2, and BCL6 and IPI score, only partially capture the mortality impact of PTLD in this population. To the best of our knowledge this is the first paper addressing this issue in a cohort of adult MVTx recipients.

Methods

This retrospective cohort study analysed 21 adults (≥ 18 yrs) diagnosed with PTLD after multivisceral transplants at the Sir Roy Calne Transplant Unit in the Addenbrookes Hospital in Cambridge, UK, between December 10th 2013 and August 31st, 2022. The data was retrieved from the intestine transplant program research database maintained at our centre. This project was registered and approved as a service evaluation by the Trust Audit Department (PRN/IRB: ID5099). In total 88 adult patients had been consecutively transplanted with Small-Bowel-containing grafts in that period of time in our Unit. No patient was excluded from this study. The types of operation performed were: (i) Full Multivisceral Transplant n=7 (33%); (ii) Modified Multivisceral Transplant (liver not included) n= 3 (14.28%) and (iii) isolated small bowel (SB) n=11 (52.38%). All the donor and recipient operative procedures adhered to our unit protocol and have been previously described (7). Three of the isolated SB recipients (14.28%) received concomitant kidney transplants from the same donor. All patients received 1 or 2 doses of Alemtuzumab as Immunosuppression Induction. The indications for intestine transplant consisted of: chronic intestinal failure with complications related to long-term TPN; cirrhosis with extensive porto-mesenteric thrombosis; unresectable abdominal desmoids and acute abdominal vascular catastrophes. Maintenance of Immunosuppression was achieved with Tacrolimus, mycophenolate mofetil and Prednisolone. Target trough tacrolimus levels in the first 3 months post Tx were between 8 and 12 ng/dL. Subsequently, these were decreased to 6 to 10 ng/dL. Episodes of ACR or renal impairment due to tacrolimus nephrotoxicity prompted changes to this protocol. Mild ACR was pulsed with 1 g methylprednisolone/day X 3 and a taper, while severe ACR was treated with a further dose of Alemtuzumab or a course of up to 14 days of ATG. In order to mitigate the relative risk of graft versus host disease, none of the grafts included the donor spleen. Nevertheless, a splenectomy was performed in 6 patients (28.57%) at the time of transplant, while 1 patient had had a splenectomy in childhood. Additional details of each patient's EBV history included number, duration, and degree of EBV viremia episodes. The degree of EBV viremia was determined by the number of cycle thresholds (CT) required for the virus to be detectable. EBV viremia was further categorized based on peak PCR across all pre-treatment episodes. All demographic and clinical data inputted in the electronic hospital records (or paper before 2014) was used in the

analysis. All missing data was due to non-systematic lack of imputation in records.

Definitions

B symptoms were defined as at least one characteristic symptom (weight loss/fever/drenching night sweats) documented within 1 month of the PTLD diagnosis. EBV viremia was defined as positive EBV PCR in plasma within 1 month of the date of the PTLD diagnosis (8). All patients who developed EBV-viremia as per our unit cutoff threshold (CT<34) were diagnosed with PTLD. None of the EBV-viremia patients above the cutoff developed PTLD. PTLDs were either histologically diagnosed by expert pathologists with excisional biopsies (16 cases) or via [18F]-fluorodeoxyglucose positron emission tomography (PET)/CT when the diagnosis could be established in the absence of a biopsy (5 cases – all with EBV viremia). Patients with high EBV viral load, unexplained fever, or lymphadenopathy underwent weekly EBV DNA measurements and this was continued until resolution of symptoms and of disease. Thereafter EBV DNA monitoring was performed as per the Clinic appointments of the patients. The management of PTLD was always undertaken in conjunction with the haematology team. Initial treatment consisted of 4 weekly doses (375 mg/m^2 4 weekly iv) of Rituximab until FDG-PET evidence of disease regression. Second line treatments included further doses of Rituximab (n=7), chemotherapy (n=3) or EBV-specific cytotoxic T cells (n=2).

Statistical Analyses

Categorical risk factors were presented as counts and percentages, and continuous risk factors were presented as means with SDs, all stratified by PTLD death status. Completeness of each risk factor was reported as counts and percentages. All analyses were complete case analysis. Cox regression was used to estimate age- and sex-adjusted hazard ratios (HRs), assessing associations between PTLD death and individual risk factors in adults (≥18y) measured at the time of PTLD diagnosis, August 31st 2022. Follow-up began at PTLD diagnosis and ended at the earliest of death, date of last follow up, or August 31st, 2022, when the study ended. The small sample size prevented fitting of a multivariable model to simultaneously adjust for all confounding variables. Likelihood ratio tests (LRT) compared age- and sex-adjusted Cox models with and without each categorical risk factor or continuous risk factor. Continuous risk factors were modelled as penalised splines to allow non-linear associations with PTLD mortality. Predictor complexity (degrees of freedom) was chosen using the Akaike Information Criterion. Multilevel categorical risk factors were modelled without amalgamating levels to assess the collective association of the multilevel risk factor with PTLD mortality. We employed the Benjamini-Hochberg method to adjust the P-values for multiple comparisons, controlling the false discovery rate. To investigate potential cutpoints for dichotomizing the EBV peak viral load (Ct) in relation to PTLD death, we employed a maximally selected log-rank statistic test and plotted Martingale residuals plots, hazard ratios comparing EBV peak viral load below each potential cutpoint to that above or equal to that cutpoint, and the proportion of cases with EBV peak viral load less than each potential cutpoint. $P \leq 0.05$ was considered to be statistically significant. All statistical analyses were done in R version 4.0.2 (2020-06-22) -- "Taking Off Again".

Results

This study represents a single-centre report over a 9 year period on potential risk factors associated with increased mortality in adult MVTx recipients who developed EBV-driven PTLD, and reveals several clinically-significant findings. Baseline characteristics are shown in *Table 1*. The median follow-up time in days for individuals who either died or did not die due to PTLD were 49 (IQR 26-112) and 778 (IQR 485-1827), respectively. *Fig. 1* shows a Kaplan-Meier plot of the survival probability

Table 1. Characteristics of individuals diagnosed with PTLD after multivisceral transplants at the Addenbookes Hospital from 10 December 2013 to 31 August 2022, stratified by whether or not they died of PTLD during follow-up. (N=21)

