Full title: The Value of EBV DNA in Early Detection of Post-Transplant Lymphoproliferative Disorders among Solid organ and Hematopoietic Stem Cell Transplant Recipients.

Running title: The value of EBV DNA to predict PTLD

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose.

Contributors

NEW designed the study, assisted in data collection and analysis, and drafted and edited the manuscript.

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1. INTRODUCTION

Post-transplantation lymphoproliferative disorders (PTLD) is a rare but severe and often life-threatening complication after solid organ (SOT) or hematopoietic stem cell transplantation (HSCT) [1, 2]. The overall mortality has been reported as high as up to 80% [2-5] depending on disease progression and transplant type.

Clinically and histologically, PTLD consists of a heterogeneous spectrum of disorders ranging from benign proliferation of B lymphocytes to fulminant lymphoma, where the latter can resemble those seen in immunocompetent patients [6-8]. Furthermore, PTLD is associated with Epstein Barr Virus (EBV) in the majority of the cases [1, 8]. Following diagnosis, response to currently available treatments such as chemotherapy or Rituximab is often poor and the clinical strategy is thus to detect the disease at early stages and prevent progression to fulminant lymphoma [1, 8]. Detection of EBV DNA in plasma or whole blood is considered an early sign of PTLD and this biomarker is thus widely used to guide pre-emptive treatment of PTLD [2, 9]. However, there is limited clinical evidence to support that screening for EBV DNA and detecting EBV DNAemia in an otherwise healthy transplant recipient can detect those with subsequent PTLD [10]. Acknowledging this, current international guidelines only recommend regular screening of EBV DNA in the first year after transplantation among recipients considered as high risk of PTLD, i.e. EBV seronegative children who receive a solid organ from a seropositive donor [9] and HSCT treated with T-cell depleting agents or HSCT with a mismatched donor [11]. Thus, since only a subgroup of patients with detectable EBV progress to develop PTLD, more precise prognostic models are required to identify those who are most likely to progress to PTLD, and thus who will most likely benefit from pre-emptive treatment.

The aim of this study was to determine the clinical utility of EBV DNAemia as a screening tool to detect emerging PTLD and furthermore to determine risk factors associated with PTLD among SOT and HSCT adults and children registered in a national transplant cohort.

2. MATERIALS AND METHODS

2.1 Study design and participants

In this retrospective cohort study we included all HSCT and SOT recipients transplanted at Rigshospitalet, Copenhagen between January 2004 and December 2014 and registered in the MATCH cohort [12]. This includes all liver and lung transplantations in Denmark and all HSCT, renal and heart transplantations in the eastern region of Denmark. HSCT consisted of recipients undergoing myeloablative conditioning regimens (MAC HSCT) or non-myeloablative conditioning regimens (Mini HSCT) with stem cells from related or unrelated donors and umbilical cord blood stem cells (UCB HSCT). Immunosuppressive regimens have previously been described by Ekenberg et al [13].

2.2 EBV serological status, protocol for EBV DNA screening and management of EBV DNAemia

Pre-transplant EBV IgG serostatus from donors (D) and recipients (R) were used to stratify the cohort into the following combinations D+/R-, D+/R+, D-/R+, and D-/R-. For SOT D+/R- was considered high risk status whereas for HSCT this was the case for D-/R+, the remaining were considered standard-/low risk.

EBV DNA was measured in EDTA plasma by real-time polymerase chain reaction (PCR). This was performed by three different methods in the cohort period; Artus EBV LC PCR Kit (Qiagen, Hamburg, Germany) from 2004 to 2013, LightCycler® EBV Quant Kit (Roche, Albertslund, Copenhagen) from 2014 to 2015, and EBV R-GENE® (Argene, Biomerieux, Lyon, France) from 2015 and onward. Importantly, before changing methods, the new method was calibrated to ensure that the lower level of detection and quantification was comparable. As such, the lower limit of detection of EBV DNA was throughout the study period 500 copies/mL; detectable levels below this threshold were non-quantifiable and set to be at the lower limit of quantification. The purification of EBV DNA was performed using standard methods (EasyMag, Biomerieux, Lyon, France). Cell lysis and thus, the risk of overestimating the EBV DNA load was taken into account by comparing the total genomic DNA in the plasma sample to a reference standard based on total genomic DNA measured in plasma samples from a healthy population.

Regular EBV DNA screening was scheduled in recipients considered as high-risk. This was scheduled every 1-2 weeks in UCB HSCT and EBV IgG seronegative SOT recipients and less intensive in MAC HSCT during the first six months of follow up. Beyond the first six months, intervals between screenings

were gradually increased until one year after transplantation where the screening was terminated. Beyond the first post-transplant year, the decision of screening was based on an individual level. This was performed in the entire cohort period for HSCT whereas screening was introduced for SOT recipients in 2010-11. Prior to 2010-11 SOT recipients were screened for EBV DNA only at the discretion of the clinicians. The remaining recipients not considered as high-risk had EBV DNA measured as part of the diagnostic procedure. If EBV DNAemia was detected, defined as a detectable EBV DNA, the intensity of testing would increase with repeated testing on each visit and usually once a week initially. In case of stable low-copy viremia, intervals would gradually increase. Thus, EBV measurements in this cohort consisted of both screening and those performed as part of the diagnostic procedure of suspected EBV related disease.

The clinical management of recipients with detected EBV DNAemia but no overt signs of PTLD usually included reduction of the daily doses of immunosuppression in the absence of graft rejection or graft versus host disease (GvHD) and depending on the clinical signs and degree of viral load, pre-emptive therapy with an anti-CD20 antibody (typically rituximab) was used.

