

The IPTA Nashville Consensus Conference on Post-Transplant lymphoproliferative disorders after solid organ transplantation in children: III – Consensus guidelines for Epstein-Barr virus load and other biomarker monitoring

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Abbreviations: Anti-EBNA, Anti-Epstein-Barr nuclear antigen; Anti-VCA, Anti-viral capsid antigen; ATP, adenosine triphosphate; AUC, area under the curve; BAL, bronchoalveolar lavage; CMI, cell-mediated immunity; CNS, central nervous system; CSF, cerebral spinal fluid; CVLP, chronic high viral load phenotype; CXCL13, chemokine (C-X₂-X motif) ligand 13; ELISpot, Enzyme-linked immunosorbent spot; HLA, human leukocyte antigen; HSCT, hemopoietic stem cell transplantation; IFN- γ , interferon gamma; Ig, immunoglobulin; IL, interleukin; IM, infectious mononucleosis; IV, intravenous; miRNA, micro RNA; NK, natural killer; NVP, negative predictive value; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PHA, phytohemagglutinin; PPV, positive predictive value; QEBV, Quantitative Epstein-Barr virus; sCD30, soluble CD30; SOT, solid organ transplant; TNF- α , tumor necrosis factor alpha; VL, viral load; WB, whole blood; WHO, World Health Organization.



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Abstract

The International Pediatric Transplant Association convened an expert consensus conference to assess current evidence and develop recommendations for various aspects of care relating to post-transplant lymphoproliferative disorders after solid organ transplantation in children. In this report from the Viral Load and Biomarker Monitoring Working Group, we reviewed the existing literature regarding the role of Epstein-Barr viral load and other biomarkers in peripheral blood for predicting the development of PTLD, for PTLD diagnosis, and for monitoring of response to treatment. Key recommendations from the group highlighted the strong recommendation for use of the term EBV DNAemia instead of “viremia” to describe EBV DNA levels in peripheral blood as well as concerns with comparison of EBV DNAemia measurement results performed at different institutions even when tests are calibrated using the WHO international standard. The working group concluded that either whole blood or plasma could be used as matrices for EBV DNA measurement; optimal specimen type may be clinical context dependent. Whole blood testing has some advantages for surveillance to inform pre-emptive interventions while plasma testing may be preferred in the setting of clinical symptoms and treatment monitoring. However, EBV DNAemia testing alone was not recommended for PTLD diagnosis. Quantitative EBV DNAemia surveillance to identify patients at risk for PTLD and to inform pre-emptive interventions in patients who are EBV seronegative pre-transplant was recommended. In contrast, with the exception of intestinal transplant recipients or those with recent primary EBV infection prior to SOT, surveillance was not recommended in pediatric SOT recipients EBV seropositive pre-transplant. Implications of viral load kinetic parameters including peak load and viral set point on pre-emptive PTLD prevention monitoring algorithms were discussed. Use of additional markers, including measurements of EBV specific cell mediated immunity was discussed but not recommended though the importance of obtaining additional data from prospective multicenter studies was highlighted as a key research priority.

KEYWORDS

biomarkers, child, Epstein-Barr virus, PTLD, solid organ transplantation

1 | INTRODUCTION

Since their introduction two and a half decades ago, assays that quantitatively (Q) measure Epstein-Barr virus (EBV) DNA in peripheral blood using nucleic acid amplification technology (QEBV) assays have been utilized extensively by transplant clinicians for surveillance of patients at high risk for EBV positive (+) PTLD as part of pre-emptive programs for PTLD prevention, for PTLD and EBV disease diagnosis in symptomatic patients and to monitor response to PTLD therapy.¹ EBV viral load (VL) testing is also sometimes performed in non-peripheral blood compartments. EBV DNAemia surveillance has been suggested for safety monitoring in clinical trials of new immunosuppressive agents² and even explored for tailoring immunosuppression in individual patients.^{3,4}

Concurrently, our understanding of the biology of acute and persistent EBV infection and its pathophysiologic role in the development of EBV+ PTLD has increased significantly^{5,6} providing information regarding EBV cell tropism, EBV DNA dynamics and the biologic forms of EBV DNA in the cellular and acellular fractions of peripheral blood during infection and lymphoproliferation. Technologic advancements have made EBV VL assays more sensitive and precise⁷; global initiatives to standardize assays are in progress.⁸ Despite the extensive use of QEBV DNA assays, evidence to support their clinical utility in specific clinical settings after pediatric solid organ transplantation (SOT) are lacking or limited. How to both choose the matrix (whole blood (WB) versus plasma) for testing and interpret results in these settings remains uncertain. Because of QEBV DNA assay limitations, investigators have elucidated and evaluated viral and host cellular, molecular and genetic characteristics associated with EBV infection and/or EBV+ PTLD as possible biomarkers that might be used alone or as adjuncts to EBV VL testing^{9,10} to support EBV+ PTLD screening, diagnosis, and management.

We reviewed evidence and make recommendations regarding the use of EBV Quantitative Nucleic Acid Testing (QNAT) assays and other biomarkers for EBV+ PTLD prevention, diagnosis, and management in pediatric SOT recipients.

2 | METHODS

The International Pediatric Transplant Association Consensus Conference on PTLD after Pediatric Solid Organ Transplantation took place in Nashville, Tennessee on March 12–13, 2019, and the work of conference members continued until the end of December 2021. The goal was to produce evidence-based consensus guidelines on the definitions, prevention, and management of PTLD and related disorders based on critical review of the literature and consensus of experts. The consensus conference was comprised of 29 international experts in organ transplantation and PTLD from the fields of hepatology, nephrology, cardiology, pulmonology, infectious diseases, immunology, hematology-oncology, surgery, virology, pathology, and epidemiology. The Working Group was supported by experts in systematic reviews from the Vanderbilt University

Medical Center's Center for Knowledge Management (<https://ckm.vumc.org/ckm/>).

Prior to the conference, each writing group discussed Conference goals, drafted key questions to be addressed by their group, and commenced literature reviews. The key questions to be addressed were discussed at the conference and further refined by consensus. After the conference, each writing group met via teleconference on multiple occasions. The first task was to finalize the structured literature search terms for each of their key questions with the assistance of the experts from the Vanderbilt University Medical Center's Center for Knowledge Management. Expert search strategies were defined for each of the key question subtopics by an information scientist and executed in MEDLINE® via the PubMed® interface. Each writing group finalized the list of articles for detailed data extraction and review.

Finally, all writing groups were provided with the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) guidelines for classification of the direction and strength of their recommendations. The groups were instructed to classify each of their recommendations as either "strong" or "weak" and the quality of evidence as high, moderate, low, or very low based on the GRADE system recommendations, which rates evidence quality across the body of evidence for an outcome rather than each study as a single unit.^{11–14}

Final approval of each recommendation was provided by both the Viral Load and Biomarker working group and by all members of the full Consensus Conference. The final manuscripts reporting recommendations of the Consensus Conference were reviewed and approved in sequence by all working group members, all participating members of the Conference, and finally by the International Pediatric Transplant Association Board.

The working group acknowledges the challenges of drawing recommendations across all organ systems when many differences exist between the pediatric organ transplant populations and the literature available may be focused on particular organ transplant populations. Nonetheless, the recommendations listed here are intended to apply across all pediatric solid organ transplant populations, unless otherwise stated.

3 | RESULTS/CONSENSUS OUTCOMES

3.1 | Technical issues related to EBV viral load (VL) surveillance and monitoring

3.1.1 | Question 1A: Can quantitative measurements of EBV (QEBV) DNAemia be compared between different institutions/assays?

Recommendations

- QEBV DNAemia assay results that are not calibrated to WHO international standard are not reliably comparable. Accordingly, we

recommend that QEBV DNA measurement should be performed using assays calibrated to the WHO international standard for EBV DNA, expressed as $\log_{10}$ IU/ml.

Evidence Grade: Strong recommendation/moderate-quality evidence.

- QEBV DNAemia assay results, even when calibrated to the WHO international standard, may not be reliably comparable. Accordingly, we recommend that patients should be monitored using the same sample type and the same assay in a single laboratory when possible. If inter-laboratory result comparison is required particularly in the setting of clinical trials or data pooling for cohort analyses, formal cross-referencing of results by testing a panel of clinical samples by all assays being used should be undertaken.

Evidence Grade: Strong recommendation/moderate-quality evidence.

- We recommend that only changes in VL exceeding $0.5 \log_{10}$ IU/ml (3-fold) be considered clinically significant differences in QEBV DNAemia.

Evidence Grade: Strong recommendation/low-quality evidence.

Supporting evidence/information

Status of result harmonization

The real-time qPCR assays commonly used in diagnostic laboratories are more precise, less prone to effects of inhibitors and have a broader linear range (6–7 orders of magnitude) compared to earlier generations of competitive end point PCR assays.⁷ Historically, even when using real-time qPCR assays the large variance in reported results resulted in poor result harmonization for all sample types (plasma, peripheral blood mononuclear cells (PBMC) or WB)^{15–22} making it very difficult to directly compare QEBV DNA results in the historical literature. The WHO Expert Committee on Biological Standardization approved the first WHO international standard for EBV DNA as a common assay calibrator in October 2011.⁸ Assays calibrated to the international standard report results in IU/ml (a consensus value not directly related to genome copies/ml). The conversion factor from copies to IU is assay specific. Although international standard calibration can improve result harmonization, it can also make agreement worse²³ and have a different impact in one matrix versus another (i.e., WB versus plasma²⁴). Because assays may demonstrate excellent result harmonization when testing samples created using whole virus preparations, but still show marked result variation when testing clinical samples, the latter must be tested to determine the true impact of WHO international standard calibration on result harmonization.