Characteristics	Did not die of PTLD during follow-up (n=15)		Died of PTLD during follow-up (n=6)	
	% (n) for categorical; mean (SD) for continuous	Missing % (n)	% (n) for categorical; mean (SD) for continuous	Missing % (n)
Sex	F: 40.0 % (6), M: 60.0 % (9)	0.0 % (0)	F: 50.0 % (3); M: 50.0 % (3)	0.0 % (0)
ECOG	0: 100.0 % (15), 3: 0.0 % (0)	0.0 % (0)	0: 50.0 % (3); 3: 50.0 % (3)	0.0 % (0)
Type of transplant (liver containing multivisceral graft or not)	no_liver: 80.0 % (12), liver: 20.0 % (3)	0.0 % (0)	no_liver: 33.3 % (2), liver: 66.7 % (4)	0.0 % (0)
Acute cellular rejection (Y/N)	0: 73.3 % (11), 1: 26.7 % (4)	0.0 % (0)	0: 33.3 % (2), 1: 66.7 % (4)	0.0 % (0)
Treatment for acute cellular rejection (Y/N)	0: 86.7 % (13), 1: 13.3 % (2)	0.0 % (0)	0: 50.0 % (3), 1: 50.0 % (3)	0.0 % (0)
Number of immunosuppression induction doses (Alemtuzumab)	1: 46.7 % (7), 2: 53.3 % (8) 3: 0.0 % (0)	0.0 % (0)	1: 33.3 % (2), 2: 50.0 % (3) 3: 16.7 % (1)	0.0 % (0)
Multivisceral retransplant (Y/N)	0: 93.3 % (14), 1: 6.7 % (1)	0.0 % (0)	0: 66.7 % (4), 1: 33.3 % (2)	0.0 % (0)
Number of extranodal sites involved	0: 33.3 % (5), 1: 46.7 % (7) 2: 20.0 % (3)	0.0 % (0)	0: 0.0 % (0), 1: 50.0 % (3) 2: 50.0 % (3)	0.0 % (0)
PTLD affecting: 1 - graft only; 0 - extragraft; 2 - both graft and extragraft	0: 33.3 % (5), 1: 40.0 % (6) 2: 26.7 % (4)	0.0 % (0)	0: 16.7 % (1), 1: 16.7 % (1) 2: 66.7 % (4)	0.0 % (0)
PTLD multiorgan - extragraft localisation	0: 80.0 % (12), 1: 20.0 % (3)	0.0 % (0)	0: 33.3 % (2), 1: 66.7 % (4)	0.0 % (0)
B symptoms (Y/N)	0: 46.7 % (7), 1: 53.3 % (8)	0.0 % (0)	0: 16.7 % (1), 1: 83.3 % (5)	0.0 % (0)
EBV positive (Y/N)	0: 6.7 % (1), 1: 93.3 % (14)	0.0 % (0)	0: 16.7 % (1), 1: 83.3 % (5)	0.0 % (0)
Splenectomy (Y/N)	0: 73.3 % (11), 1: 26.7 % (4)	0.0 % (0)	0: 16.7 % (1), 1: 83.3 % (5)	0.0 % (0)
Donor/recipient EBV serology	D+R+: 46.7 % (7), D-R+: 20.0 % (3), D?R+: 6.7 % (1), D+R-: 20.0 % (4)	0.0 % (0)	D+R+: 16.7 % (1), D-R+: 33.3 % (2), D?R+: 16.7 % (1), D+R-: 33.3 % (2)	0.0 % (0)
Donor/recipient CMV serology	D+R+: 13.3 % (2), D-R-: 53.3 % (8), D- (0) R+: 6.7 % (1), D?R-: 6.7 % (1), D?R+: 0.0 % (0), D+R-: 20.0 % (3)	0.0 % (0)	D+R+: 0.0 % (0), D-R-: 33.3 % (2), D- (0) R+: 33.3 % (2), D?R-: 0.0 % (0), D?R+: 16.7 % (1), D+R-: 16.7 % (1)	0.0 % (0)
IPI Stage	0: 30.8 % (4), 1: 46.2 % (6) 2: 23.1 % (3), 3: 0.0 % (0) 4: 0.0 % (0)	13.3 % (2)	0: 16.7 % (1), 1: 33.3 % (2) 2: 0.0 % (0), 3: 16.7 % (1) 4: 33.3 % (2)	0.0 % (0)
Age at first Tx (years)	45.4 (14.2)	0.0 % (0)	43.8 (13.0)	0.0 % (0)
Donor age (years)	32.6 (15.0)	20.0 % (3)	21.2 (10.6)	33.3 % (2)
Duration of immunosuppression overdose (days)	11.7 (4.6)	0.0 % (0)	11.2 (9.5)	0.0 % (0)
LDH tumorlysis (Y/N)	321.8 (154.4)	20.0 % (3)	520.3 (422.8)	0.0 % (0)
EBV peak viral load (CT)	29.4 (3.5)	0.0 % (0)	23.0 (4.0)	0.0 % (0)
EBV peak viral load duration (days)	29.2 (30.2)	0.0 % (0)	29.3 (26.2)	0.0 % (0)
Time from Tx to PTLD diagnosis (days)	229.1 (210.4)	0.0 % (0)	471.5 (937.6)	0.0 % (0)
Follow up time (days)	1111.9 (866.6)	0.0 % (0)	449.8 (971.5)	0.0 % (0)
Age at PTLD diagnosis (years)	46.0 (14.2)	0.0 % (0)	45.1 (13.2)	0.0 % (0)

in this group of patients. There was a 28.6% mortality associated with PTLD (6/21) (*Table 1*). As expected, a majority (19/21, 90.47%) of the patients had EBV-driven PTLD. The study group had a slight female predominance (57.14%). However, in the group of patients who died with PTLD, the M/F ratio was 1:1. There was no difference between the two

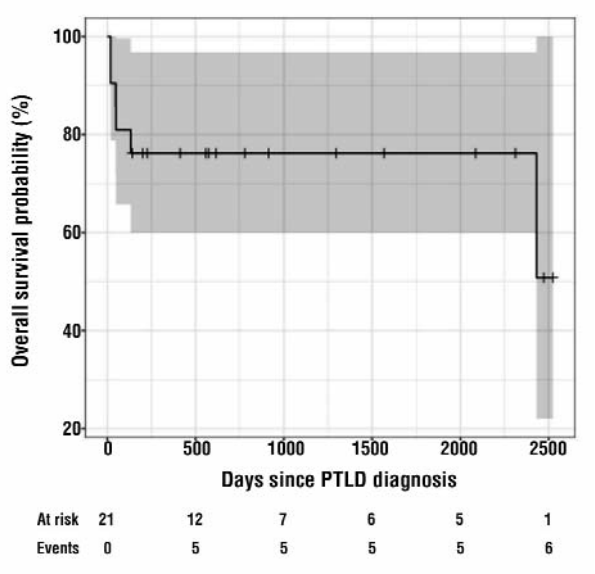

Figure 1. Kaplan-Meier curve showing time from PTLD diagnosis to PTLD death in multivisceral transplant recipients

groups in terms of age (years ± standard deviation) at first transplant: 45.4 (± 14.2) in the survivor group and 43.8 (± 13.0) in the death group, respectively. While PTLD survivors received grafts from older older donors (32.6 yrs ± 15.0) compared to the PTLD deaths group (21.2 yrs ± 10.6), the large number of missing donor age data (5/21, 23.8%) would preclude drawing any conclusion from this particular finding. *Table 2* shows age and sex- adjusted associations between PTLD mortality and binary clinical characteristics. Age- and sex-adjusted HRs indicated risk of PTLD mortality was significantly decreased for lower values of peak EBV load [expressed in cycle thresholds (p=0.009; HR 0.63; 95%CI 0.45-0.89)] (*Table 2*), and was significantly elevated for Stage 3 ECOG performance score (p=0.005; HR 34.77; 95%CI 2.94- 410.91), if the recipients had a splenectomy (p=0.036; HR 14.36; 95%CI 1.19-172.89), or required retransplantation (p=0.039; HR 11.23; 95% CI 1.13-112.12). However the wide confidence intervals for the three latter variables due to small sample size should be noted. *Table 3* shows age and sex-adjusted associations between PTLD mortality and categorical clinical characteristics. Likelihood ratio tests

Table 2. Associations between risk factors and PTLD mortality. Age- and sex-adjusted hazard ratios (95 % CI) for PTLD mortality from univariate (other than age and sex) linear Cox PH regression. Not calculated indicates insufficient data to perform the calculation