2.3 Definition and ascertainment of PTLD

The definition of PTLD was based on the WHO criteria, which requires a biopsy confirmed diagnosis [14]. However, it was considered to be insufficient using only this criterion since cases treated empirically would likely not have a biopsy performed, and thus would not be captured by this definition. We therefore expanded the definition by including the certainty criteria "definite", "probable" and "possible" PTLD to be able to also ascertain non-biopsy confirmed PTLDs. The ascertainment was performed through an extensive review of a variety of sources by a single trained clinician including lab data, death causes and journal records (Online Resource 1). Rituximab use was based on prescription data including start and end dates, ascertained using the electronic medication records.

2.4 Statistical analysis

Factors associated with screening for EBV DNA were determined using logistic regression models.

Among those with EBV DNA screening, factors associated with a positive EBV DNA (including those measured at the limit of detection), were determined using Kaplan-Meier analyses and Cox Proportional hazards models. Persons were left censored at first EBV DNA measurement. The model was adjusted for the time between transplant and first EBV DNA. Person follow-up was censored at the earliest of positive EBV DNA, death or last visit plus 60 days. Additional analyses investigated factors associated with an EBV DNA above the limit of detection, where the relationship between a positive EBV DNA at the limit of detection and subsequent EBV DNA above the limit of detection was investigated by including a positive EBV DNA at the limit of detection as a time-updated variable.

Kaplan-Meier analyses and Cox proportional hazards models were used to investigate factors associated with PTLD; persons were censored at PTLD, last visit plus 60 days or death, whichever occurred first. A priori, because of the likely biologic causal relationship between EBV DNAemia and PTLD, we included EBV DNAemia in all our models. Persons were classified as EBV DNA unknown until a value became available during follow-up thus EBV DNA was included as a time-updated covariate. The EBV DNA result was lagged by 28 days to reduce the impact of EBV DNA testing in connection with clinical symptoms. AUC ROC curves were constructed to evaluate the diagnostic performance of an EBV DNAemia, again lagging the EBV DNA result by 28 days. Models were adjusted for date of transplant, age, type of transplant and number of transplants (as a time-updated covariate). ROC curves were repeated including only definite PTLD cases.

A wide range of sensitivity analyses were performed including stratification by calendar year, age group, and certainty of PTLD diagnosis, in SOT or HSCT and limiting analyses to after 2011 when routine screening was available for both transplant types. Furthermore, models were repeated by including information on T-cell depleting treatment, acute GvHD and donor relation (related vs unrelated) for HSCT. Models were also constructed including a variety of laboratory data, such as hemoglobin, leukocytes, lymphocytes, neutrocytes, thrombocytes, creatinine, alanine transaminase (ALT), albumin, C-reactive protein (CRP), and lactate dehydrogenase (LDH). Each laboratory variable was divided into quartiles and fitted as time-updated variables. The association with PTLD was tested in univariate analyses; those with a

global p-value <0.1 were included in multivariate models, and categories were combined where results were similar across quartiles. All P values are 2-sided. A P value < .05 indicates statistical significance. Statistical analysis were conducted using SAS statistical software version 9.4 (SAS institute, Cary, NC, USA).

2.5 Approvals

The research is conducted after approval of the National Data Protection Agency (2012-58-0004, RH-2015-67, with I-Suite number: 03787).

3. RESULTS

3.1 Patient characteristics and measurement for EBV DNA

In total 2642 consecutive adults and children underwent SOT or HSCT since January 2004 and were included in the study; these recipients were mainly males (59.8%), above 50 years of age (42.9%) and transplanted with a solid organ (74.2%). A total of 1784 (67.5%) recipients had been measured for EBV DNAemia at least once during follow-up (Figure 1); first EBV DNA was performed within 4 weeks from transplantation in 429 (24.1% (95% confidence intervals (CI) 20.1-28.1)) and between 4-26 weeks in additional 873 (48.9% (95% CI 45.6-52.2)) recipients.

Patient characteristics according to those with and without EBV DNA measurement are summarized in table 1. EBV DNA measurements were more likely to be performed among younger (adjusted odds ratio (aOR) 3.1 (95% CI 2.00-4.90)) and less likely among older recipients (0.66 (95% CI 0.51-0.87)). As expected, persons transplanted in 2010 or later were more likely to be tested for EBV DNA than those transplanted earlier (2.4 (1.9-3.0).

3.2 Incidence and factors associated with EBV DNAemia among those measured for EBV DNA

Among 1784 recipients measured for EBV DNA, EBV DNAemia was observed in 331 (18.6% (95% CI 16.8-20.4)) (Figure 1). The cumulative incidence of EBV DNAemia at 52 weeks after first measurement was 16.9%

(95% CI 15.1-18.7) (Figure 2A). Viral loads at first measurement were mostly at the lower limit of detection (67.4%) whereas in 1 out of 10 (9.7%) viral loads at first measurement was $\geq$5000 copies/mL.

After adjustment, recipients aged $\leq$16 years were 4 times more likely to have a positive EBV DNA (adjusted hazard ratio (aHR) 4.14 (95% CI 2.89-5.93)), compared to those aged 17-35. The other factor associated with EBV DNAemia was type of transplant. Compared to HCT MAC, HCT Mini (0.49 (0.30-0.79)), heart (0.29 (0.11-0.80)), live kidney (0.48 (0.30-0.77)), and deceased kidney (0.44 (0.29-0.66)) were all less likely to have a positive EBV DNA. There was no association with gender (female: 0.82 (0.65-1.03)), EBV serostatus (low-risk: 0.82 (0.51-1.30)) and importantly year of transplant ($\geq$2010: 1.16 (0.90-1.50). When EBV DNA was defined only as a value above the lower limit of detection, a prior positive EBV DNAemia level at the lower limit of detection was associated with over an 8-fold higher rate of EBV DNAemia above the limit of detection compared to negative test results (8.18 (5.72 – 11.68)). Low age and type of transplant was also associated with an EBV above the limit of detection, although with wider confidence intervals.