Most information regarding the impact of the WHO international standard on result agreement comes from testing on national or international proficiency panels that do not include clinical samples.^{25–27} These studies all concluded that use of the international standard for assay calibration markedly reduced result variability, with >80% results reported by multiple laboratories falling within

$\pm 0.5 \log_{10}$ IU/ml, for WB and plasma samples.^{25,27} However, when four US laboratories participating in a clinical transplant trial reported results for the WHO international standard serially diluted in plasma, only 62% of values for dilutions above $4 \log_{10}$ were within $\pm 0.5 \log_{10}$ IU/ml of expected values.²⁸ When clinical samples were tested using assays calibrated to the WHO IS, excellent result agreement was often found when only two assays were compared, but greater variability was sometimes reported among specific assay pairs when a larger number of assays were included in the analysis.^{29–34} A recent study comparing results for 41 EBV DNA plasma samples using 10 different assays found that although most results fell within $0.5 \log_{10}$ IU of the mean of all results, differences in results of the same sample could be as large as $1.5 \log_{10}$ IU for some assay pairs.³⁵ Overall, use of WHO international standard calibrated EBV DNA assays appears to have improved result harmonization and should be widely adopted at a global level in order to reduce the impact of calibration as a source of result variability. However, harmonization is not sufficiently predictable to allow direct result comparison without cross-validation of specific assay results. Potential additional sources of persistent variability requiring ongoing quality improvement include nucleic acid extraction techniques, commutability of secondary and tertiary calibrators, choice of gene targets and other factors (reviewed by ref 36).

Viral load assay result precision

It is not clear how much precision is achievable or clinically acceptable using real-time qPCR assays, but variation of $\pm 0.5 \log_{10}$ is often used, extrapolated from data in HIV³⁷; precision is poorest at low VLs. It is therefore suggested that changes in values should differ by at least 3-fold or ($0.5 \log_{10}$) and as much as 5-fold ($0.7 \log_{10}$) near the assay's limit of detection to be considered biologically important changes.^{37,38} Although not currently available in most clinical laboratories, digital PCR has been proposed as an alternate and perhaps better reference technology for EBV VL measurement as it provides absolute quantification without need for a standard curve and is more precise when quantifying low copy numbers.^{35,38}

Research priorities for knowledge gaps

- Result harmonization of QEBV DNA assays should continue to be improved through international collaboration.

3.1.2 | Question 1B: What is the optimal specimen type for EBV DNA measurement in peripheral blood?

Recommendations

- We recommend that either WB or plasma can be acceptable matrices for EBV DNA measurement; optimal specimen type may be clinical context dependent.

Evidence Grade: Strong recommendation/low-quality evidence.

- WB surveillance may be preferred to maximize sensitivity for early detection of QEBV DNAemia in patients serially monitored after transplant, while plasma testing may be more specific in disease detection and monitoring of treatment response.

Evidence Grade: Weak recommendation/very low-quality evidence.

Supporting evidence/information

What is being measured?

Optimal specimen choice and matrix-specific result interpretation requires understanding the number and type of cells in peripheral blood that are either lytically or latently EBV-infected, the biologic form of EBV DNA in plasma (circulating cell free DNA vs. DNA encapsulated in virions) and how this varies depending on clinical context (acute primary infection, persistent infection, reactivation infection and PTLD). Because data in SOT populations are limited, extrapolation of important information from studies of immunocompetent hosts³⁹⁻⁴⁴ and other immunocompromised populations⁴⁵ is required; these data have been reviewed in detail by Preiksaitis and Burton.³⁶

In both immunocompetent and immunocompromised hosts EBV DNA in plasma likely almost always exists as fragmented circulating cell free DNA, released predominantly from latently or lytically infected normal or malignant cells outside the circulation with no clear evidence that EBV DNA in plasma is encapsulated in virions.^{39,41,46,47} The very short half-life of ~2 hours of circulating cell free EBV DNA in plasma,⁴⁸ makes dynamic changes in its measurement more rapidly responsive to treatment interventions than changes in cellular EBV DNA. EBV DNA is almost always detected in the plasma of immunocompetent patients with infectious mononucleosis (IM) but only for <30 days from symptom onset^{41-43,49,50} although it can persist significantly more than one year in pediatric SOT recipients with primary infection.^{51,52} Most SOT patients with chronically elevated VL studied late after transplant do not have detectable EBV DNA in plasma. When detected, it is generally found in patients with higher VL in PBMC, is present in small amounts, and is transient.⁵³⁻⁵⁵

Little is known about the type of cells infected in early primary EBV infection post-transplant. However, studies later after transplant of adult kidney recipients and pediatric recipients with variable allograft types and persistent detectable EBV VL, suggest EBV is present primarily in resting memory B cells as in immunocompetent adults⁵⁵⁻⁵⁷ containing 1–2 genomes/infected cell.⁵⁷ Schauer et al⁵⁷ found pediatric SOT recipients with high EBV VL load >1 year after transplant also had highly atypical, usually Ig-null EBV-infected cells containing 30–60 genome copies/cell, which disproportionately contributed to total VL. In a study of adult transplant recipients with high VL Fink et al confirmed that genome copies/cell is highly variable across samples and even among cells in the same sample.⁵⁸ Despite these observations, most investigators, although not all,⁵⁸ have found a reasonable correlation between total VL measured directly in peripheral blood of patients with persistent high loads and the number of EBV-infected cells detected by in situ hybridization.⁵⁹⁻⁶¹ Using immune-FISH, Ito et al⁶⁰ found that in pediatric liver transplant recipients with elevated VL EBV-infected

cells were predominantly CD20⁺ IgM⁺, IgD⁺CD27⁺ memory B cells, with 16.7 genome copies/cell; this cell subset usually represents only a minor infected population in the immunocompetent host.⁶²

Epstein-Barr virus DNA in the peripheral blood of SOT patients exists as complex mixtures of forms in the cellular and plasma fractions with proportional distribution varying during stages of acute and persistent infection, inter-current illness, and evolving malignancy. It is therefore more appropriate to use the term EBV DNAemia rather than EBV viremia as the latter term implies infectivity which is almost never present in plasma. EBV DNA is found surprisingly often in atypical B cells as well as in non-B cells in the peripheral blood, particularly in the immunocompromised host.^{54,59,63} In transplant patients with persistent elevated VL detectable in WB, it may be important to differentiate patients who have only an altered viral “set point” (see section on EBV VL kinetics) from patients who carry clonally abnormal B, T, or NK EBV-infected cells in their circulation as is seen in chronic active EBV infection in the immunocompetent host.⁶⁴

Choosing peripheral blood specimen type and reporting units

Although there is consensus that peripheral blood is the preferred sampling site in SOT patients, the optimal sample type remains unresolved. Cellular specimen types including WB, leukocytes, PBMC, and isolated B cells, as well as acellular fractions (plasma and serum) have been evaluated.^{51,54,65-82} Quantitative EBV DNA measurements in different cellular specimen types (WB, PBMC, isolated B cells) are highly correlated when tested using the same assay.^{67,74,83,84} Moreover, normalization of results in cellular sample types to cell number or genomic DNA using either total DNA or housekeeping genes did not significantly change this correlation or alter dynamic trending in serially followed patients compared to reporting the results on a /volume (ml) basis.^{66,67,74} However, the presence of severe lymphocytosis or leukopenia should be reviewed when interpreting dynamic changes in WB results.^{66,85} Because of reduced processing steps and lower blood volumes required, WB has become the preferred cellular specimen with VL reported in IU/ml, without normalization. Serum is sometimes used as an alternative to plasma.^{65,71,72,75,76} To avoid contamination from EBV DNA in circulating cells,⁸⁶ particularly when cellular VL is high, plasma is preferred over serum as the non-cellular fraction of choice.