Characteristic	HR (95 % CI)	P-value	FDR adjusted p-value
Easter Cooperative Oncology Group (ECOG) stage 3	34.77 (2.94,410.91)	0.005	0.0629
EBV peak viral load cycle threshold	0.63 (0.45,0.89)	0.009	0.0629
LDH tumour lysis syndrome	1.00 (1.00,1.01)	0.019	0.087
Splenectomy	14.36 (1.19,172.89)	0.036	0.1454
Multivisceral retransplantation	11.23 (1.13,112.12)	0.039	0.1454
PTLD treatment with Rituximab	0.59 (0.35,1.00)	0.050	0.1708
Age at first transplant	0.62 (0.37,1.03)	0.067	0.178
Duration from transplant to PTLD diagnosis	1.00 (1.00,1.00)	0.067	0.178
Liver included in transplanted organs	6.54 (0.85,50.34)	0.071	0.178
Acute rejection episode posttransplant	6.95 (0.78,61.62)	0.082	0.178
Treatment of acute rejection episode	5.42 (0.70,42.13)	0.106	0.2173
Presence of B symptoms	7.04 (0.62,80.56)	0.117	0.2284
Positive crossmatch	3.79 (0.62,23.15)	0.148	0.2545
EBV donor/recipient mismatch	5.55 (0.54,56.71)	0.149	0.2545
PTLD detected in more >1 organ	3.34 (0.54,20.82)	0.197	0.3107
Donor age	0.94 (0.83,1.05)	0.278	0.4071
International prognostic index (IPI) stage >1	2.83 (0.29,27.11)	0.368	0.5029
PTLD in intestinal transplant	1.95 (0.21,18.17)	0.556	0.6908
CD20 positive	0.56 (0.05,6.73)	0.650	0.7838
Induction immunosuppression doses >2	1.47 (0.25,8.63)	0.670	0.7849
Duration of abnormal EBV viremia	1.00 (0.96,1.03)	0.866	0.9376
Duration of immunosuppression overdose (days)	0.99 (0.85,1.15)	0.869	0.9376
More than 1 extranodal sites involved by PTLD	not calculated	0.999	0.999
CMV donor/recipient mismatch	not calculated	0.999	0.999
PTLD morphology (monomorphic or other than polymorphic)	not calculated	0.999	0.999

Table 3. Likelihood ratio tests (LRT) compared age- and sex-adjusted Cox models with and without each categorical risk factor. We present hazard ratios and their 95% confidence intervals for each contrast level compared to the reference level of each variable. The unadjusted and FDR-adjusted p-values correspond to the LRT. Not calculated indicates insufficient data to perform the calculation

Characteristic	Characteristic level	HR (95% CI)	P-value	FDR adjusted p-value
Number of doses of Alemtuzumab, induction immunosuppression (vs dose = 1)	Dose = 2	1.33 (0.20, 8.66)	0.843	0.938
	Dose = 3	2.31 (0.14, 38.24)		
Number of extranodal sites (vs number = 0)	Number = 1	Not calculated	-	-
	Number = 2	Not calculated		
PTLD graft (vs PTLD graft = 0)	PTLD graft = 1	0.78 (0.04, 14.39)	0.401	0.531
	PTLD graft = 2	2.99 (0.30, 29.93)		
PTLD morphology (vs plasmacytic hyperplasia)	Infectious mononucleosis	Not calculated	-	-
	Monomorphic	Not calculated		
	Polymorphic	Not calculated		
EBV status (vs donor positive recipient positive)	Donor negative recipient positive	5.01 (0.38, 66.64)	0.291	0.412
	Donor indeterminate recipient positive	17.87 (0.62, 511.46)		
	Donor positive recipient negative	6.12 (0.45, 83.37)		
IPI stage (vs stage = 0)	stage = 1	1.77 (0.25, 12.43)	0.019	0.087
	stage = 2	Not calculated		
	stage = 3	476.39 (28.64, 7924.37)		
	stage = 4	19.96 (2.51, 158.58)		
Donor/recipient CMV status (vs donor positive recipient positive)	Donor negative/ recipient negative	Not calculated	-	-
	Donor negative/ recipient positive	Not calculated		
	Donor indeterminate/ recipient negative	Not calculated		
	Donor indeterminate/ recipient positive	Not calculated		
	Donor positive recipient negative	Not calculated		

further identified significant associations between PTLD mortality and International Prognostic Index, (IPI) although confidence intervals were wide (Stage 1 HR 1.77 (0.25-1.24), Stage 3 HR 476.39 (28.64-7924.37), Stage 4 HR 19.96 (2.51-158.58). *Fig. 2* shows the association of PTLD mortality with continuous clinical characteristics. The width of confidence intervals precludes the finding of clinical meaningful results for most characteristics, except for a significant trend for increased risk of PTLD mortality with lower peak EBV load (p=0.008), longer time from MVTx to PTLD diagnosis (p<0.001), and donor age (p<0.001). PTLD patients in our study group who succumbed with their disease were more likely to have had at least one episode of acute rejection (66.7% vs 26.7%, NS) and therefore exposed to more immunosuppression. Moreover, although this did not reach statistical significance, 50% of the PTLD patients who died (3/6) had been treated for acute rejection, compared to only 13.3% (2/15) in the PTLD survivors' group. Nevertheless, survival was not influenced by the number of induction doses (1 vs 2 doses of Alemtuzumab) (HR=1.47, 95% CI 0.25 to 8.63, p=0.670). All patients in this analysis had immunosuppression reduction at the time of diagnosis and were started on a front-line treatment defined as a Trappe (9) approach with rituximab monotherapy induction. We hypothesised that over- immunosuppression might not only drive the occurrence of PTLD, but also