3.3 Incidence of and factors associated with PTLD among all recipients

Among the 2642 recipients included in the cohort, PTLD was identified in 79 (3%) (incidence rate 7.0/1000 person-years of follow-up (PYFU) (95% CI 5.5 – 8.6)) during a median follow-up of 3.5 years (Interquartile Range (IQR) 1.2 – 6.7) (Figure 1). The vast majority of the cases developed PTLD within two years of transplantation (1.8% (95% CI 1.3 – 2.4) at 12 months; 2.4% (95% CI 1.7-3.0) at 24 months) (Figure 2B). Characteristics of recipients with PTLD according to certainty of the diagnosis are presented in table 2. The majority of those developing PTLD were males (70%), median age at transplantation was 39.1 (IQR 11.6-57.9), and half of the cases (n=41) were definite PTLD.

Among the 331 with EBV DNAemia, 16.2% (95% CI 12.1 – 20.3) developed PTLD 52 weeks after first positive EBV DNA whereas seven (2%) received pre-emptive rituximab in relation to EBV DNAemia.

After adjustment, younger recipients (aged ≤16 years vs. 17-35 years: aHR 2.51 (95% CI 1.14-5.49) and those transplanted in more recent years (≥2010 vs ≤2009: 1.99 (1.14-3.47)) were more likely to

develop PTLD whereas females (0.61 (0.37-0.99)) and those with low- vs. high-risk EBV serostatus (0.42 (0.18-0.98)) were less likely. There was no association with type of transplant (1.33 (0.82 – 2.16) SOT vs. HSCT). Recipients with a negative EBV DNA were less likely to develop PTLD (0.09 (0.05-0.16)) compared to those with EBV DNA at the lower limit of detection. Those with an EBV DNA of 501-5000 copies/mL had a non-significant increased risk of PTLD (2.03 (0.83-4.95)) while a viral load >5000 copies/mL was significantly associated with PTLD (5.78 (1.57-21.25).

Analyses of risk of PTLD were repeated testing a wide variety of laboratory parameters. In sensitivity analyses, hemoglobin, thrombocytes and CRP were associated with PTLD, and were therefore added to our multivariate model. Similar to the results above, after adjustment, low age (2.87 (1.31-6.29)) was more whereas female gender (0.61 (0.37-1.00)) and low-risk EBV serostatus were less associated with PTLD. Also, as above,, a negative EBV DNA was less associated with PTLD (0.11 (0.06-0.19) whereas the association between a high EBV DNA (>5000 copies/mL) compared to a positive EBV DNA at the lower limit of detection and PTLD was weaker (3.59 (0.97-13.35)). Later calendar year of transplantation was no longer significantly associated with PTLD (1.05 (0.58-1.91)). Type of transplant did not influence risk of PTLD. High levels of CRP (8-31.5 and >31.5 vs. ≤8 mg/L, unknown values, respectively: 2.17 (1.18-3.98) and 5.16 (2.83-9.41)), low levels of thrombocytes (≤89 vs. >89x10^9/L, unknown values: 2.14 (1.08-4.23)), and low levels of hemoglobin (≤5.8 vs. 5.8-7.7 mmol/L: 1.89 (1.01-3.54)) were more whereas high levels of hemoglobin (>7.7, unknown values vs. 5.8-7.7 mmol/L: 0.52 (0.29-0.95)) were less associated with PTLD.

3.4 Findings according to type of transplant: SOT and HSCT

Risk of a positive EBV DNA for age and gender was similar for SOT and HSCT when considered separately. However, while there was no association between year of transplant (≥2010 compared to ≤2009) and a positive EBV DNA for HSCT (aHR 0.88 (95% CI 0.64 – 1.20)), as expected, there was for SOT (1.97 (1.25 – 3.12), p<0.0001, test for interaction). Similar results were seen for EBV DNAemia above the limit of detection, although the test for interaction was no longer statistically significant (p=0.19).

Risk of PTLD among HSCT was assessed adjusting for additional risk factors, such as T-cell depleting treatment, acute GvHD and donor relation (related vs unrelated) in addition to the factors included in the main analyses. After adjustment, there was no longer an association between any of the factors from the main analyses and PTLD ((a negative vs. positive EBV DNA at the lower limit of detection: aHR 0.45 (95% CI 0.13-1.56)), younger age: 1.40 (0.39-5.05), female gender: 0.75 (0.33-1.72), low-risk EBV serostatus: 0.52 (0.15-1.84)). There was a non-significant trend towards increased risk of PTLD in those transplanted in 2010 and after (2.13 (0.89-5.09)). Importantly, T-cell depleting treatment led to an almost 10-fold increased risk of PTLD (9.53 (2.58-35.18)). Donor relation (1.83 (0.52-6.39 for unrelated vs related donor) and aGvHD (1.52 (0.67-3.44)) did not influence risk of PTLD.

Additional models included laboratory parameters. For HSCT, these results were similar to the main results, although with wider confidence intervals.

Among SOT, low-risk EBV serostatus (0.25 (0.07-0.88)) and EBV DNA (a negative vs. positive EBV DNA at the lower limit of detection: 0.07 (0.03-0.16)) were less associated with PTLD whereas al other risk factors no longer influenced risk of PTLD, although these were all with wide confidence intervals.

3.5 Sensitivity and specificity of EBV DNA to predict PTLD among those measured for EBV DNA

Among those with a measurement of EBV DNA (Figure 1), ROC plots demonstrated that an EBV DNAemia had an area under the curve (AUC) of 66% (95% CI 60-72%) for identifying recipients with subsequent PTLD, demonstrating a sensitivity and specificity of 47% and 85%, respectively (Table 3). Furthermore, positive- and negative predictive values were 12.5% and 97.2%, respectively. Increasing viral load cut-off values to higher levels did not improve AUC. When relevant clinical information, including gender, age, year of transplantation, transplant type, number of transplants, and high-risk EBV serostatus were included in addition to EBV DNAemia (full model) to the ROC, AUC increased to 72% (95% CI 66-78%). Considering SOT separately, AUC of the ROC for EBV DNA alone for predicting PTLD was 72% (95% CI 64 – 79%) increasing to

82% (76-88%) in the full model. AUC increased to 83% (75-90%) when adjusting for the laboratory parameters hemoglobin, thrombocytes, and CRP, (Figure 3A).