Although, generally, EBV DNA becomes detectable in plasma or serum as EBV VL rises in matched WB samples or PBMC and is linearly correlated, the correlation coefficient between EBV VL measured in WB or PBMC versus plasma is relatively low.^{54,65,70,71,74,80,87} When detection is discordant, the pattern most commonly observed is EBV DNA detection in WB or PBMC while plasma is negative, particularly when VL in cellular sample types is low. Quantitative differences between WB and plasma may be >2 log₁₀ copies/ml; differences appear smaller in symptomatic than asymptomatic patients.⁵² The biologic forms present and distribution of EBV DNA in the cellular component vs plasma differs over time in individual patients and in different clinical settings, likely explaining these observations. Available studies of longitudinally monitored patients comparing contemporaneous results in different sample types have limitations that make it difficult to determine the preferred sample type in specific clinical settings. These

study limitations are due to the small patient numbers in each study often with few or no PTLD cases,^{65,68,74,82,87} monitoring of very low risk groups,^{65,81} or sampling not starting at transplant in PTLD patients.⁷⁴

The choice of specimen type in pediatric SOT recipients may depend on the reason for testing; one size may not fit all settings. If the goal is to detect EBV infection/reactivation as early as possible in patients at high risk for developing PTLD, with interventions to re-establish control of the infection assuming this will lower future PTLD risk, then the more sensitive WB sample may be preferred. EBV VL is most often significantly higher, usually by more than a log₁₀ in cellular peripheral blood fractions than in acellular fractions^{65,70,71,79,81,87} and higher during primary infection than reactivation infection in both fractions.^{65,88,89} In the small number of EBV mismatched SOT, for whom longitudinal contemporaneous monitoring in both specimen types was reported, EBV DNA was first detected in WB relative to plasma in only three of 10 patients studied.^{51,65,68} and in a recent study of EBV mismatched pediatric liver transplant recipients, all first detection of EBV DNAemia in WB was concordant with its detection in serum.⁵² Some investigators observed increasing levels of EBV DNA in plasma/serum but not WB occur just before PTLD diagnosis and found testing in both WB and plasma/serum may be additive optimizing sensitivity and specificity for PTLD prediction.^{71,74} Although either specimen type could be used in pre-emptive PTLD prevention programs, monitoring both might be a better strategy, although this is associated with increased cost and more complex laboratory logistics. If the goal of EBV DNA testing is early EBV+PTLD diagnosis, treatment monitoring or prediction of relapse, plasma may be the preferred specimen type (see Question 4 and 5).

Research priorities for knowledge gaps

- We recommend study of contemporaneous plasma and WB EBV DNA measurement in longitudinally followed patients in specific clinical settings (post-transplant monitoring, PTLD diagnosis, treatment monitoring) to assist in result interpretation and matrix optimization including evaluation of the clinical utility of measuring EBV DNA in both matrices either simultaneously or sequentially taking into account sensitivity and specificity, cost and laboratory logistics.

3.1.3 | Question 1C: Are specific strains of EBV associated with PTLD risk? Do specific EBV gene or miRNA (viral and host) expression in peripheral blood or EBV proteins in serum predict PTLD risk?

Recommendations

- Ascertainment of EBV strain is not recommended at this time as there is insufficient evidence to support that certain EBV strains confer specific PTLD risk.

Evidence Grade: Weak recommendation/very low-quality evidence.

- The use of EBV gene or miRNA (viral or host) expression analyses are not recommended for PTLD surveillance, diagnosis or prognosis.

Evidence Grade: Weak recommendation/very low-quality evidence.

Supporting evidence/information

Genetic variability among EBV strains

Since 2014, more than 200 whole genome sequences of EBV have become available⁹⁰ including from the WB of PTLD patients.⁹¹ A large number of single nucleotide polymorphisms^{92,93} have been identified, more commonly in latent genes than viral lytic genes.^{94,95} Although definitive links between specific gene polymorphisms and risk of EBV disease or EBV associated cancer risk including PTLD have not been established, several variants are being further studied⁹⁰ including LMP1 variants⁹⁶ in a prospective clinical trial of pediatric transplant recipients ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02182986), NCT02182986).

EBV gene expression

EBV gene expression of latent and lytic genes in blood has been investigated as alternative or adjunct biomarkers of PTLD risk or PTLD diagnosis in both SOT^{53,54,56,97-102} and HSCT patients.^{45,54,99,102,103} Results have been variable, likely due to factors including non-standardization of assays, use of pooled cells which precludes determination of specific expression patterns among individual cells,⁶ populations studied^{45,53,54,56,97-103} and differences in study design.^{53,54,99-102}

No consistent peripheral blood EBV gene expression patterns have been found that clearly associated with quantitative EBV DNA measurement^{98,102} or are predictive for, or diagnostic of, PTLD although only small numbers of PTLD patients have been studied.^{54,97,99,102} Currently there is no clear additional benefit of EBV gene expression profiling over measurement of EBV DNAemia alone in SOT recipients.

miRNAs

While EBV infection impacts host miRNA expression, the virus also encodes ~44 miRNAs.¹⁰⁴ Because miRNAs are highly stable and easily quantified in either PBMC or plasma they are attractive as potential biomarkers for PTLD. Analysis of miRNA in transplant recipients with primary EBV infection has not yet been done. However, preliminary studies have identified serum miR-BART22 as a potential biomarker of EBV reactivation in pediatric liver transplant recipients¹⁰⁵ and plasma miBART2-5p as possibly having a role in sustaining persistent high set points in pediatric kidney transplant recipients.¹⁰⁶ These interesting observations suggest further study of host and EBV miRNA profiling in peripheral blood is warranted as an alternative or adjunct marker for PTLD or PTLD risk.

Research priorities for knowledge gaps

- We recommend study of the biologic form of EBV DNA in plasma (target sequence fragment profiling) and cells (immuno-FISH or other technology) during phases of EBV infection/PTLD to understand pathogenesis and develop future biomarkers.
- We recommend exploration of the detection of viral and host cell miRNA in conjunction with VL measurements as: a predictor of EBV+PTLD.

3.2 | Question 2: What is the role of EBV serology screening before and after transplantation for risk and early detection of PTLD?

Recommendations

- We recommend that all donors and transplant candidates be serologically screened for evidence of past EBV exposure to enable donor/recipient (D/R) PTLD risk stratification.
Evidence Grade: Strong recommendation/moderate quality of evidence.
- We recommend differentiation of recently from remotely infected seropositive transplant candidates for risk stratification. Anti-VCA IgG and IgM and anti-EBNA-1 is a preferred screening panel in transplant candidates; QEBV DNA measurement may further refine classification (see Figure 1).

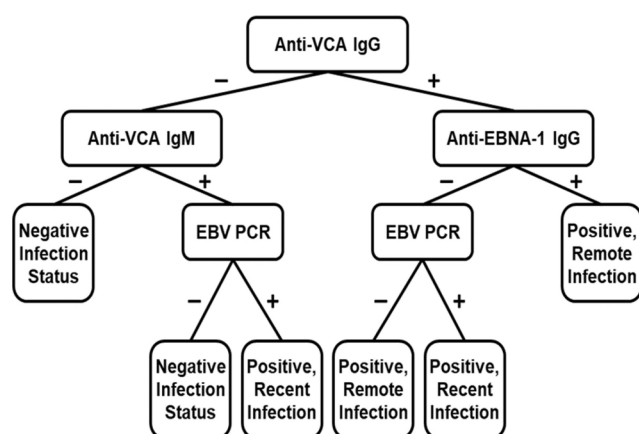


FIGURE 1 Interpretation of EBV serology in pediatric SOT candidates >12 months of age. Notes about EBV Serology: PCR testing in whole blood remains positive longer than in plasma during acute primary infection. Transfusion history should be reviewed to ensure that serology is reliable. When anti-VCA IgG is positive, anti-VCA IgM may be positive in recent infection (expect anti-EBNA IgG to be negative) or in remote infection with reactivation (expect anti-EBNA IgG to be positive). While most children with remote EBV infection will have a positive anti-EBNA IgG, some will remain anti-EBNA IgG negative (anti-EBNA non-seroconverters). A serology result that is anti-EBNA IgG positive, and Anti-VCA IgG Negative is biologically improbable: Likely false positive anti-EBNA IgG result. Repeat serology.

Evidence Grade: Weak recommendation/low-quality evidence.

- Serial serologic rescreening of EBV-negative SOT candidates on the waitlist (e.g., q6–12) months and at the time of transplantation is recommended (see Table 1).

Evidence Grade: Strong recommendation/low-quality evidence.

- Screening should occur remote from receipt of blood products when possible.

Evidence Grade: Strong recommendation/low-quality evidence.

- We recommend that seronegative SOT recipients at risk of donor-derived primary EBV infection (D+R-) and patients at risk of community acquired infection (D-R-) be considered at high (D+R-) and moderately increased (D-R-) risk for PTLD at least for the first three post-transplant years with respect to implementation of prevention strategies; monitoring algorithms may differ in the two subgroups (see question 3, Table 4 for preferred QEBV DNAemia monitoring); seronegative patients and their families should be counselled pre-transplantation about higher risk of PTLD development post-transplantation.

Evidence Grade: Weak recommendation/low-quality evidence.

- We do not recommend routine post-transplant serologic monitoring to predict PTLD risk; serologic monitoring to document ongoing susceptibility to infection in D+/R- and D-/R- who have no documented EBV DNAemia may be considered.

Evidence Grade: Weak recommendation/very low-quality evidence.

Supporting evidence/information

Pre-transplant EBV serostatus

Epidemiologic community seroprevalence studies in developed countries demonstrate ~40% of the population becomes EBV infected by age 5, rising to 80% in 18–19-year-olds.^{107–109} Infants may be protected from natural EBV infection by maternal antibodies during the first six months of life.^{110,111} with the duration of protection dependent on quantitative maternal antibody levels and decay kinetics.¹¹² However, whether maternal antibody prevents transmission from truly seropositive donors to seropositive infants <6 months of age is unknown as transmission in this setting is iatrogenic rather than natural; these recipient infants should be considered seronegative when stratifying risk. (Table 2) A recent Canadian study¹¹³ found that infant EBV seropositive donors <six months of age almost never transmit EBV to seronegative recipients (i.e. only have passive maternal antibody). Donors <six months of age are most commonly used in infant pediatric heart transplantation due to size-matching constraints, explaining why EBV mismatched (D+R-) infants in an International Society for Heart Lung Transplantation registry were at significantly lower risk of PTLD in the first six post-transplant months than older children.¹¹⁴ Therefore, very young seropositive donors (conservative estimate <3 months) could be considered seronegative when risk stratifying for pre-emptive strategies (Table 3).