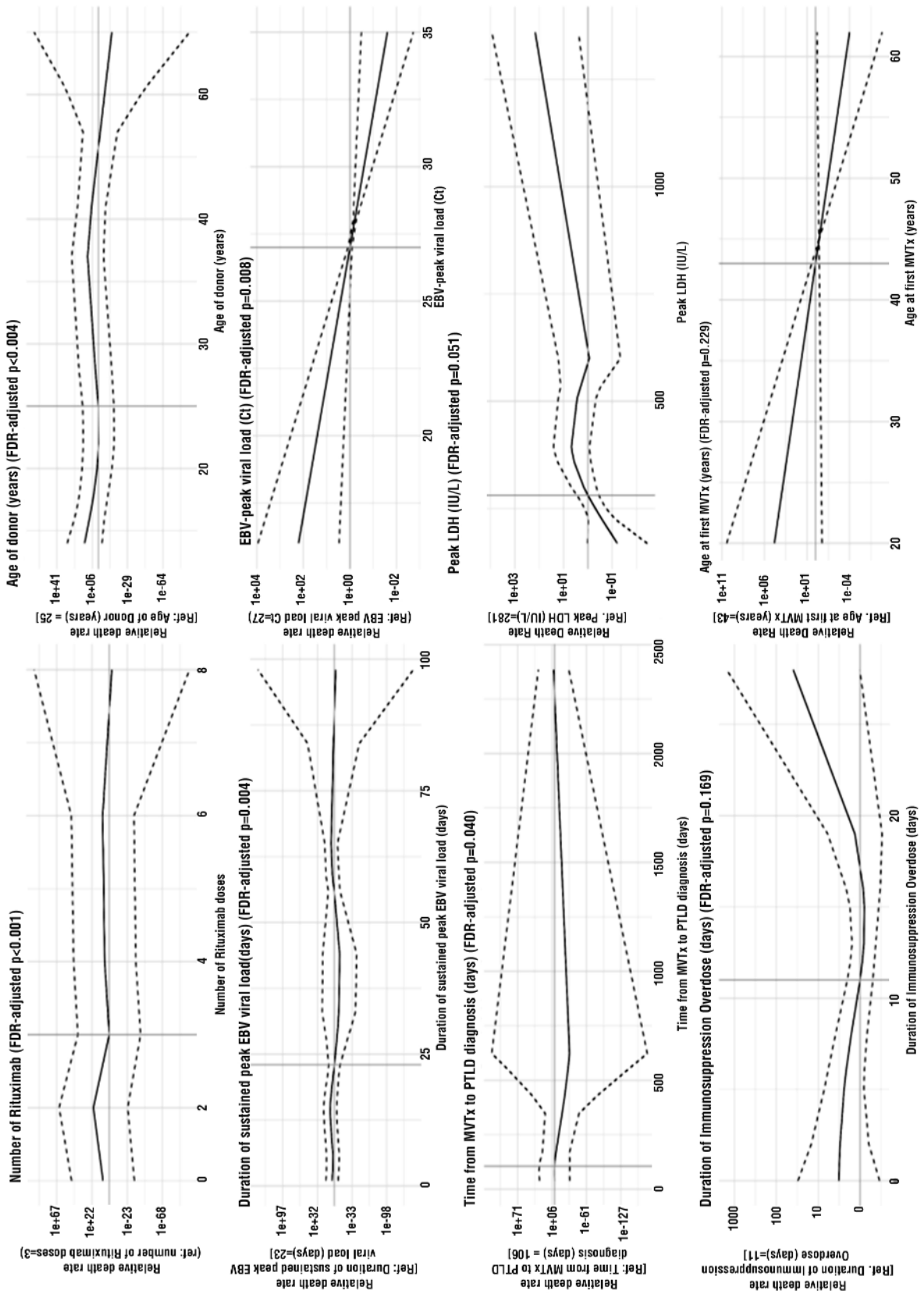

Figure 2. Likelihood ratio tests (LRT) with FDR-adjusted P-values to compare ageand sex-adjusted Cox models with and without each continuous risk factor. The continuous risk factors were modelled as penalised splines to allow non-linear associations with PTLD mortality. Predictor complexity (degrees of freedom) was chosen using the Akaike Information Criterion. Multilevel categorical risk factors were modelled without amalgamating levels to assess the collective association of the multilevel risk factor with PTLD mortality.

contribute to its severity, expressed in an increased risk of dying. However, our study did not confirm this, as there was no statistically significant difference between the number of days of immunosuppression overdosing (Tacrolimus serum trough level > 12 ng/mL and/or trough Ciclosporin levels prior to administration of morning dose > 300 ng/mL) in the survival group vs the death one (11.7 days vs 11.2 days, respectively).

Prognostic indices and performance status were important indicators in this study. Patients with an IPI score > 0 were 2.83 (95% CI 0.29 to 27.11, p=0.368) times more likely to die from PTLD compared to patients with a score of 0. Moreover, all patients who survived PTLD had an ECOG score = 0, while none of the patients with ECOG = 3 survived.

Anatomical location of PTLD seems to play a role in its prognosis, as all the patients who succumbed had extranodal disease compared to 66.7% of the patients who survived. Moreover, 50% (3/6) of the patients who died had > 2 extranodal sights affected by PTLD, compared to only 20% in the survival group. This is furthermore reflected in the extent of the disease to both the graft AND extragraft anatomical locations: in the latter situation, the mortality rate was 50 % (4/8). However, in the patients with PTLD located to either the allograft OR native organs, PTLD associated mortality was only 15.38% (2/13).

One of the intriguing findings of our study is the fact that PTLD patients who have received liver-containing grafts may have fared worse compared to MMVTx or isolated small-bowel recipients: only 20% of the patients who survived PTLD (3/15) had liver containing grafts compared to 66.6% (4/6) in the death group. Only 14.2% of the PTLD patients with non-liver containing liver grafts expired, compared to a majority (57.14%) of the liver-containing-graft recipients. Again, this trend did not reach statistical significance (HR 6.54 95% CI 0.85 to 50.34, p=0.071). Moreover it may also reflect functional immunosuppression due to splenectomy, as 71.42% (5/7) of the liver-containing graft recipients were splenectomised, compared to 14.28% (2/14) in the non-liver containing grafts recipients.

In terms of the clinical manifestations of PTLD, the mortality risk was higher in those who developed B symptoms compared to those who did not. However, due to the low number of patients, this did not reach statistical significance (HR 7.04, 95% CI 0.62 to 80.56, p=0.117). Peak LDH before treatment commencement (triggered by a diagnosis of CT<34) was also found to be significantly raised in the mortality group vs the survival group (321.8 ± 154.4 IU/L vs 520.3 ± 422.8 IU/L; HR 1.00, 95%CI 1.00 to 1.01, p=0.019). Peak viral load recorded before treatment initiation (Cycle Threshold cutoff = 32 in our Pathology Laboratory) was found to correlate with the relative risk of death in MVTx patients who developed PTLD, with the survivors having a mean CT value of 29.4 (3.5) compared to 23.0 (4.0) in the mortality group. Nevertheless, the duration of abnormally high EBV titres (CT < 32) did not correlate with risk of death in our cohort: 29.2 (30.2) vs 29.3 (26.2) in survivor vs death cases, respectively.

Discussion

The majority of PTLD cases derive from reactivation of latent EBV infection of the recipient in B cells, facilitated by immunosuppressive treatment. EBV seronegative recipients, however, may develop PTLD following primary EBV infection through passenger lymphocytes in the engrafted organs (10). If primary EBV infection occurs after SOT, diminished EBV-directed CD8+ T-cell responses allow for EBV infection to establish in a larger B-cell reservoir than when EBV infects immunocompetent hosts (11).

EBV promotes PTLD by either: (i). immune system dysregulation via downregulating the MHC I & II expression, thus effectively escaping the immune system or (ii). upregulating the checkpoint inhibitors, such as PD-1 inhibitory receptors on the surface of T lymphocytes, which leads to T-cell exhaustion, conversely impinging optimal infection control and favouring anergy

against EBV (12). Nevertheless, other forms of PTLD can include T cell (13) or NK cell lymphoproliferations (14). It is important to note, however, that PTLD in SOT patients most frequently derives from the recipient, whereas HSCT-related PTLD is usually transmitted from the donor (15). The highest incidence of EBV+PTLD occurs in the first 1–2 years post-transplant (16). Over the past two decades, there has been an increase in the incidence of PTLD attributed to an increased number of SOTs, introduction of novel and more potent immunosuppressive regimens, and improved diagnosis (17). The marked reduction of EBV viral load after commencement of antiCD20 treatment is most likely due to B-lymphocyte removal from the peripheral blood by rituximab (18). A plethora of PTLD risk factors have been identified (EBV mismatch, CMV mismatch, type of graft, age at diagnosis, increased immunosuppression et al). However, given the large variability in terms of PTLD manifestation and prognosis, robust data lacks in regards to prognostic factors associated with an increased risk of death in MVTx recipients who develop PTLD. PTLD-related mortality after liver and intestinal transplantation has historically been reported to be approximately 50% at 5 years (19), which is substantially higher compared to any other type of SOT. Several poor outcome risk factors in PTLD patients have been identified: increased LDH (20), hypoalbuminemia (21), CNS, graft and BM involvement, monomorphic pathology (22), poor performance status, age>45 years at the time of transplant (23) and EBV- and CD20-negativity assessed by immunohistochemistry of the tumour (24). Additionally, a clinical score assessing the RR of developing PTLD which comprises 5 variables (high-risk pre-transplant EBV, IgG donor/recipient serostatus,positive plasma EBV DNA, abnormal hemoglobin and CRP levels) was recently published (25). However, these studies pooled together pediatric and adult PTLD patients who received a wide variety of solid organ transplants, including but not limited to small bowel. Moreover, although pediatric PTLD patients have a higher incidence and earlier occurrence of the disease (22), they have been shown to fare better compared to adult SOT who develop PTLD. The highest rejection rates in solid organ transplantation occur in transplantation of the small intestine and the enhanced immunosuppression therefore needed is arguably the most important contributor to the increased PTLD rate in this cohort of patients (26). PTLD may occur early after lung transplantation as well, where transplanted lymphoid tissue is also abundant. Nevertheless, as antibody induction is not used in lung recipients compared to MVT ones, early PTLD incidence rate in the latter group has been consistently higher (12,27,28). Calcineurin-inhibitors seem to play augmented role in the pathogenesis of PTLD vs other types of immunosuppression regimens (10). Pre-emptive reduction of immunosuppressive therapy at the first evidence of increasing EBV titers decreases the incidence of PTLD and it may also contribute to a milder PTLD phenotype and improved clinical outcomes (29). While our study did not detect any association between PTLD outcome and the number of doses of Alemtuzumab induction, this regimen per se was associated with a twofold-increased incidence of PTLD after pediatric ITx when compared with rATG (30). The Authors sub-sequently decided to abandon Alemtuzumab induction altogether, therefore underscoring the important role cumulative over immunosuppression might play in the development of PTLD. A relatively novel approach employs IS induction with Rituximab in SOT recipients, which appears to preclude the occurrence of PTLD, and the benefit remained consistent when specific organ subanalysis was performed (31). This strategy may prove especially useful in selected MTVx recipients with risk factors of death from a potential PTLD, such as where splenectomy is necessary at the time of transplant or previously resected. Furthermore addition of Rituximab to a rATG induction regimen may remove B cells more effectively, therefore mitigating the risk of preformed/de novo DSAs against the multivisceral graft (32). Since