Among HSCT, AUC of the ROC for EBV DNA alone for predicting PTLD was 59% (95% CI 51 – 68%), increasing to 77% (70-84%) in the full model. This increased further when adjusting for the laboratory parameters to 84% (CI 79-89%). Adding additional risk factors of T-cell depleting treatment, acute GvHD, and donor relation increased AUC to 85% (78 – 91%), (Figure 3B).

3.6 Sensitivity analyses

Sensitivity analysis excluding EBV PCR results performed within 7 days from CT and PET/CT scans were performed to exclude the EBV DNAemia which was detected as part of the diagnostic procedure in relation to PTLD diagnosis. These results were similar to the main results although with wider confidence intervals.

Repeating analysis including only definite PTLD also led to similar results as the main results, although with wider confidence intervals given the smaller number of cases. The AUC of a positive EBV DNA for predicting PTLD were higher compared to when including all PTLD cases; 68% (60 – 76%) for EBV DNA alone increasing to 82% (95% CI 76 – 89%) in the full model.

4. DISCUSSION

This cohort study shows that an EBV DNAemia developed after SOT or HSCT carried an increased risk of progressing to PTLD even after adjusting for relevant factors such as demographics, type of transplantation, serostatus for EBV, and laboratory values at time of transplantation. However, less than 1 out of 5 of recipients with EBV DNAemia progressed to develop PTLD one year after first positive EBV DNA and the ability of an EBV DNAemia to predict PTLD had low sensitivity. Conversely, when EBV DNAemia was assessed together with other clinically relevant information, such as gender, age, transplant year, transplant type, number of transplantations, and high-risk EBV serostatus, the AUC for the ROC increased significantly and we were better able to identify those with PTLD. Our prognostic model performed

particularly well among SOT, and among HSCT when including further risk factors such as T-cell depleting treatment, with AUC of the ROC above 80% in both groups.

Risk factors of PTLD determined in the present study were generally consistent with what have been reported previously, namely young males with high-risk donor-recipient EBV serostatus among SOT [1, 15, 16] and T-cell depleting treatment and increased risk in more recent years among HSCT [17, 18]. In fact, T-cell depleting treatment led to a 10-fold increased risk of progressing to PTLD and this treatment modality seemed to have a much higher importance in regards of predicting those with PTLD compared to EBV DNAemia.

Although previous reports support our finding in regards of the low sensitivity of an EBV DNAemia alone to predict PTLD among SOT [19-21] and HSCT [22, 23] EBV DNA screening is still a widely recommended and used tool [9, 11, 24]. The test is minimally invasive, applicable to most centers and currently the only known biomarker to be used in the follow-up after SOT or HSCT to early diagnose PTLD. To our knowledge, our study is the first to propose a model where, if used in this context, information routinely available in addition to EBV DNA screening could be used to better predict those who would most likely develop PTLD and thus identify those who would benefit from early management or more intensive follow-up. External validation of our findings in a separate cohort is needed and upon good performance, this model could have clinical implications and be used as part of routine management of transplant recipients in the first year after transplantation.

Our results should be seen in the light of their limitations. EBV DNA measurements included both those performed as part of the screening protocol and as part of a diagnostic procedure and thus, differed between patient groups and over calendar time. However, we performed several sensitivity analyses to address this, including excluding EBV PCR results performed in connection with CT and PET/CT scans to exclude those not performed as part of screening, which showed similar results, and adds strength to our findings. Further, all EBV DNA results were lagged to 28 days to reduce the impact of reverse causality. Excluding recipients transplanted prior to 2010 led to similar results as our main findings, although with notably less precision. To address the heterogeneity of our patient population we stratified

analyses by type of transplant and could confirm the differences in both SOT and HSCT recipients. We also performed sensitivity analyses. Our classification of PTLD included both probable and possible cases, possibly including other disease entities than PTLD. Sensitivity analyses including only definite PTLD showed similar results. Probable and possible cases largely depended on EBV DNA results which could potentially increase the strength of association between EBV DNAemia and PTLD development, but our data do not support this bias as EBV DNAemia was a poor predictor when assessed alone.

Conversely, use of pre-emptive Rituximab or reduction of immunosuppression could prevent potential PTLD development and thus underestimate the diagnostic performance of detected EBV DNA. However, by including the definition of possible and probable PTLD, we believe that we likely captured most of those cases where if left untreated would likely progress to fulminant PTLD.

The major strengths of this study include the size and the heterogeneous transplant recipients who were all transplanted at a tertiary hospital and closely monitored during the first year following transplantation. Furthermore, we ensured a comprehensive clinical review across the entire cohort for ascertainment of PTLD including definite PTLD, ascertained independent of EBV DNA results

In summary, this study provides novel information of the clinical course of EBV DNAemia following SOT or HSCT and the clinical value of this test for identifying recipients with risk of subsequent PTLD. Based on our results, testing for EBV DNAemia has a low clinical value and cannot be recommended alone. However, including relevant clinical characteristics such as gender, age, year and type of transplantation, number of transplantations and high-risk EBV serological status, in addition to EBV DNAemia as proposed in this study increased the sensitivity of this test, and may provide important information in the clinical management of transplant recipients.

Contributors

NEW designed the study, assisted in data collection and analysis, and drafted and edited the manuscript.

JDL designed the study, assisted in data collection and analysis, and edited the manuscript.