Single center studies studying pre-transplant donor and recipient EBV serology as risk factors for PTLD are often limited by small

TABLE 1 Example of an algorithm for follow-up testing on the waitlist and at transplant in SOT candidates >12 months of age^a

EBV infection status (see Flowchart)	Follow-up testing ^b
Negative	Repeat EBV serology every 6–12 months while on waitlist and at Transplant - If serology changes, ensure no recent transfusion (Rule out passive antibody causing false positive serology) and then consult flowchart for interpretation EBV PCR at Transplant - If EBV PCR negative: maintain “Negative Infection status”. See Table 4 for post-transplant monitoring - If EBV PCR positive classify as “Positive, Recent infection”. See Table 4 for post-transplant monitoring
Positive, Remote infection	No further EBV PCR or serology required
Positive, Recent infection	Repeat serology in 1–3 months or at transplant if less than three months If serology changes, ensure no recent transfusion (Rule out passive antibodies causing false positive serology) and then consult flowchart for interpretation Obtain EBV PCR at transplant

^aNote this algorithm is an example and does represent specific recommendations or consensus as some authors recommend testing more or less frequently while on the waitlist.

^bEBV PCR in whole blood preferred over plasma.

PTLD case numbers and registry data have issues with PTLD case undercounting, missing and misinterpreted EBV serology data.¹¹⁵ Despite this, an overwhelming risk factor for EBV+ PTLD occurring in the early years after transplant, in almost all analyses across all non-intestinal SOT allograft types in both pediatric and adult populations is EBV seronegativity pre-transplant and primary EBV infection post-transplant.^{88,114,116–122} In a multivariate analysis of the Collaborative Transplant Study database which includes both pediatric and adult SOT recipients an increased risk for non-Hodgkin lymphoma was observed in kidney and heart recipients EBV seronegative pre-transplant (adjusted hazard ratio (HR) 6.5 and 3.6, respectively) but not in liver recipients (HR 0.6).¹¹⁶ However, an analysis of the SRTR data in the United States not only confirmed an increased risk associated with being EBV-seronegative in kidney (HR 3.6), and heart (HR 4.0) but also found a smaller but significantly increased risk in seronegative liver recipients (HR 1.5).¹¹⁹ In pediatric liver transplant this risk was further confirmed in a multi-center study where EBV seronegativity pre-transplant was associated with an increased risk of both EBV disease and PTLD (HR 3.51 and 2.94 respectively).¹²² Although EBV seronegativity pre-transplant is a predominant risk factor for EBV+PTLD in adults within the first five years after transplant, the influence of this risk factor wanes and is lost as EBV-negative PTLD equals or surpasses EBV+ PTLD in frequency late after transplant.^{117,123,124} This has been less studied in pediatric SOT recipients. In pediatric recipients PTLD incidence is highest in the first post-transplant year making this the most important period in which to target VL monitoring. A second smaller peak was described 3–5 years post-transplant in an analysis of a German multicenter pediatric PTLD registry; pre-transplant seronegativity was observed in 87% of patients who developed PTLD in the first post-transplant year but in only 62% with PTLD more than 5 years after transplant.¹²⁵ EBV seronegativity remained an independent risk factor for late PTLD (>3 years after transplant) with a HR of 1.65 (95%CI 1.06–2.55) in a recent analysis of a pediatric heart transplant study database.¹²⁶

EBV infection rates are high in the first transplant year in EBV mismatched (D+R-) patients as a result of donor-transmitted infection, with transmission assessed by WB EBV DNAemia varying from

29.3% in heart recipients to 62.4% in liver recipients in one study.¹¹³ Not unexpectedly, when post-transplant follow-up periods are short, studies have demonstrated that risk for PTLD is higher in D+R- than in D-R- recipients in both pediatric heart¹¹⁴ and liver¹²² recipients and for symptomatic EBV infection (combined disease and PTLD) in pediatric kidney recipients.⁸⁸ However, at three years post-transplant the risk of PTLD in D+R- and D-R- pediatric kidney recipients in an Organ Procurement and Transplantation Network/United Network for Organ Sharing database analysis was comparable.¹²⁰ Given the decline in the incidence of PTLD occurring in the first post-transplant year in more recently studied cohorts,^{120,122} the differential importance of donor-derived (D+R-) versus community acquired (D-R-) primary EBV infections as a risk factor for EBV+PTLD in pediatric populations may be decreasing, although the timing of donor-derived infection is more predictable. Therefore, both D-R- and D+R- pediatric subgroups should be considered for inclusion in pre-emptive prevention programs although monitoring algorithms may need to differ.

Intestinal transplant recipients, appear to have a high risk of PTLD development, independent of pre-transplant EBV serostatus.^{127–129} Children with non-intestinal transplants seropositive pre-transplant are at lower risk of PTLD than those seronegative, although the difference may be less marked than in adults.^{116,127,130} This may reflect a greater risk of serostatus misclassification in children because of passive maternal antibody or blood products. Donor EBV serostatus does not impact PTLD risk in seropositive heart transplant recipients¹¹⁴ but data regarding this issue are limited.

In contrast to EBV seropositive adults, most of whom were infected in the remote past, a proportion of seropositive children who are being transplanted are likely recently infected (i.e., less than one year ago) because of higher rates of community-acquired infection. This is particularly true for seropositive children between 1–2 years of age, a common age for pediatric liver transplantation. Even in healthy immunocompetent subjects experiencing primary EBV infection VLs generally approach but do not achieve equilibrium and a quantitative “set point” comparable to VLs observed in EBV seropositive asymptomatic children and adults until approximately one

TABLE 2 Pre-Transplant EBV Infection Status Classification and example of testing on the waitlist and at transplant in candidates <12 months of age^a

Anti-VCA IgM	Anti-VCA IgG or Anti-EBNA-1 IgG	EBV PCR	EBV infection status	Monitoring while on waitlist
Positive or Negative	Positive or Negative	Positive	Positive, Recent Infection	Monitor EBV PCR monthly while on wait list and serology and PCR at Tx
Positive or Negative	Positive or Negative	Negative	Negative Infection status	Monitor with repeat serology and EBV PCR every six months while on wait list and at Tx

^aChildren born to EBV seropositive mothers will have passive antibody (anti-VCA IgG and/or anti-EBNA IgG) which will wane over the first year of life. Children born to both seronegative and seropositive mothers can have high rates of acute infection between 6–12 months of life; infection prior to six months is uncommon and is quite rare prior to 3 months of age. EBV PCR is a more sensitive marker of acute infection than anti-VCA IgM. All candidates <12 months of age should have EBV PCR screening as well as EBV serology.

TABLE 3 Interpretation of EBV serology in donors

Donors <3 months of age	Donors >3 months of age
- Consider donor EBV negative regardless of anti-VCA IgG or anti-EBNA IgG result - Consider donor EBV positive if anti-VCA IgM is positive	- Consider donor EBV negative if: both anti-VCA IgG and anti-EBNA IgG result negative - Consider EBV positive if anti-VCA IgG, anti-EBNA IgG, or anti-VCA IgM or anti-VCA are positive - In living donors with unclear EBV serology (anti-VCA IgG Negative and either anti-EBNA IgG OR anti-VCA IgM are Positive) repeat serology if possible.

Note: Assumption: donors do not have EBV PCR testing performed.

year after infection onset.^{131–133} Recently infected children with incompletely developed EBV-specific immune responses may represent an intermediate risk group between seronegative and seropositive children with respect to PTLD risk, although this has not been systematically studied. A more extensive EBV serology panel that includes markers such as anti-VCA IgG and IgM and anti-EBNA1 IgG, readily available in most clinical laboratories along with EBV VL testing, preferably using WB (see Figure 1) could be used to more precisely determine the infection status of children pre-transplant and while on the waitlist.¹³⁴ Immunoblotting or VCA IgG avidity testing is also used in some centers to identify recent acute infection and resolve indeterminate serology results.¹³⁵ Classification of pediatric recipients into three categories: (1) seronegative and naïve (uninfected), (2) seropositive and acutely (recently) infected, and (3) seropositive and remotely infected to inform monitoring algorithms for preventive measures may be useful as outlined in Figure 1 and Tables 1–4.

Post-transplant serology

In SOT recipients who are EBV seronegative pre-transplant, seroconversion is significantly delayed relative to EBV DNAemia in blood, sometimes occurring concomitantly with PTLD presentation.^{88,136–139} In pediatric seronegative kidney transplant recipients with EBV DNAemia, Hocker et al⁸⁸ found seroconversion occurred at 7.5 ± 2.3 months, 4.9 ± 3.3 months after EBV DNAemia; 19% of patients with EBV DNAemia had not seroconverted by one-year post-transplant. In seropositive recipients, serologic parameters were also not correlated with EBV DNAemia.^{81,88} Therefore, serology is not useful as a primary surveillance tool in pre-emptive PTLD prevention strategies early after transplant. However, it could

be used to identify patients at ongoing risk of community-acquired infection among those seronegative prior to transplant without documented EBV DNAemia.