antiviral prophylaxis in EBV naive patients has proven to exert no effect on the incidence of PTLD (33), it currently seems that the most effective way of mitigating PTLD-related deaths is focusing on identifying risk factors and tailoring timing and dosage of treatment, rather than prophylaxis of EBV infection. Dechu et al (34) reported good results when administering valganciclovir while concomitantly decreasing MMF by 30% in patients with EBV besides the decrease in CNI dosage. Antimetabolite treatment may be discontinued altogether when early PTLD is diagnosed (35). CMV infection exerts an indirect pathogenic effect by further immunosuppressing an already susceptible transplant recipient via downregulating HLA expression, T-cell proliferation, and NK cell activity (36). CMV primary infection and recipient CMV seronegativity have been shown to lead to poorer outcomes in a PTLD cohort of pediatric liver transplant recipients (37). Furthermore, CMV-EBV coinfection in SOT recipients increases both the RR of developing PTLD as well as the rate of ACR compared with patients with only one of the infections (38). Whereas our analysis did not find any association between CMV infection and PTLD occurrence, it should be noted that CMV has been involved in the pathogenesis of early PTLD (39) and should be taken into consideration in any EBV D/R -/- patient, suggesting prompt antiCMV treatment. In EBV-driven PTLD clinical presentations are protean and not specific. This is compounded by the fact that a significant heterogeneity of histopathological forms has been noted, from non-destructive to destructive PTLDs (40), which conversely has hampered detailed assessments and pooling of smaller similar studies in meta analyses of these critical complications. Our study did not identify any prognostic association of severity with any clinical presentation, bar an increased association with B symptoms in patients who died of PTLD. However, while not reaching statistical significance, we noticed a trend towards a higher mortality RR monomorphic PTLD patients compared to non-monomorphic histology. 3/4 PTLD patients who died and had a histological diagnosis presented the monomorphic subtype, compared with 4/12 (33%) in the survival group, corroborating Tajima et al's 22 findings in their large cohort of PTLD post LTx. The statistically significant increased LDH in the group of patients who died with PTLD in our study complements an earlier study conducted by Tsai et al (41) (2001), who identified LDH, organ dysfunction and multi-organ involvement as risk factors for failure of reduction of Immunosuppression in successfully treating PTLD. This relatively inexpensive and widespread laboratory marker, if validated in larger cohorts, may be used as a surrogate predictor for PTLD mortality in MVTx patients. Consistent with our data that any IPI>0 is associated with a 3-fold higher risk of mortality (42), found a 1.56 increased RR of mortality in their 88 mixed SOT patients. Of note, only 6 of their patients were MTVTx recipients. Corroborating other authors' findings (43), re-transplantation correlated with an increased risk of death in our group of patients, and this may be attributed to the cumulative effect of supplementary IS). Re -transplantation was associated with higher risk of death from PTLD in our cohort of patients. Although the available data is quite scarce, Kubal et al (44) have shown that multi-visceral re-transplant recipients have a higher rate of severe ACR which necessitates increased IS, and this correlates with the number and severity of previous episodes of ACR. This may explain a potentially accentuated and earlier dysfunction of CD4 T cells and thereby a propensity to develop more severe EBV-driven PTLD. It is noteworthy that surgical removal of a single resectable PTLD tumor (8), as well as radiation therapy in localized disease (45), are still considered as part of the therapeutic armamentarium. One postulated solution meant to decrease the rate of ACR in MVTx retransplants and thereby indirectly the rate of PTLD consists of an IS-free interval after a graft enterectomy, which allows for reconstitution of the recipient immune system (44). Nevertheless, this latter solution should

be employed judiciously, given that any potential sensitization episode may decrease the pool of organs available for retransplantation.

Administration of the full course (4 doses) of Rituximab was clearly associated with survival in our cohort of patients (p=0.05). While the benefits of Rituximab should be weighed against its increased risk of infections (10), it nevertheless remains the treatment of choice in this situation. Choquet et al (46) have identified a survival benefit from Rituximab monotherapy in patients aged < 60 years, ECOG performance status < 1 and normal LDH, suggesting that patients outside these criteria should receive Rituximab + chemotherapy. However, patients in complete remission after 4 doses of Rituximab, as well as those in partial remission but with IPI < 3 may receive Rituximab consolidation vs chemotherapy, with the same EFS and OS, as the PTLD-2 trial recently showed (28). This probably due to the decreased TRM that rituximab offers vs conventional chemotherapy (27). In our cohort, 2/6 patients who succumbed due to PTLD received chemotherapy (1) or CTLs (1) vs 2/15 in the survival group. The addition of these treatments does not seem to have influenced the rate of survival in our experience, though these may have been given too late in the disease course to have afforded any benefit. Jain et al (47) reported that the addition of CHOP chemotherapy in their group of patients who had Rituximab treatment failure offered only a marginal survival benefit. The Authors suggested that progression under rituximab treatment predicts a poor outcome in diffuse large B cell-type PTLD and might preclude adding chemotherapy in this subset of patients altogether. In agreement with our findings that a higher number of rituximab doses is associated with lower mortality, the PTLD-1 Trial proved that 25% of the recipients treated with Rituximab do not require chemotherapy and suggested that rituximab consolidation (8 rather than 4 weekly doses) prevents relapse (48). Moreover, the rate of response to rituximab regardless of EBV status is a predictor for overall survival (4). Allogeneic cryopreserved EBV-specific CTLs are commercially available in blood banks in the United Kingdom (49). They use healthy EBV seropositive donors and cover the most common HLA types matched with the recipients. CTLs treatment (adoptive immunotherapy) is postulated on the fact that PTLD is most often of host-origin and EBV-specific host CTL are dysfunctional due to immunosuppression of the host recipient. CTLs are infused from HLA-matched donors or autologous lymphocytes to recipients. Their clinical benefit in patients who have progressive disease under Rituximab relies on their potential to restore cellular immunity after EBV infection, and eradicate EBV-infected B cells, while avoiding immune-ablation and organ toxicity seen in chemotherapy (50). In our experience, of the only 2 patients who received CTLs after Rituximab had failed to achieve remission, one survived and one died. Corroborating our findings, Prockop et al (51) reported a 54% rate of complete response or durable partial response Rituximab-failure SOT PTLD patients who received CTLs. The risk of allograft loss resulting from activation of cytotoxic T cells by cytokine signalling seems to constitute a legitimate target of novel research in order to ameliorate the results of adoptive immunotherapy in PTLD patients. Previous studies have revealed a decreased relative risk of developing PTLD in recipients who had their IS dose halved at a certain EBV viral load threshold (52). However, to the best of our knowledge, our study is the first one to allude to a certain threshold of EBV viral load (CT<24) beyond which the risk of death increases threefold (*Supplementary Fig. 1*). Although testing for EBV DNAemia as a blanket rule in all adult SOT recipients has a low clinical value per se and is only recommended in certain high risk subgroups (53), we found it appropriate in MVTx recipients, who have a significantly increased baseline risk of PTLD. Nevertheless, it should be noted that in a pediatric setting, peak EBV viremia was correlated with a dose-dependent risk of mortality in a recent study by Chang et al (54). Further studies creating an international registry of PTLD in SBTx recipients would