AM designed the study, performed data analysis and edited the manuscript.

CDB, NK, FG, MI, AR, HS, SSS, CH assisted in data collection and edited the manuscript.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article.

Supporting information include definition of PTLD (Online Resource 1).

Data availability

The datasets generated during and/or analyzed during the current study are available from the

corresponding author on reasonable request.

Compliance with Ethical Standards

Funding

Conflict of interest

NEW declares that she has no conflict of interest. AM declares that she has no conflict of interest. CDB

declares that he has no conflict of interest. NK declares that he has no conflict of interest. FG declares that

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conflict of interest. MI declares that he has no conflict of interest. CH declares that he has no conflict of

interest. SSS declares that he has no conflict of interest. JDL declares that he has no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical

standards of the institutional and/or national research committee and with the 1964 Helsinki declaration

and its later amendments or comparable ethical standards.

Informed consent

All relevant approval for this project was obtained from the Danish Health and Medicines Authorities

according to Danish legislation on retrospective studies. For this type of study, formal consent is not

required.

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Figure Legends

Figure 1. Flow diagram of recipients in the study according to EBV DNA measurement and PTLD diagnosis.

Table 1. Characteristics at time of transplantation, of recipients with and without EBV DNA measurement during follow-up and according to EBV DNA results.

Figure 2A. Kaplan Meier time from first measurement to first EBV DNAemia among those measured at least once following transplantation

Figure 2B. Kaplan Meier time to PTLD from first positive EBV DNA among those tested at least once following transplantation

Table 2. Characteristics of recipients with PTLD (N=79) according to transplant type and certainty of diagnosis[1].

Table 3. AUROC of EBV DNAemia (a positive vs. negative PCR) for identification of subsequent PTLD; EBV DNAemia alone vs including relevant clinical characteristics (full model).

Figure 3A. Receiver operating characteristics curve of EBV DNAemia for identifying SOT recipients with subsequent PTLD; EBV DNAemia alone vs full model including relevant clinical characteristics.

Figure 3B. Receiver operating characteristics curve of EBV DNAemia for identifying HSCT recipients with subsequent PTLD; EBV DNAemia alone vs full model including relevant clinical characteristics.

Table 1. Characteristics at time of transplantation, of recipients with and without EBV DNA measurement during follow-up and according to EBV DNA results.

		All		EBV DNA[1]		Positive		Negative		No EBV DNA	
Characteristics		**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
	All	2642	100.0	1784	67.5	331	18.6	1453	81.4	858	32.5
Gender	**Male**	1581	59.8	1088	61.0	215	65.0	873	60.1	493	57.5
	Female	1061	40.2	696	39.0	116	35.1	580	39.9	365	42.5
Age, years	**<=16**	319	12.1	287	16.1	146	44.1	141	9.7	32	3.7
	17-35	471	17.8	335	18.8	42	12.7	293	20.2	136	15.9
	36-50	718	27.2	477	26.7	56	16.9	421	29.0	241	28.1
	>50	1134	42.9	685	38.4	87	26.3	598	41.2	449	52.3
Tx type	**HSCT MAC**	552	20.9	488	27.4	152	45.9	336	23.1	64	7.5
	HSCT MINI	399	15.1	285	16.0	27	8.2	258	17.8	114	13.3
	HSCT UCB	41	1.6	40	2.2	10	3.0	30	2.1	1	0.1
	HEART	134	5.1	54	3.0	4	1.2	50	3.4	80	9.3
	Renal_D	509	19.3	324	18.2	33	10.0	291	20.0	185	21.6
	Renal_L	262	9.9	200	11.2	22	6.7	178	12.3	62	7.2
	LIVER	434	16.4	238	13.3	55	16.6	183	12.6	196	22.8
	LUNG	311	11.8	155	8.7	28	8.5	127	8.7	156	18.2
N Tx	**1**	2545	96.3	1715	96.1	315	95.2	1400	96.4	830	96.7
	>=2	97	3.7	69	3.9	16	4.8	53	3.7	28	3.3
Year Tx	**<=2009**	1271	48.1	719	40.3	137	41.4	582	40.1	552	64.3
	>=2010	1371	51.9	1065	59.7	194	58.6	871	59.9	306	35.7
D/R risk[2]	**High**	105	4.0	87	4.9	21	6.3	66	4.5	18	2.1
	Low	1249	47.3	972	54.5	163	49.2	809	55.7	277	32.3
	Unknown	1288	48.8	725	40.6	147	44.4	578	39.8	563	65.6
Age, years	**Median, IQR**	47.0	31-58	45	26 - 56	23	8 - 51	46	31 - 57	51	39 - 59

Abbreviations: IQR, interquartile range; HSCT, haematopietic stem cell transplantation; includes myeloablative (MAC), non-myeloablative (Mini) and umbilical cord transplantation (UCB); Renal_D, deceased renal donor; Renal_L, living renal donor; EBV, Epsten-Barr virus.

1. Regular screening with EBV DNA was introduced in 2010 for solid organ recipients (SOT) whereas HSCT recipients had regular screening in the entire study period.

2. D+/R- among SOT and D-/R+ among HSCT are considered high risk. All other combinations are considered standard or low risk.

Table 2. Characteristics of recipients with PTLD (N=79) according to transplant type and certainty of diagnosis[1].