Specific patterns of EBV-specific antibodies described in a relatively small number of patients provide preliminary evidence for use as possible adjunct biomarkers to EBV VL for PTLD risk stratification. These require further validation and include markedly delayed or non-seroconversion^{138,140,141} or lack of antibody evolution (i.e. non-seroconversion to anti-EBNA 1 or anti-EA)¹⁴² in seronegative patients and falling EBNA-1 antibody titers in seropositive patients.^{137,143}

Research priorities for knowledge gaps

- We recommend further studies to determine allograft-specific risks of early EBV+ PTLD in recipients who are EBV seropositive (R+) pre-transplant.
- We recommend further studies of EBV-specific serology profiles post transplant as predictors of EBV+ future PTLD.

3.3 | Question 3: Can QEBV DNAemia kinetic parameters in peripheral blood be used to identify risk for EBV+ PTLD and inform pre-emptive interventions?

This question addresses the diagnostic algorithm; details on pre-emptive intervention measures are discussed in the “consensus guidelines for prevention” in the same issue covering these

TABLE 4 Example of an algorithm for post-transplant EBV PCR and serology monitoring^{a,b}

Donor (D)/Recipient (R) EBV Infection status	PTLD risk status	Post-Transplant Monitoring algorithm
D-R-	At risk of community acquired EBV infection, most common in younger children and teenagers PTLD Risk: intermediate	EBV PCR at transplant, then - Monthly x6 months or until maintenance immunosuppression is achieved, whichever is longer - Then q3-6 months at routine follow-up visits AND at time of any subsequent illness with symptoms suggestive of infectious mononucleosis - If EBV PCR becomes positive, consider obtaining EBV serology. Monitor EBV PCR weekly until dynamic pattern is determined (see specifics in D+R- section). - EBV serology - At 6 months and 1 year and then annually until seroconversion to positive anti-VCA IgG - If seroconversion to positive anti-VCA IgG occurs with no prior positive EBV PCR, repeat serology to confirm, and obtain EBV PCR
D+R- All R+ intestinal / multivisceral transplant recipients	High risk of donor transmitted EBV Infection, may be recipient acquired in R+ intestinal/multivisceral recipients PTLD risk: High	EBV PCR at transplant, then Weekly to 3 months, biweekly until 6 months, and then monthly or at follow-up routine bloodwork to 12 months, modifying algorithm as below if required EBV PCR most commonly becomes positive between 2-4 months after transplant If EBV PCR becomes positive - Continue weekly EBV VL monitoring until peak VL has been reached (Usually 2-4 weeks from first detection) and VL has decreased after peak by at least 1 log₁₀ (example: if peak VL is 4.5 log₁₀ IU/ml then gradually decrease monitoring once EBV VL <3.5 log₁₀ IU/ml) - Subsequently incrementally decrease the frequency of EBV VL monitoring to biweekly, monthly, then q 3 months depending on follow-up visits until VL is undetectable or "set point" VL is established (generally 1-2 years after first detection) - Pediatric Transplant infectious disease physician can provide more specific guidance - EBV serology in R- recipients only - At 6 months, 1 year and then annually until seroconversion to positive anti-VCA IgG. - If seroconversion to positive anti-VCA IgG occurs with no prior positive EBV PCR, repeat serology to confirm, and obtain EBV PCR
R+, classified as Recent infection	Full EBV-specific immune response may not yet be established PTLD Risk: unknown; may be Intermediate	EBV PCR at transplant then weekly to biweekly until 3 months - If EBV PCR becomes positive monitor EBV PCR weekly until dynamic pattern is determined (see specifics in D+R- section). - EBV Serology - If anti-EBNA IgG negative and/or anti-VCA IgM positive at transplant, repeat EBV serology at 6 months, and 1 year to assess seroconversion to positive EBNA IgG and/or disappearance of anti-VCA IgM.
R+ Remote infection: All organs except Intestinal transplant recipients (see above)	PTLD risk: Low	No EBV VL monitoring or repeat EBV serology required
Recipient who was R-preTx, has primary EBV infection and now has persistent high QEBV DNAemia ^c >1 year after first detection	PTLD risk: unknown; appears low for all non-cardiac recipients	EBV PCR and EBV serology 18 months, 2 years then annually (optional)

^aNote this algorithm is an example and does represent specific recommendations.

^bAdditional for cause EBV PCR testing should be performed at clinician's discretion when symptoms suggestive of infectious mononucleosis are present.

^cMost common with WB testing.

conference reports.¹⁴⁴ Similarly, treatment of PTLD is reviewed in a separate guideline.¹⁴⁵

Recommendations

- We cannot recommend specific quantitative EBV DNA levels or quantitative changes in peripheral blood that identify PTLD risk and should trigger interventions. However, we recommend immunosuppression review on initial qualitative detection of EBV DNA (above limit of detection of assays used in clinical laboratories) in the peripheral blood in patients seronegative pre-transplant.

Evidence Grade: Strong recommendation/low-quality evidence.

- We recommend QEBV DNAemia surveillance to identify risk for EBV+ PTLD and to inform pre-emptive interventions in patients who are EBV seronegative pre-transplant and receive organs from an EBV seropositive donor (D+R-) donor (for details, see Table 4).

Evidence Grade: Strong recommendation/low-quality evidence.

- We recommend QEBV DNAemia surveillance to identify risk for EBV+ PTLD and to inform pre-emptive interventions in patients who are EBV seronegative and receive an EBV seronegative organ (D-R-; for details, see Table 4).

Evidence Grade: Weak recommendation/very low-quality evidence.

- QEBV DNAemia surveillance is not recommended for recipients who are EBV seropositive pre-transplant with remote infection except for intestinal transplant recipients and patients undergoing re-transplantation after PTLD (for details, see Table 4).

Evidence Grade: Strong recommendation/low-quality evidence.

- We recommend QEBV DNAemia surveillance to identify risk for EBV+ PTLD and to inform pre-emptive interventions in seropositive recipients with evidence of recent infection prior to transplantation.

Evidence Grade: Weak recommendation/low-quality evidence (for details, see Table 4).

Supporting evidence/information

QEBV DNAemia kinetics: implications for surveillance and monitoring algorithms

In addition to absolute quantitative levels of EBV DNAemia we look to parameters related to EBV VL kinetics to inform implementation of pre-emptive PTLD prevention strategies and to monitor response to prevention and treatment interventions.¹ Defining "abnormal" QEBV DNAemia kinetic patterns in SOT recipients requires understanding "normal" EBV VL dynamics in different fractions of peripheral blood during acute and persistent infection in immunocompetent people of varying ages. These data are recent, limited and are primarily derived from studying adolescents and young adults both seroconverting to EBV and with IM in developed nations^{131,146-149} and infants infected very early in life in Africa.^{112,150,151} QEBV DNAemia during primary infection is characterized by an incubation or "eclipse phase"

after exposure, an acute and convalescent phase during which EBV DNAemia is detected, accelerates to a peak with a specific doubling time and then regresses, followed by an equilibrium state with an individual "set point" marking persistent infection. Time to first detection, time from first detection to peak, time from peak to set-point and area under the QEBV DNAemia time curve (AUC) are additional parameters that might be measured. Available data related to primary EBV infection in immunocompetent hosts are reviewed in greater detail elsewhere.³⁶

Incubation or "eclipse" phase

The exact timing of EBV exposure is known in EBV mismatched D+/R- patients; QEBV DNAemia is being monitored during the eclipse phase. In an era prior to the routine use of anti-viral medications for CMV prophylaxis, the median time to EBV DNA detection in saliva during primary donor-derived EBV infection after SOT was 6 weeks,¹⁴⁰ similar to the IM incubation period of 32-49 days.^{147,149,152,153} A more recent study in EBV-mismatched pediatric and adult SOT patients monitored serially for QEBV DNAemia using real-time qPCR assays, noted later initial detection at a median of 84 days with only rare detection in the first post-transplant month; initial detection occurred in months 2-4 in most patients.¹¹³ The use of anti-viral prophylaxis appeared to delay the onset of EBV DNAemia in a cohort of adult kidney transplant recipients,¹⁵⁴ but not in a similar pediatric cohort.¹⁵⁵

Acute and convalescent infection phase

Acceleration phase and peak: In immunocompetent hosts WB QEBV DNAemia rises explosively becoming detectable by diagnostic assays 7-10 days before symptom onset and rising rapidly to peak over the next two weeks.^{146,147,149,156} A similar explosive rise is seen in transplant patients.^{45,70,76,157} Modeling of data from the Kenyan infant study found very short WB QEBV DNAemia doubling times of 1.6 to 2.1 days.¹⁵⁸ Although doubling times as rapid as 56 hours have been described in the lung transplant setting⁷⁶ data on time to peak are limited after SOT. This very rapid initial VL rise makes the logistics of pre-emptive therapy challenging; frequent weekly monitoring during the highest risk period for donor-derived infection (1-4 months)¹¹³ is therefore preferred for EBV-mismatched SOT recipients. However, pre-emptive surveillance frequencies have not been compared and there are no data to suggest that less frequent monitoring (i.e., biweekly or even longer intervals later in the first year after transplant) negatively impacts pre-emptive strategy outcomes. In evaluating interventions during the acceleration phase of primary infection reduction in doubling time or lower peak or set point could be considered positive endpoints; absolute quantitative reduction in load may not be an appropriate measurement.