facilitate comparison of outcomes after treatment. Another important domain of research would be the study of viral and cellular genome expression in pathologic specimens of PTLD biopsies, in order to develop biomarkers (EBV miRNAs) that might predict treatment response (4,55). Switching the calcineurin-based regimen to Sirolimus at the onset of PTLD (56,57), or even when EBV viremia is detected, has been reported with very good results, however it needs to be assessed in larger cohorts of patients. Lastly, a potential EBV- gp350-Ferritin nanoparticle vaccine with a saponin-based Matrix-M adjuvant is being currently investigated by the National Institutes of Health in the United States in a phase I clinical trial in healthy adults (58). The eagerly awaited results may translate into a significant reduction of PTLD disease burden for MVTx recipients.

The limitations of our study should be noted: the sample size of this observational cohort was small and from a single centre. A multicentre study with a larger cohort is required to validate our findings and to adjust for all confounders using a multivariable analysis. Furthermore, our study was limited by missing data from patients transplanted before our electronic database (EPIC) was implemented in late 2014. Standardized assays for EBV quantitation and multiple PCR-based platforms have not been introduced in clinical practice yet. Consequently the determination of cut-offs of elevated EBV viral load is established by each individual laboratory, which hinders the ability to compare the results across centres (16). We thereby consider further studies are needed in order to confirm the association we have detected in our study group between high EBV viral loads in peripheral blood and mortality rate. Our comprehension of risk stratification, therapeutic algorithms, and response will benefit from more uniform data collection and reporting.

Conclusion

In conclusion, this retrospective analysis provides real world data on 21 PTLD cases in adult MVTx recipients, to the best of our knowledge the first cohort published in the literature in this type of transplant. Our findings may prove useful in establishing the most relevant risk factors for death in MVTx patients who develop EBV-driven PTLD. Early identification of these factors may be useful not only in establishing prognosis but in mitigating the mortality rate by offering timely treatment to high-risk patients.

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Author's Contributions

Participated in research design: LMS, AJB, MII, GF.
Participated in the writing of the paper: MII, IP, JKB, LMS.
Participated in the performance of the research: MII, LMS, IP, JKB, AJB.
Participated in data analysis: MII, IP, JKB.

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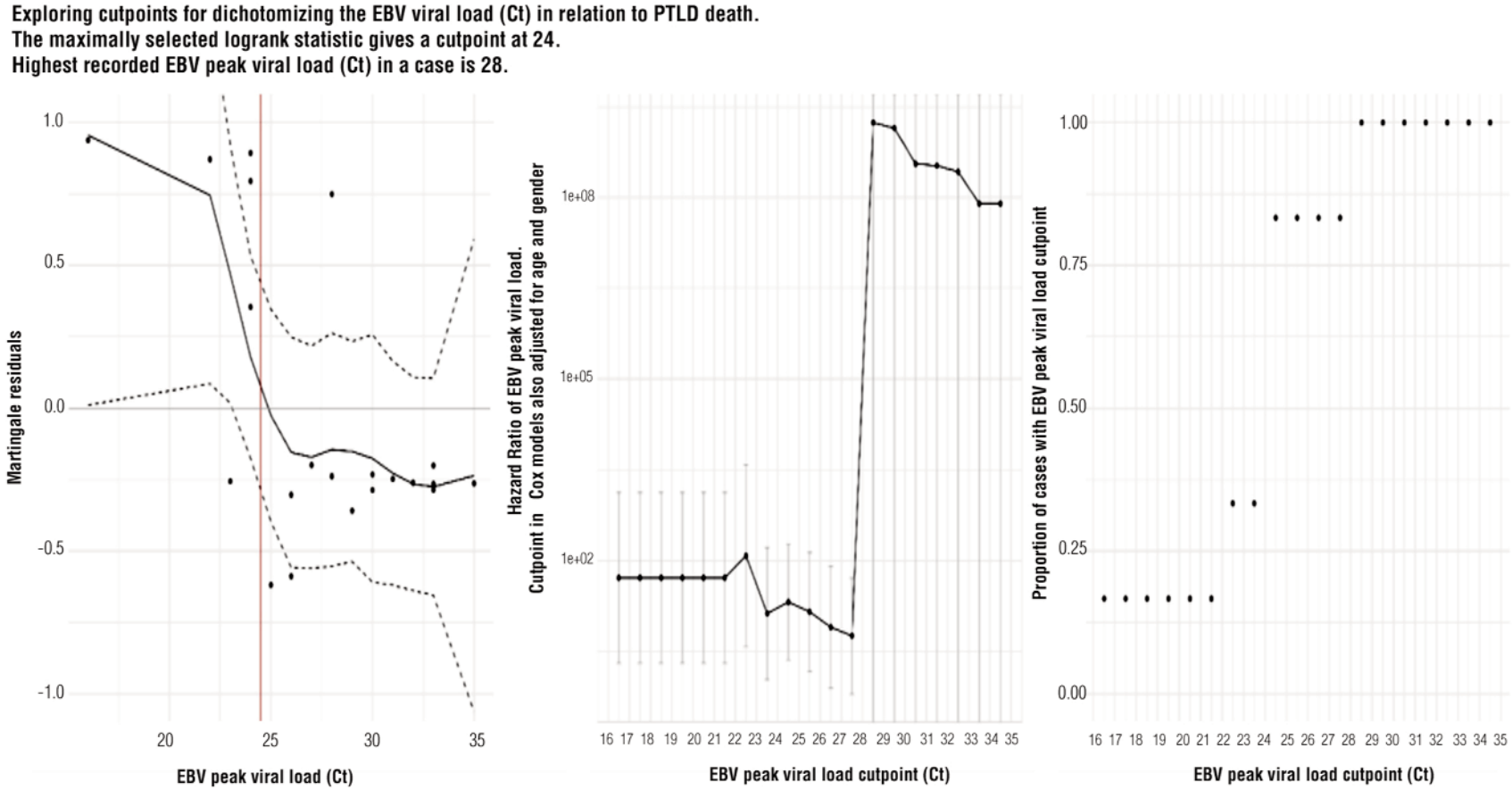
References