Characteristics	All PTLD	SOT			HSCT		
	N (%)	Definite PTLD N (%)	Probable PTLD N (%)	Possible PTLD N (%)	Definite PTLD N (%)	Probable PTLD N (%)	Possible PTLD N (%)
All recipients	79 (100)	32 (71)	4 (9)	9 (20)	9 (26)	12 (35)	13 (38)
Male gender	55 (70)	19 (59)	3 (75)	7 (78)	9 (100)	9 (75)	8 (62)
Median age at transplantation, years (IQR)	39.1 (11.6-57.9)	51.1 (33.2-62.6)	33.1 (11.7-53.7)	14.2 (8.6-46.0)	43.6 (26.4-63.4)	11.9 (9.6-26.9)	14.3 (1.8-51.9)
Type of transplantation							
HSCT	34 (43)				9 (100)	12 (100)	13 (100)
Renal	20 (25)	17 (53)	1 (25)	2 (22)			
Liver	13 (17)	5 (16)	3 (75)	5 (56)			
Lung	9 (11)	7 (22)	0 (0)	2 (22)			
Heart	3 (4)	3 (9)	0 (0)	0 (0)			
Year of transplantation[2]							
≤2009	32 (41)	19 (59)	1 (25)	4 (44)	1 (11)	4 (33)	3 (23)
≥2010	47 (59)	13 (41)	3 (75)	5 (56)	8 (89)	8 (67)	10 (77)
Median time-span from transplantation to PTLD diagnosis, months (IQR)	8.4 (2.7-35.0)	27.1 (11.7-67.7)	10.2 (5.9-51.3)	11.3 (6.6-35.3)	6.3 (2.2-17.9)	2.0 (1.9-3.0)	4.4 (2.1-11.6)
B symptoms							
Yes	55 (70)	14 (44)	2 (50)	9 (100)	8 (89)	11 (92)	11 (85)
No	19 (24)	14 (44)	2 (50)	0 (0)	1 (11)	0 (0)	2 (15)
Unknown	5 (6)	4 (12)	0 (0)	0 (0)	0 (0)	1 (8)	0 (0)
Palpable lymphadenopathy							
Yes	24 (30)	4 (13)	2 (50)	1 (11)	7 (78)	9 (75)	1 (8)
No	52 (66)	25 (78)	2 (50)	8 (89)	2 (22)	3 (25)	12 (92)
Unknown	3 (4)	3 (9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Symptoms of extra-nodal involvement							
Yes	34 (43)	22 (69)	1 (25)	2 (22)	2 (78)	1 (8)	6 (46)
No	44 (56)	10 (31)	3 (75)	7 (78)	7 (22)	10 (84)	7 (54)
Unknown	1 (1)	0 (0)	0 (6)	0 (0)	0 (0)	1 (8)	0 (0)
Extra-nodal sites[3]							
CNS	10 (31)	8 (33)	1 (50)	0 (0)	1 (33)	0 (0)	0 (0)
Bone or bone marrow	5 (16)	4 (17)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)
Liver	5 (16)	2 (8)	1 (50)	0 (0)	1 (33)	1 (50)	0 (0)
Kidney	1 (3)	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
GI tract	8 (25)	6 (25)	0 (0)	0 (0)	1 (33)	1 (50)	0 (0)
Lung	1 (3)	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Mamma	1 (3)	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tongue	1 (3)	1 (4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Ann Arbor stage							
I	15 (19)	6 (19)	0 (0)	1 (11)	4 (44)	3 (25)	1 (8)
II	2 (3)	0 (0)	0 (0)	0 (0)	0 (0)	2 (17)	0 (0)
III	10 (13)	2 (6)	2 (50)	0 (0)	2 (22)	4 (33)	0 (0)

IV	31 (39)	23 (72)	2 (50)	1 (11)	3 (33)	2 (17)	0 (0)
Unknown	21 (27)	1 (3)	0 (0)	7 (78)	0 (0)	1 (8)	12 (92)
Empiric treatment with Rituximab[4]							
Yes	26 (33)	N.A	4 (100)	1 (11)	N.A	11 (92)	10 (77)
Outcome during the study period	23 (29)	11 (34)	0 (0)	2 (22)	4 (44)	1 (8)	5 (38)
Dead							

Abbreviations: IQR, Interquartile range; HSCT, hematopoietic stem cell transplantation; CNS, central nervous system; GI, gastrointestinal; N.A, non-applicable.

1. definite PTLD= biopsy-confirmed according to WHO criteria [14]; probable PTLD=significant lymphadenopathy (or other end-organ disease) with EBV DNAemia with the absence of another cause; possible PTLD=relevant EBV-related symptoms and EBV DNAemia without evidence of probable or proven disease.

2. Regular screening with EBV DNA was introduced in 2010 for solid organ recipients (SOT) whereas HSCT recipients had regular screening in the entire study period.

3. Sites of PTLD were determined by either biopsy specimens or imaging, such as FDG PET/CT scans, N=32;definite=27;probable=4; possible=1.

4. Definite PTLD cases were diagnosed appropriately before initiating treatment.

Table 3. AUROC of EBV DNAemia (a positive vs. negative PCR) for identification of subsequent PTLD; EBV DNAemia alone vs including relevant clinical characteristics (full model).

	AUROC, % (95% CI)		
	All recipients	**SOT**	**HSCT**
EBV DNA alone	66 (60-72)	72 (64-79)	59 (51-68)
EBV DNA in full model[1]	72 (66-78)	82 (76-88)	77 (70-84)
EBV DNA in full model, incl. laboratory parameters[2]	76 (71-82)	83 (75-90)	84 (79-89)
EBV DNA in full model, incl. laboratory parameters[2] and additional risk factors[3]			85 (78-91)

Abbreviations: Area under the Receiver operating characteristics curve, AUROC; acute graft vs. host disease, aGvHD; C-reactive protein, CRP.

[1]Full model includes relevant clinical characteristics (age, gender, type of transplant, number of transplantations, year of transplantation), and high-risk of EBV infection (Donor (D)+/Recipient (R) - for SOT, D-/R+ for HSCT)). EBV DNA tests performed ≤28 days from the PTLD diagnosis were excluded.

[2]Laboratory parameters include hemoglobin, thrombocytes, and CRP.