Although there is overlap, SOT recipients with PTLD appear to have higher VLs measured in both plasma^{69,159} and PBMC^{61,69,143,160} than those observed in immunocompetent IM patients. For reference, median peak loads (not calibrated to the WHO international standard) reported in IM studies varied from 3.0 log₁₀ copies/ml of WB to 7350 copies/10⁶ PBMC or ~4.2 log₁₀ copies/ml of

WB.^{131,146,148,149} Peak VL is similar in both IM patients and asymptomatic patients with primary infection^{131,161} suggesting IM symptoms result from an exaggerated immune response rather than a higher VL.¹⁴⁸ Earlier age of infection (mean 7.28 months) was associated with higher and more persistent WB QEBV DNAemia in a Kenyan infant study, suggesting infants infected earlier in life have poorer control of infection.¹⁵¹

Historically pre-emptive strategies were based on older studies using non-standardized assays and sample types that found high peak VL was a sensitive but not specific marker of EBV+ PTLD occurring early, usually in the first year after transplant; QEBV DNAemia most often peaked before symptom onset (reviewed by¹⁶²). Most analyses were univariate using single center data with small numbers of PTLD events. Result interpretation was sometimes complicated by the heterogeneity of the populations studied, with high and low risk populations and SOT and HSCT populations pooled for analysis.

More recent studies have provided more conflicting data. No clinically useful association was found between high WB peak VL and PTLD in German pediatric kidney transplant recipients⁸⁸ or a Korean SOT population (predominantly pediatric liver recipients).¹⁵⁷ However, high peak VL was identified as an independent predictor of PTLD in a multi-center study of pediatric kidney transplant recipients using multivariate analysis¹³⁰ and early peak load, was a PTLD risk factor in a single center study of pediatric heart transplant recipients.¹⁶³ In a study of pediatric kidney transplant recipients, the AUC of EBV VL during the first post-transplant year was also not predictive of symptomatic EBV infection that included PTLD.⁸⁸ Moreover, almost all historical and more recent studies were not natural history studies, as clinicians implemented interventions based on available results. These data question whether any specific level of peak QEBV DNAemia is associated with PTLD risk and precludes making recommendations regarding trigger points for interventions.

Regression from peak load: QEBV DNAemia regression patterns are variable among patients and may depend on patient age and the “immunosuppressive environment” in which primary infection occurs. In a study of adolescents and young adults the median duration of WB EBV DNAemia was 17 days¹⁴⁷ but was as long as 164 and 202 days in some patients. A biphasic pattern of WB/PBMC VL regression has been observed as adaptive immune responses develop.^{164,165} In younger children regression may be slower¹⁶⁵; the median duration of WB EBV DNAemia during primary infection was 4.9 to 6.3 months in Kenyan infants.¹⁵⁸

In the transplant setting, observations regarding QEBV DNAemia regression kinetics in the absence of interventions are extremely limited. The half-life of the biologic form of EBV DNA being measured, in the order of hours for circulating cell free EBV DNA in plasma versus weeks for latently infected cells should be considered in interpreting dynamic changes observed. Unlike IM patients and asymptomatic non-HIV infected African infants with primary EBV infection in whom EBV DNAemia in plasma becomes undetectable in <1 month^{41–43,49,50} and <3 months,¹⁶⁶ respectively, EBV DNA is often detected in the plasma in asymptomatic pediatric liver transplant

recipients with primary infection for at least a year.^{51,52} It is difficult to assess the impact of interventions using viral kinetic data without a control group not receiving the intervention or controlling for the timing of the intervention relative to the EBV DNAemia onset. When evaluating interventions implemented during the regression phase of acute primary infection half-life or time to clearance (for diagnostic assays) may be a better measurement of efficacy than an absolute quantitative reduction in VL.

Recurrent episodes of EBV DNAemia: Acute primary EBV infection may include recurrent episodes or waves of EBV DNAemia that are not necessarily associated with short-term adverse events in the immunocompetent subject^{49,158} Rebound events after initial regression have also been observed in the SOT setting after primary infection managed by reduction of immunosuppression or antiviral therapy^{167,168} with no apparent adverse effects.¹⁶⁷

Equilibrium or set point VL: All EBV seropositive patients have persistent peripheral blood EBV infection even though EBV DNA levels are often below the level of detection of real-time qPCR assays for specimen types and assays used in clinical laboratories in the transplant setting. The term “clearance” should be interpreted from this perspective. This host-virus equilibrium or set point is estimated to take up to a year to achieve after acute infection^{131–133} and is unique to an individual and relatively stable for at least 3.5 years. “Set point” VL may also be geographically variable. Healthy children and adults in Gambia have a set point (median 850 copies/10⁶ PBMC) >10-fold higher than adults in the United Kingdom (median 79 copies/10⁶ PBMC); some had set points similar to those seen in IM patients in the United Kingdom.¹⁴⁸ Interventions occurring after set point has been established may differ and may need to be analyzed separately from those earlier during acute primary infection as they likely occur at very different stages of host-EBV immune response development. There is some stochastic variation in values around the set point over time estimated at $\pm 25\%$ and this should be considered when defining clinically meaningful changes.¹⁶⁹

The time required to establish an EBV viral set point after donor-derived primary infection in SOT recipients may be longer because exogenous immunosuppression may delay the development of EBV-specific adaptive immune responses. Colombini et al¹³⁰ observed that the 38% of patients experiencing primary infection after kidney transplantation who “cleared” their EBV DNAemia did so at a median of 22.1 months after detection. Similar protracted regression to relatively stable but persistently elevated WB VL was observed by Kullberg-Lindh et al⁵² on withdrawal of all immunosuppression in pediatric liver transplant recipients with “set-point” WB VLs sometimes not observed until two years after initial detection, often concordant with clearance of EBV DNAemia in serum. A significant proportion of SOT recipients experiencing primary EBV infection have detectable WB QEBV DNAemia, sometimes at very high levels that is sustained for many years; this appears to represent an “abnormally high” viral set point and is observed less frequently in SOT patients seropositive pre-transplant.^{53,88,100,155,170–174} The immunopathogenesis and factors that influence setting and sustaining EBV viral

set point are uncertain.^{175,176} Young age at infection, immunodeficiency and intercurrent infections such as malaria are believed to elevate set-points in African infants^{150,177}; warm ischemia time and high graft to recipient weight ratio have been identified as factors in pediatric liver transplant recipients.¹⁷³

Investigators have often defined SOT patients as having a chronic high VL phenotype (CVLP) when VL was sustained (>50%) with WB EBV VLs in the range of 5,000–16,000 copies/ml (assays not calibrated to WHO international standard) for >6 months.^{53,88,100,130,155,170–174} High EBV viral set point is the terminology used for a similar biologic state in HIV patients.¹⁷⁸ It is not known whether six months after first detection is sufficient time for equilibrium or set point to be achieved in SOT recipients and what specific quantitative set point, if any, is associated with increased later PTLD risk. Although studies of heart transplant recipients with CVLP suggest they may be at significantly increased risk of late onset EBV-positive PTLD,^{170,171} this risk appears in part to be organ-specific with intermediate risks observed in intestinal transplants¹⁷⁴ and low to negligible risk in pediatric liver^{53,172,173} and kidney^{88,130,155,176} transplant recipients. Whether any intervention, such as immune reconstitution (typically following reduction in immunosuppression in SOT recipients)^{178–180} or prolonged antiviral therapy,¹⁸¹ can alter EBV set point when established is uncertain.

QEBV DNAemia in seropositive subjects with remote infection

Transient EBV DNAemia can be detected using clinical assays even in asymptomatic immunocompetent seropositive subjects with remote infection^{39,40}; and is detected at a higher rate in WB than plasma when contemporaneously samples are collected from the same patient.⁴⁰ Critical illness and sepsis increases the prevalence of EBV DNA detection.⁴⁰ In acute malaria a 5- to 6-fold increase in PBMC EBV VL was observed that persists for at least 4–6 weeks.¹⁴⁸ Whether similar dynamics in set point disturbances occur during other acute infections in either the transplant or non-transplant setting is not known.

Although peak loads are usually lower than in primary infection and many EBV+ PTLD cases, the high prevalence of EBV DNA detection (13–72% in WB) and the low incidence of PTLD in remotely infected adult seropositive non-intestinal SOT recipients questions the benefit of routine QEBV DNAemia surveillance of this patient group.^{77,89,182–184} EBV DNAemia prevalence is lower when plasma is monitored.^{73,185} Data from pediatric recipients are more limited but show similar patterns. In two pediatric kidney cohorts seropositive pre-transplant, EBV DNA was detected in WB above a “significant level (3000 copies/ml)” in 19.9%¹³⁰ and at any level in 44.4%.⁸⁸

Based on available evidence of QEBV DNAemia dynamics in specific risk groups, an example of monitoring algorithms that might be used for pre-emptive strategies is illustrated in Table 4. Center-specific algorithms should be designed considering local factors including routine patient follow-up intervals, access to specific types of laboratory testing, health care delivery systems and cost as well as regulatory issues related to donor screening.

Research priorities for knowledge gaps

- We recommend studies evaluating ongoing surveillance of QEBV DNAemia in WB versus plasma for pre-emptive therapy when the recipient is on stable maintenance immunosuppression years after transplant and “set point” has been established even if elevated.
- We recommend studies of all QEBV DNAemia kinetic parameters (mathematical modeling) of EBV infection after transplant and evaluation of these parameters as risk factors for PTLD development.
- We recommend studies of the natural history of high VL set points with respect to dynamics of clearance and future risk of EBV+ PTLD.