1. Raghu VK, Beaumont JL, Everly MJ, Venick RS, Lacaille F, Mazariegos GV. Pediatric intestinal transplantation: analysis of the intestinal transplant registry. Pediatr Transplant. 2019;23(8):e13580.
2. Peters AC, Akinwumi MS, Cervera C, Mabilangan C, Ghosh S, Lai R, et al. The Changing Epidemiology of Posttransplant Lymphoproliferative Disorder in Adult Solid Organ Transplant Recipients Over 30 Years: A Single-center Experience. Transplantation. 2018;102(9):1553-1562.
3. L'Huillier AG, Dipchand AI, Ng VL, Hebert D, Avitzur Y, Solomon M, et al. Posttransplant lymphoproliferative disorder in pediatric patients: Survival rates according to primary sites of occurrence and a proposed clinical categorization. Am J Transplant. 2019;19(10):2764-2774.
4. Allen UD, Preiksaitis JK, AST Infectious Diseases Community of Practice. Post-transplant lymphoproliferative disorders, Epstein-Barr virus infection, and disease in solid organ transplantation: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33(9):e13652.
5. Hakim B, Myers DT, Williams TR, Nagai S, Bonnett J. Intestinal transplants: review of normal imaging appearance and complication s. Br J Radiol. 2018 Oct;91(1090):20180173. doi: 10.1259/bjr.20180173. Epub 2018 Jun 5.
6. Voorhees TJ, Kannan KK, Galeotti J, Grover N, Vaidya R, Moore DT, et al. Identification of high-risk monomorphic post-transplant lymphoproliferative disorder following solid organ transplantation. Leuk Lymphoma. 2021; 62(1):86-94.
7. Vianna RM, Mangus RS, Fridell JA, Kazimi M, Hollinger E, Tector J. Initiation of an intestinal transplant program: the Indiana experience. Transplantation. 2008;85(12):1784-90.
8. Abdulovski R, Møller DL, Knudsen AD, Sørensen SS, Rasmussen A, Nielsen SD, et al. Early- and lateonset posttransplant lymphoproliferative disorders among adult kidney and liv er transplant recipients. Eur J Haematol. 2022;109(4):343-350.
9. Trappe RU, Choquet S, Dierickx D, Mollee P, Zaucha JM, Dreyling MH, et al. International prognostic index, type of transplant and response to rituximab are key parameters to tailor treatment in adults with CD20-positive B cell PTLD: clues from the PTLD-1 trial. Am J Transplant. 2015;15(4):1091-100.
10. Lauro A, Arpinati M, Pinna AD. Managing the challenge of PTLD in liver and bowel transplant recipients. Br J Haematol. 2015;169(2):157-72.
11. Lindsay J, Othman J, Heldman MR, Slavin MA. Epstein-Barr virus post-transplant lymphoproliferative disorder: update on management and outcomes. Curr Opin Infect Dis. 2021;34(6):635-645.
12. Markouli M, Ullah F, Omar N, Apostolopoulou A, Dhillon P, Diamantopoulos P, et al. Recent Advances in Adult PostTransplant Lymphoproliferative Disorder. Cancers (Basel). 2022;14(23):5949.
13. Martinez OM, Krams SM. The Immune Response to Epstein Barr Virus and Implications for Posttransplant Lymphoproliferative Disorder. Transplantation. 2017;101(9):2009-2016.
14. Nakid-Cordero C, Choquet S, Gauthier N, Balegroune N, Tarantino N, Morel V, et al. Distinct immunopathological mechanisms of EBV-positive and EBV-negative posttransplant lymphoproliferative disorders. Am J Transplant. 2021;21(8):2846-2863.
15. Lee C, Vincentelli H, Visuri J, Knight S, Ploeg R. Epstein-Barr Virus-Negative Diffuse Large B-Cell Post-transplant Lymphoma in an Epstein-Barr Virus-Positive Recipient. Cureus. 2021;13(9):e18134.
16. Martinez O.M. Biomarkers for PTLD diagnosis and therapies. Pediatr Nephrol. 2020;35(7):1173-1181.
17. Shahid S, Prockop SE. Epstein-Barr virus-associated post-transplant lymphoproliferative disorders: beyond chemotherapy treatment. Cancer Drug Resist. 2021;4(3):646-664.
18. Codeluppi M, Cocchi S, Guaraldi G, Di Benedetto F, Bagni A, Pecorari M, et al. Rituximab as treatment of posttransplant lymphoproliferative disorder in patients who underwent small bowel/multivisceral transplantation: report of three cases. Transplant Proc. 2005;37(6):2634-5.
19. Abu-Elmagd KM, Mazariegos G, Costa G, Soltys K, Bond G, Sindhi R, et al. Lymphoproliferative disorders and de novo malignancies in intestinal and multivisceral recipients: improved outcomes with new outlooks. Transplantation. 2009;88(7):926-34.
20. Romero S, Montoro J, Guinot M, Almenar L, Andreu R, Balaguer A, et al. Post-transplant lymphoproliferative disorders after solid organ and hematopoietic stem cell transplantation. Leuk Lymphoma. 2019;60(1): 142-150.
21. Vergote VKJ, Deroose CM, Fieuws S, Laleman W, Sprangers B, Uyttebroeck A, et al. Characteristics and Outcome of Post-Transplant Lymphoproliferative Disorders After Solid Organ Transplantation: A Single Center Experience of 196 Patients Over 30 Years. Transpl Int. 2022;35:10707.
22. Tajima T, Hata K, Haga H, Nishikori M, Umeda K, Kusakabe J, et al. Post-transplant Lymphoproliferative Disorders After Liver Transplantation: A Retrospective Cohort Study Including 1954 Transplants. Liver Transpl. 2021;27(8):1165-1180.
23. Mucha K, Staros R, Foroncewicz B, Ziarkiewicz-Wróblewska B, Kosieradzki M, Nazarewski S, et al. Comparison of Post-Transplantation Lymphoproliferative Disorder Risk and Prognostic Factors between Kidney and Liver Transplant Recipients. Cancers (Basel). 2022;14(8):1953.
24. Ghobrial IM, Habermann TM, Ristow KM, Ansell SM, Macon W, Geyer SM, et al. Prognostic factors in patients with post-transplant lymphoproliferative disorders (PTLD) in the rituximab era. Leuk Lymphoma. 2005;46(2):191-6.
25. Dos Santos Q, Wareham NE, Mocroft A, Rasmussen A, Gustafsson F, Perch M, et al. Development and Validation of a Risk Score for PostTransplant Lymphoproliferative Disorders among Solid Organ Transplant Reci pients. Cancers (Basel). 2022;14(13):3279.
26. Kubal C, Mangus R, Saxena R, Lobashevsky A, Higgins N, Fridell J, et al. Prospective Monitoring of Donor-specific Anti-HLA Antibodies After Intestine/Multivisceral Transplantation: Significance of De Novo Antibodies. Transplantation. 2015;99(8):e49-56.
27. Santarsieri A, Rudge JF, Amin I, Gelson W, Parmar J, Pettit S, et al. Incidence and outcomes of post-transplant lymphoproliferative disease after 5365 solid-organ transplants over a 20-year period at two UK transplant centres. Br J Haematol. 2022; 197(3):310-319.
28. Zimmermann H, Koenecke C, Dreyling MH, Pott C, Dührsen U, Hahn D, et al. Modified risk-stratified sequential treatment (subcutaneous rituximab with or without chemotherapy) in B-cell Post-transplant lymphoproliferative disorder (PTLD) after Solid organ transplantation (SOT): the prospective multicentre phase II PTLD-2 trial. Leukemia. 2022;36(10):2468-2478.
29. Glotz D, Chapman JR, Dharnidharka V, Hanto DW, Castro MC R, Hirsch HH, et al. The Seville expert workshop for progress in posttransplant lympho-proliferative disorders. Transplantation. 2012;94(8):784-93.
30. Devine K, Ranganathan S, Mazariegos G, Bond G, Soltys K, Ganoza A, et al. Induction regimens and posttransplantation lymphoproliferative disorder after pediatric intestinal transplantation: Single-center experience. Pediatr Transplant. 202;24(5):e13723.
31. Walti LN, Mugglin C, Sidler D, Mombelli M, Manuel O, Hirsch HH, et al. Association of antiviral prophylaxis and rituximab use with posttransplant lymphoproliferative disorders (PTLDs): A nationwide cohort study. Am J Transplant. 2021;21(7):2532-2542.
32. Vianna R, Farag A, Gaynor JJ, Selvaggi G, Tekin A, Garcia J, et al. Association of More Intensive Induction With Less Acute Rejection Following Intestinal Transplantation: Results of 445 Consecutive Cases From a Single Center. Transplantation. 2020;104(10):2166-2178.
33. AlDabbagh MA, Gitman MR, Kumar D, Humar A, Rotstein C, Husain S. The Role of Antiviral Prophylaxis for the Prevention of Epstein-Barr VirusAssociated Posttransplant Lymphoproliferative Disease in Solid Organ Transplant Recipients: A Systematic Review. Am J Transplant. 2017;17(3): 770-781.
34. Puliyanda DP, Jordan SC, Kim IK, Patel M, Murthy A, Huang E, et al. Use of Rituximab for persistent EBV DNAemia, and Its effect on donor-specific antibody development in pediatric renal transplant recipients: A case series. Pediatr Transplant. 2021; 25(8):e14113.
35. Reiche W, Tauseef A, Sabri A, Mirza M, Cantu D, Silberstein P, et al. Gastrointestinal manifestations, risk factors, and management in patients with post-transplant lymphoproliferative disorder: A systematic review. World J Transplant. 2022;12(8):268-280.
36. Freeman RB. The 'indirect' effects of cytomegalovirus infection. Am J Transplant. 2009;9(11):2453-8.
37. Huang JG, Tan MYQ, Quak SH, Aw MM. Risk factors and clinical outcomes of pediatric liver transplant recipients with post-transplant lymphoproliferative disease in a multi-ethnic Asian cohort. Transpl Infect Dis. 2018;20(1).
38. Anderson-Smits C, Baker ER, Hirji I. Coinfection rates and clinical outcome data for cytomegalovirus and Epstein-Barr virus in post-transplant patients: A systematic review of the literature. Transpl Infect Dis. 2020; 22(6):e13396.
39. Mañez R, Breinig MC, Linden P, Wilson J, Torre-Cisneros J, Kusne S, et al. Posttransplant lymphoproliferative disease in primary Epstein-Barr virus infection after liver transplantation: the role of cytomegalovirus disease. J Infect Dis. 1997 Dec;176(6):1462-7.