[3]Additional risk factors include T-cell depleting treatment, aGvHD, donor match (unrelated vs related).

Figure 1. Flow diagram of recipients in the study according to EBV DNA measurement and PTLD diagnosis.
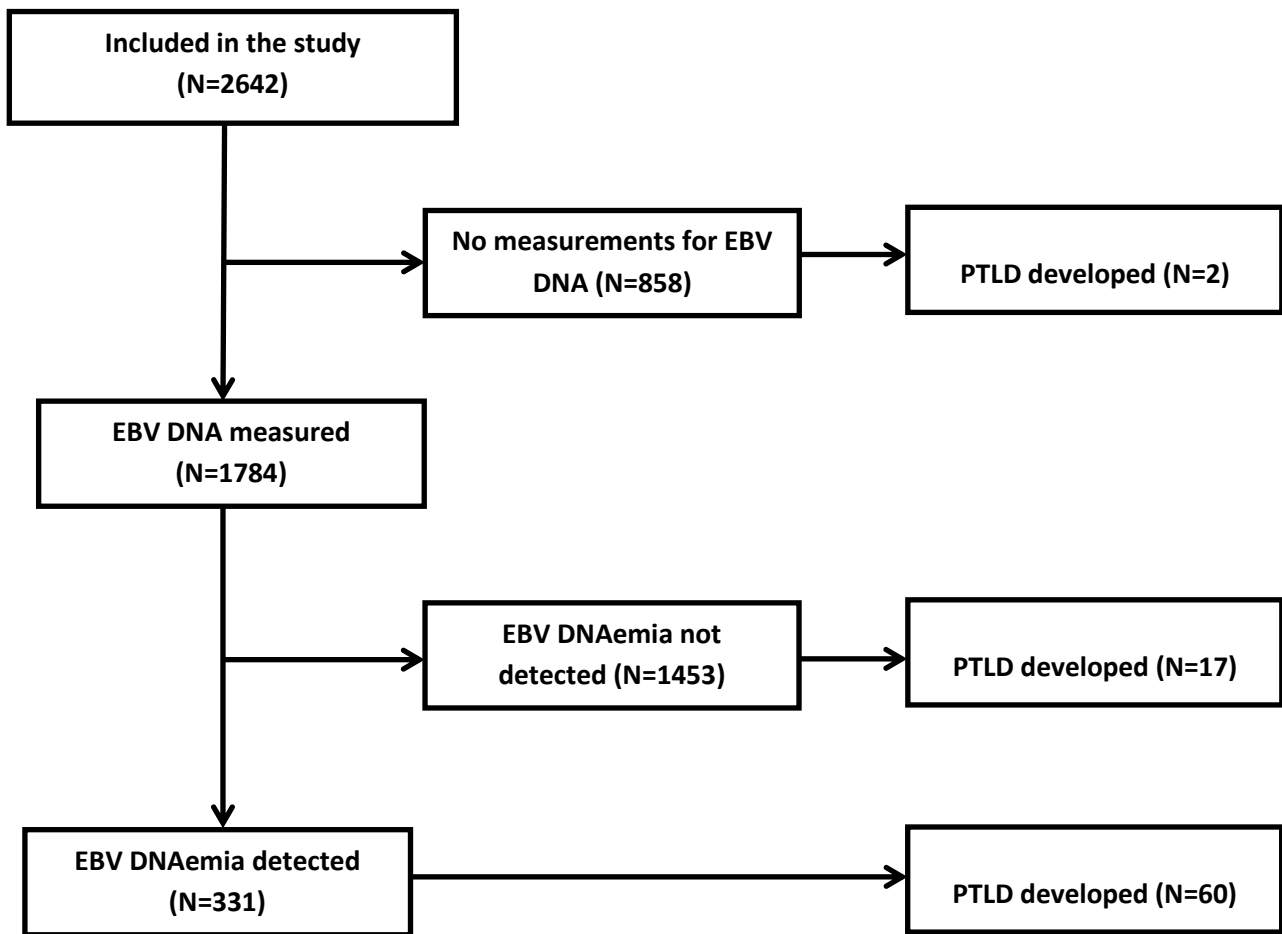
Figure 2A. Kaplan Meier time from first measurement to first EBV DNAemia among those measured at least once following transplantation
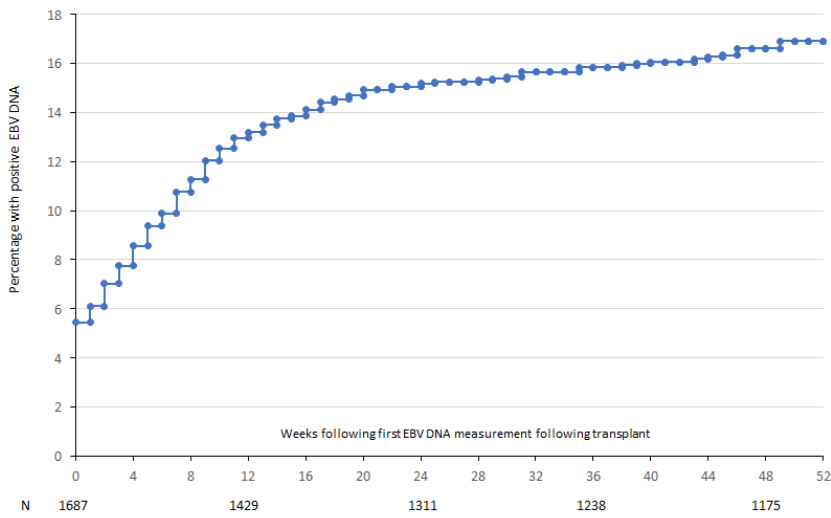