3.4 | Question 4: Can QEBV DNAemia be used alone as a test to diagnose EBV+ PTLD?

Recommendations

- QEBV DNAemia should not be used alone to diagnose PTLD in pediatric SOT recipients.
Evidence Grade: Strong recommendation/high-quality evidence.
- We recommend that non-detection of EBV DNA in peripheral blood in serially monitored patients can be used as a screening test with good negative predictive value (NPV) in patients at increased risk for EBV+ PTLD occurring early after transplant but does not exclude EBV-negative PTLD and some cases of EBV+ PTLD.
Evidence Grade: Strong recommendation /low quality evidence.

Supporting evidence/information

Pathology remains the gold standard for PTLD diagnosis. The highest risk pediatric SOT recipients are most often being serially monitored for QEBV DNAemia in the early post-transplant period. Only limited pediatric SOT data exists with respect to direct comparison of QEBV DNAemia with pathology as a single evaluation only at the time a patient presents with symptoms and/or signs of possible PTLD.^{52,88,130}

The advocacy for WB over plasma as the preferred sample type in SOT dates back to early studies that failed to detect EBV DNA in the plasma of a significant number of PTLD cases, despite detection in WB samples.^{75,76} However, these studies were performed using less sensitive pre-real-time qPCR technology with large amplicons and older nucleic acid extraction methods that may have failed to identify the small EBV DNA fragments in plasma.¹⁸⁶ In contrast, more recent studies using real-time qPCR assays and testing predominantly adult populations found detectable plasma EBV DNA in almost all cases of EBV+ PTLD with the exception

of localized CNS PTLD and EBV+ mucocutaneous ulcer.^{69,72,77,159} EBV DNA detection in plasma has also been shown to be a more specific marker of EBV disease, including EBV+ PTLD, than WB or PBMC detection.^{69,70,74,77}

Several studies of pediatric SOT recipient cohorts serially monitored for QEBV DNAemia in WB or PBMC after transplant found a positive test had poor specificity for PTLD diagnosis but persistent non-detectable EBV DNAemia throughout monitoring had a good NPV for EBV+ PTLD occurring early after transplant.¹⁸⁷⁻¹⁸⁹ However, two of these studies^{187,188} used older, less sensitive semi-quantitative assays and the NPV of testing only at the time of diagnosis was not specifically studied. EBV DNA has also been found to be non-detectable in WB in some pediatric thoracic transplant recipients at the time lung PTLD was diagnosed.¹⁹⁰ Whether the sensitivity or specificity of WB or plasma EBV DNA measurement as a diagnostic test can be improved by evaluating specific quantitative levels as cut-off values has not been studied.

A major confounder for the use of QEBV DNAemia as an initial screening test for PTLD diagnosis is the occurrence of EBV-negative PTLD whose pathogenesis may not be related to EBV. EBV-negative PTLD is very common in adult SOT populations making up ~50% or more of PTLD cases after the first post-transplant year.^{77,123,191} EBV DNA is detected in WB/PBMC in ~25% of EBV-negative PTLD cases, similar to the prevalence of detection in SOT recipients without PTLD.^{69,77} However, it is almost always non-detectable in the plasma of EBV-negative cases and when detected it is present at low levels and transiently.^{69,77} Therefore testing in plasma allows for better discrimination between EBV+ PTLD and EBV-negative PTLD than testing cellular blood fractions when PTLD is present.^{69,77} However, it is not useful as an initial screening test for any PTLD in adult populations after the first post-transplant year.^{77,123,191} The yearly post-transplant incidence of EBV-negative PTLD has been less well described in pediatric populations. In a German pediatric multi-organ PTLD registry analysis 11/65 (16.9%) of cases occurring after the first-post-transplant year were EBV-negative PTLD, lower than what is observed in adult SOT settings.¹²⁵ However, in a multicenter pediatric heart transplant database analysis of 110 non-recurrent PTLD cases occurring >3 years after transplant only 25% of cases had detectable EBV DNA in blood at diagnosis although the proportion tested for QEBV DNAemia and sample type used was not specified; only 35% of these late cases were found to be EBV+ PTLD.¹²⁶

Research priorities for knowledge gaps

- We recommend further studies comparing QEBV DNAemia in WB and plasma in correlation to tissue pathology, EBV status of PTLD tissue and disease localization in adult and pediatric populations.

3.5 | Question 5: Should QEBV DNAemia be used to monitor response to therapy?

Recommendations

- We do not recommend QEBV DNAemia testing alone to monitor response to PTLD treatment. In particular, monitoring of VL may be misleading for children receiving rituximab or other anti-B cell monoclonal therapies as treatment for PTLD. When using VL monitoring as part of following clinical response, we recommend plasma as the preferred sample type.

Evidence Grade: Weak recommendation/low-quality evidence.

Supporting evidence/information

In a study of seven pediatric liver recipients with EBV+ PTLD, clearance of EBV DNA in PBMC was observed within the first month after treatment with reduction of immunosuppression, IV ganciclovir and CMVIG¹⁶⁷ concomitant with clinical response but subsequent rebound of VL was frequent in the absence of disease recurrence. In HSCT PTLD patients treated with rituximab, investigators reported that persistently detectable EBV DNAemia in WB¹⁹² or in WB or plasma samples (in equal frequency with results pooled for analyses)¹⁹³ was a predictor of poor response. However, in both SOT and HSCT populations, the bulk of data suggest that plasma may be preferred over samples with cellular fractions for monitoring response to therapy and predicting relapse as VL correlations with clinical response appear better, particularly when the patient had received rituximab therapy.^{69,74,77,194-196}

Research priorities for knowledge gaps

- We recommend that standardized QEBV DNAemia monitoring in both WB and plasma be incorporated into prospective clinical trials of treatment for PTLD.

3.6 | Question 6: Is QEBV DNA testing in CSF, BAL and saliva useful to predict or diagnose PTLD?

Recommendations

- We recommend testing for EBV DNA in CSF for suspected CNS EBV disease or CNS PTLD.

Evidence Grade: Weak recommendation/very low-quality evidence.

- We do not recommend routine testing for EBV DNA in BAL samples for the diagnosis of pulmonary PTLD.

Evidence Grade: Weak recommendation/low-quality evidence.

- We do not recommend testing for EBV DNA in saliva for surveillance for pre-emptive PTLD strategies, PTLD diagnosis or treatment monitoring.

Evidence Grade: Weak recommendation/low-quality evidence.

Supporting evidence/information

CSF

Testing for EBV DNA in the CSF has been used to assist in the diagnosis of CNS PTLD or EBV encephalitis, primarily with the goal of avoiding potentially morbid invasive biopsy procedures for tissue-based diagnosis. Data addressing this question are primarily extrapolated from the HIV-infected population where qualitative detection of EBV DNA in CSF is common in a wide variety of HIV neurological diseases and has poor positive predictive value (PPV) but good NPV for diagnosis of EBV+ CNS lymphoma.¹⁹⁷⁻¹⁹⁹ Quantitative cut-offs in the range of 3.3–4.0 log₁₀ copies/ml (not calibrated to WHO international standard) in CSF improved both specificity and PPV for both primary CNS lymphoma and CNS involvement in systemic AIDS-related lymphoma when compared to qualitative results alone.^{197,199,200} In a study that included five SOT recipients, Weinberg et al²⁰¹ observed that patients with primary CNS lymphoma had high CSF VLs associated with low CSF leukocyte counts. In contrast, patients with high leukocyte counts were more likely to be diagnosed with EBV encephalitis in the presence of high VL, or post-infectious complications, when VLs were low. In the HSCT setting, Liu et al²⁰² found that levels of EBV DNA were higher in CSF than in blood and predicted CNS disease and clinical response better. These data support a potential role for the use of quantitative EBV PCR to screen for EBV+ CNS lymphoma in SOT recipients. However, data addressing this question remain very limited in pediatric SOT recipients. Importantly, large case series of CNS PTLD in SOT recipients do not report CSF EBV PCR data; of note, only 30% of patients with primary CNS lymphoma had detectable EBV DNA in blood in the most recent case series.^{203,204}

BAL

In lung and heart-lung transplant patients, the lung is often the primary site of PTLD.²⁰⁵ Although initial reports suggested that high quantitative levels of EBV DNA in BAL fluid may be a more sensitive predictor of PTLD than EBV DNA measurement in peripheral blood,¹⁹⁰ EBV VL in BAL was not predictive of PTLD in a larger multicenter study of pediatric lung transplant recipients.²⁰⁶ Moreover, EBV DNA was detected in BAL fluid, often at high levels in adult lung²⁰⁷ and other transplant as well as non-transplant recipients²⁰⁸ in the absence of PTLD. Thus, interpretation of EBV PCR results from BAL fluid is challenging and its utility for determining the presence or absence of lung PTLD in transplant recipients is low based on current data.