40. Swerdlow SH, Webber SA, Chadburn A, Ferry JA. Post-transplant lymphoproliferative disorders. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, Revised 4th edn. Lyon: IARC; 2017. p. 453-462.
41. Tsai DE, Hardy CL, Tomaszewski J, Kotloff RM, Oltoff KM, Somer BG, et al. Reduction in immunosuppression as initial therapy for posttransplant lymphoproliferative disorder: analysis of prognostic variables and long-term follow-up of 42 adult patients. Transplantation. 2001;71(8):1076-88.
42. Montes de Jesus F, Dierickx D, Vergote V, et al. Prognostic superiority of International Prognostic Index over [18F]FDG PET/CT volumetric parameters in post-transplant lymphoproliferative disorder. EJNMMI Res. 2021; 11(1):29.
43. Wozniak LJ, Mauer TL, Venick RS, Said JW, Kao RL, Kempert P, et al. Clinical characteristics and outcomes of PTLD following intestinal transplantation. Clin Transplant. 2018; 32(8):e13313.
44. Kubal CA, Pennington C, Fridell J, Ekser B, Muhaylov P, Mangus R. Challenges with Intestine and Multivisceral Re-Transplantation: Importance of Timing of Re-Transplantation and Optimal Immunosuppression. Ann Transplant. 2018;23:98-104.
45. Stanley K, Friehling E, Ranganathan S, Mazariegos G, McAllister-Lucas LM, Sindhi R. Post-transplant lymphoproliferative disorder in pediatric intestinal transplant recipients: A literature review. Pediatr Transplant. 2018;22(5): e13211.
46. Choquet S, Oertel S, LeBlond V, Riess H, Varoqueaux N, Dörken B, et al. Rituximab in the management of posttransplantation lymphoproliferative disorder after solid organ transplantation: proceed with caution. Ann Hematol. 2007;86(8):599-607.
47. Jain MD, Lam R, Liu Z, Stubbins RJ, Kahlon A, Kansara R, et al. Failure of rituximab is associated with a poor outcome in diffuse large B cell lymphoma-type post-transplant lymphoproliferative disorder. Br J Haematol. 2020;189(1):97-105.
48. Trappe R, Dierickx D, Zimmermann H, Morschhauser F, Mollee P, Zaucha JM, et al. Response to rituximab induction is a predictive marker in B-cell post-transplant lymphoproliferative disorder and allows successful stratification into rituximab or R-CHOP consolidation in an international, prospective, multicenter phase II trial. J Clin Oncol. 2017;35(5):536-543.
49. Haque T, Wilkie GM, Jones MM, Higgins CD, Urquhart G, Wingate P, et al. Allogeneic cytotoxic t-cell therapy for ebv-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. Blood. 2007;110(4):1123-31.
50. Fulchiero R, Amaral S. Post-transplant lymphoproliferative disease after pediatric kidney transplant. Front Pediatr. 2022;10:1087864.
51. Prockop S, Doubrovina E, Suser S, Heller G, Barker J, Dahi P, et al. Off-the-shelf EBV-specific T cell immunotherapy for rituximab-refractory EBV-associated lymphoma following transplantation. J Clin Invest. 2020;130(2): 733-747.
52. Chen HS, Ho MC, Hu RH, Wu JF, Chen HL, Ni YH, et al. Roles of Epstein-Barr virus viral load monitoring in the prediction of posttransplant lymphoproliferative disorder in pediatric liver transplantation. J Formos Med Assoc. 2019;118(9):1362-1368.
53. Wareham NE, Mocroft A, Sengeløv H, Da Cunha-Bang C, Gustafsson F, Heilmann C, et al. The value of EBV DNA in early detection of post-transplant lymphoproliferative disorders among solid organ and hematopoietic stem cell transplant recipients. J Cancer Res Clin Oncol. 2018; 144(8):1569-1580.
54. Chang YC, Young RR, Mavis AM, Chambers ET, Kirmani S, Kelly MS, et al. Epstein-Barr Virus DNAemia and post-transplant lymphoproliferative disorder in pediatric solid organ transplant recipients. PLoS One. 2022; 17(10):e0269766.
55. Sen A, Enriquez J, Rao M, Glass M, Balachandran Y, Syed S, et al. Host microRNAs are decreased in pediatric solid-organ transplant recipients during EBV+ Post-transplant Lymphoproliferative Disorder. Front Immunol. 2022;13:994552.
56. Cullis B, D'Souza R, McCullagh P, Harries S, Nicholls A, Lee R, et al. Sirolimus-induced remission of posttransplantation lymphoproliferative disorder. Am J Kidney Dis. 2006;47(5):e67-72.
57. Boratynska M, Watorek E, Smolska D, Patrzałek D, Klinger M. Anticancer effect of sirolimus in renal allograft recipients with de novo malignancies. Transplant Proc. 2007;39(9):2736-9.
58. Rozman M, Korac P, Jambrosic K, Židovec Lepej S. Progress in prophylactic and therapeutic EBV vaccine development based on molecular characteristics of EBV target antigens. Pathogens. 2022;11(8):864-75.

Supplementary



Supplementary Figure 1. (Left) Martingale residuals plots to investigate potential cutpoints for dichotomizing the EBV peak viral load (Ct) in relation to PTLD death, (Center) Hazard ratios comparing EBV peak viral load below each potential cutpoint to that above or equal to that cutpoint, (Right) Proportion of cases with EBV peak viral load less than each potential cutpoint. The maxstat package was used for the maximally selected log-rank statistic test, which gave a cut-off point at 24 CTs.