Figure 2B. Kaplan Meier time to PTLD from first positive EBV DNA among those tested at least once following transplantation
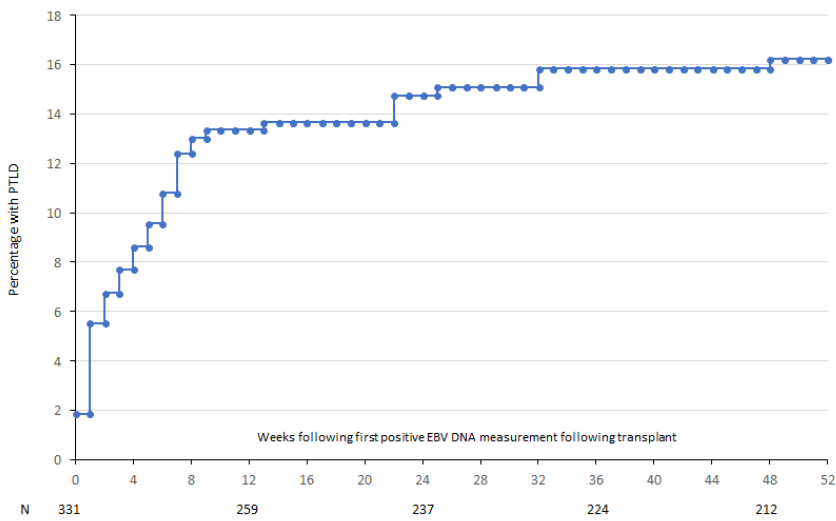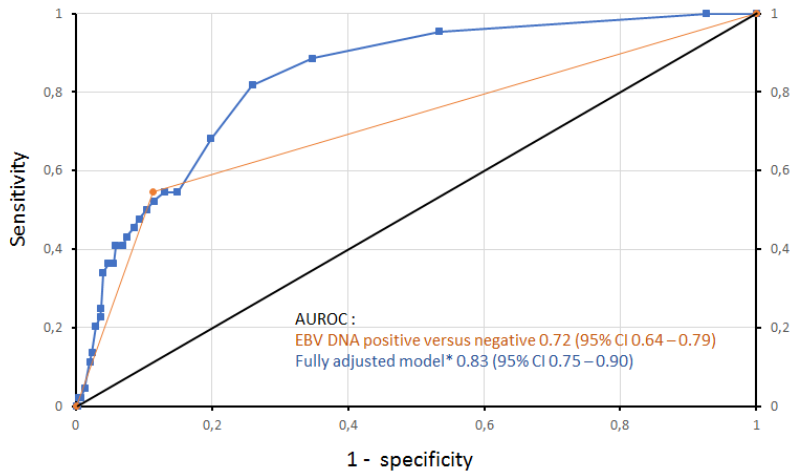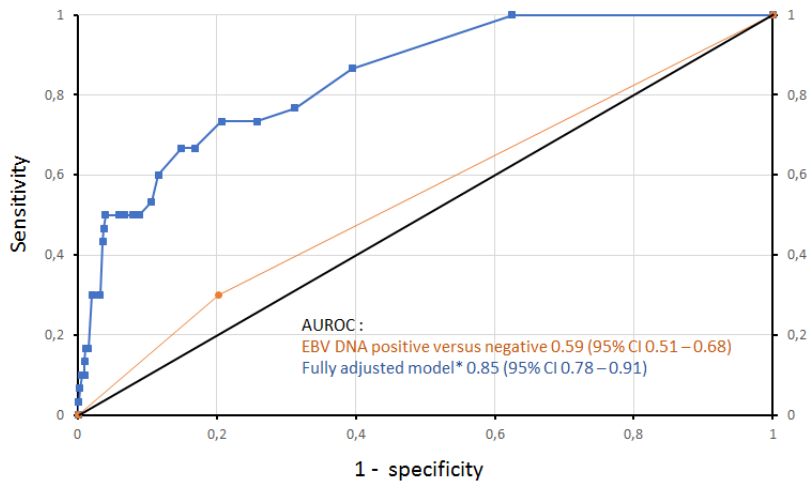
Figure 3A. Receiver operating characteristics curve of EBV DNAemia for identifying SOT recipients with subsequent PTLD; EBV DNAemia alone vs full model including relevant clinical characteristics.

AUROC :
EBV DNA positive versus negative 0.72 (95% CI 0.64 – 0.79)
Fully adjusted model* 0.83 (95% CI 0.75 – 0.90)

*Model includes adjustment for age, gender, year of transplant, number of transplants, high risk status (D+/R- for SOT), hemoglobin, thrombocytes, and CRP

Figure 3B. Receiver operating characteristics curve of EBV DNAemia for identifying HSCT recipients with subsequent PTLD; EBV DNAemia alone vs full model including relevant clinical characteristics.

AUROC :
EBV DNA positive versus negative 0.59 (95% CI 0.51 – 0.68)
Fully adjusted model* 0.85 (95% CI 0.78 – 0.91)

*Model includes adjustment for age, gender, year of transplant, number of transplants, high risk status (D-/R+ for HSCT), hemoglobin, thrombocytes, CRP, T-cell depleting treatment, aGvHD, and donor match (unrelated vs related).

Abbreviations: Area under the Receiver operating characteristics curve, AUROC; acute graft vs. host disease, aGvHD; C-reactive protein, CRP.

Online Resource 1. The definition of and ascertainment of PTLD

PTLD definition	Criteria	Ascertainment
Definite	Confirmed by a biopsy according to WHO criteria	The national pathology registry The national cancer registry
Probable	EBV DNAemia together with significant lymphadenopathy (or other end-organ disease) with the absence of another cause	Identification and review of patients with - EBV DNA tests and other laboratory data, - PET/CT scan reports, - Treatment with the anti-CD20 antibody, rituximab - Review of fatal cases - Patient records
Possible	EBV DNAemia and appropriate EBV-related symptoms, such as B-symptoms without evidence of probable or definite disease	See ascertainment of probable PTLD