Saliva

EBV DNA is predominantly cell-associated in saliva, presumably in lytically infected epithelial cells.^{140,149,169,209-212} In studies of immunocompetent subjects, EBV DNA appears in saliva explosively at high

levels approximately a week before onset of IM symptoms, peaks at 2 months after onset and remains markedly elevated over the first year; very high levels may persist for as long as 3.1 years,^{49,147,149,156} but decline over time to lower persistent levels.^{169,212} Longitudinal studies of healthy adults with remote EBV infection show nearly universal detection of EBV DNA in saliva at some time although point prevalence varies significantly from 24% to 90%.^{169,210-214}

In SOT recipients not receiving anti-viral agents with EBV activity, both the detection prevalence (83–96%) and quantity of EBV DNA in saliva increases significantly beginning in the second month post-transplant with persistence and then decline in the 6–12 months post-transplant period.^{140,215} Saliva EBV DNA levels were higher with increasing immunosuppression, in primary infection, and in patients with PTLD.¹⁴⁰ However, saliva EBV DNA detection as a potential predictor of PTLD using current real-time PCR technology has not been studied. In a recent study, pre-transplant EBV DNA detection in saliva and/or blood did not predict post-transplant EBV DNAemia²¹⁶ but no cases of PTLD were identified in the cohort. Importantly, use of IV ganciclovir, IV acyclovir, valacyclovir and valganciclovir results in clearance or a significant reduction in VL in saliva during treatment even in immunocompromised hosts but rapid rebound occurs on drug withdrawal.^{140,209,214}

Given the variability in sample collection, the frequent post-transplant use of antivirals with EBV activity that suppress the lytic replication of EBV in saliva, and the lack of conclusive studies, saliva testing is not recommended for VL surveillance in pre-emptive PTLD prevention strategies. EBV VL detection in saliva may warrant further exploration as an adjunct to serology to clarify pre-transplant EBV infection-status in patients with passive antibody and as an ongoing surveillance tool for community acquired primary infection.

Research priorities for knowledge gaps

- Studies to clarify the sensitivity, specificity, PPV and NPV of EBV DNA measurement in CSF in SOT recipients with possible evidence of CNS involvement for EBV+ PTLD diagnosis and treatment monitoring.
- Studies of saliva EBV DNA testing to detect community-acquired EBV infection, allowing more frequent monitoring in D-R- patients.
- Studies of saliva testing to clarify infection status in children with passive antibody (maternal or blood transfusion).

3.7 | Question 7: Is there evidence that non-specific immunologic tests after transplant are useful for the surveillance, diagnosis, prediction or monitoring of EBV+ PTLD?

Recommendations

- We do not recommend the use of measurement of ATP release by CD4⁺ or CD3⁺ T cells in response to PHA or EBV-Ag lysate

stimulation (Immuknow Assay) for PTLD related surveillance, diagnosis, or prognosis.

Evidence Grade: Weak recommendation/low-quality evidence.

- We do not recommend the use of functional or phenotypic measurements of NK cells for PTLD surveillance, diagnosis or prognosis outside of clinical trials.

Evidence Grade: Weak recommendation/low-quality evidence.

- We do not recommend the use of lymphocyte subpopulation assessments for PTLD surveillance, diagnosis or prognosis outside of clinical trials.

Evidence Grade: Weak recommendation/very low-quality evidence.

- We do not recommend the use of monitoring for torque teno viruses in serum or tissue for PTLD surveillance, diagnosis, or prognosis.

Evidence Grade: Weak recommendation/low-quality evidence.

Supporting evidence/information

Direct investigations for non-specific markers of immune competence or for effectors of innate immunity in SOT patients (including pediatric recipients) with impaired EBV control (presence of CVLP and/or with PTLD), have been documented extensively in the literature. One approach used the Cylex ImmunoKnow assay to detect possible over-immunosuppression, risk of CVLP or of progression to PTLD after organ transplantation. Specifically, ATP levels released by circulating CD4⁺ or CD3⁺ T cells from SOT recipients (including pediatric heart and liver recipients) in response to non-specific (PHA) or EBV-specific stimulation, inversely correlated with EBV VL and the risk of PTLD in a cohort of pediatric heart recipients.²¹⁷ In addition, PHA-stimulated ATP values under a certain low threshold (i.e., <125mg/ng/ml) were associated with a 100% probability of high EBV VL (>4000copies/μg DNA) development in pediatric liver recipients.²¹⁸

Evidence for perturbations in effectors of innate immunity with possible direct consequences on adaptive anti-EBV immune control after SOT have also been reported.⁹ The importance of NK cells in EBV control has been documented in healthy subjects during both primary infection and latency.²¹⁹ These cells produce elevated levels of cytokines (IFN-γ/TNF-α) that may interfere with EBV infectivity, while promoting type-1 immunity in CD8⁺ T cells. NK cells release perforin and granzyme B that lead to increased lysis of EBV-infected cells. Phenotypic and functional data related to NK cells changes after SOT in patients with persistently elevated VL or PTLD have also been reported. In a cohort of pediatric thoracic transplant recipients, Wiesmayr et al. detected decreased circulating CD56^{bright} and CD56^{dim}CD16⁺ NK cell subset levels, that down-regulated Nkp46 and NKG2D, and significantly up-regulated inhibitory molecule PD-1. These phenotypic changes were paralleled by NK functional impairment (low IFN-γ), resembling cellular exhaustion.²²⁰ A decrease in NK cell number accompanied by a reversed CD4:CD8 T-cell ratio was shown to predispose to recurrent EBV-PTLD in 14 pediatric PTLD cases.²²¹

In some recent reports, transplant for congenital heart disease has been associated with a higher incidence of PTLD compared to patients who suffered from acquired heart disease before transplant.^{222,223} It has been conjectured that this may be attributed to thymectomy in early life performed in association with surgery of congenital heart defects. These children may show reduced CD4⁺ and CD8⁺ lymphocyte counts, and recently persistence of these alterations was demonstrated in heart transplant patients developing PTLD.²²³ However, there remains insufficient evidence to recommend serial lymphocyte phenotyping for prospective evaluation of risk of development of PTLD.

Finally, the quantitative detection of torque teno virus DNA, a virus ubiquitously present in the blood of immunocompetent humans, has been studied as a measure of non-specific immune competence. Increased levels have been observed in the plasma of pediatric liver transplant recipients²²⁴ and significantly elevated amounts of teno virus DNA was found in paraffin embedded tissue from PTLD patients with worse survival outcomes.²²⁵

While all these findings are important, currently there is a lack of consensus on which marker or combination of markers may be of value for monitoring, risk stratification and prediction of EBV complications/PTLD after transplantation prohibiting their use in prevention strategies, diagnosis, or prognosis for PTLD at this time.

Research priorities for knowledge gaps

- We recommend that due to a poor consensus on which marker or combination of markers may be of value for non-specific/in-nate immunity monitoring, risk stratification and prediction of EBV complications/PTLD after transplantation, further research to evaluate and validate these biomarkers should be performed through multicenter studies and international collaborations.

3.8 | Question 8: Does measuring EBV-specific cell-mediated immune (CMI) responses either alone or together with EBV VL help to predict development of EBV+ PTLD?

Recommendations

- We do not recommend the routine use of EBV-specific cell-mediated immune response (CMI) measurement by multimer or cytokine-readout assays as a predictor of EBV+ PTLD.

Evidence Grade: Weak recommendation/low-quality evidence.

Supporting evidence/information

In non-immunocompromised patients EBV infection provokes a strong T-cell mediated immune response (CMI) targeting epitopes of multiple

EBV proteins by both CD4⁺ and CD8⁺ T cells leading to life-long protective immunity (reviewed in ref 226). Following a strong expansion of EBV-specific CD8⁺ and, to a lesser extent, CD4⁺ T cells during acute infection the repertoire of T cells narrows to form a memory T cell response mainly directed against focused lytic antigens BZLF and BMLF-1 and latent antigens EBNA-3B/C and LMP-2, that control both occasional lytic reactivation and latent EBV infection over time. It is hypothesized that PTLD develops as a result of an insufficient EBV-specific CMI response in the setting of medical immunosuppression.²²⁷ Remission of PTLD upon adoptive transfer of EBV-specific T cells further suggests a pivotal role of EBV-specific CMI in PTLD development and treatment.^{228,229} Thus, monitoring of EBV-specific CMI in SOT recipients either alone or in combination with EBV VL may be a tool to estimate PTLD risk in pediatric SOT patients. Few studies have been conducted to directly address this issue, and the subject is substantially complicated by the variety of laboratory techniques to detect EBV-specific T cells. EBV-specific T cells can be enumerated using either T-cell receptor detection by HLA-peptide multimers or by functional tests, most of which assess cytokine secretion after antigenic stimulation using flow cytometry, Enzyme-linked immunosorbent spot (ELISpot) analysis, or cytokine capture systems. Various sources of EBV antigen can be used for stimulation of T cells ranging from either autologous B cell lines transformed by a laboratory strain of EBV to recombinant EBV proteins or individual or overlapping peptide epitopes. A study by Smets et al. prospectively evaluated 45 pediatric liver transplant recipients from the time of transplant for EBV VL and EBV-specific CMI using ELISpot to measure IFN- γ secretion after stimulation with autologous EBV-transformed lymphoblastoid cells (B-LCLs).²³⁰ And 28 patients had primary EBV infection after SOT, 7 of whom developed PTLD. These patients displayed a low ratio of EBV-specific CMI to EBV VL predictive of PTLD. Upon reduction of immunosuppressive medication EBV-specific T cells increased thereby allowing re-introduction of immunosuppressive drugs to protect organ function. Similar results were obtained in a second study of 16 pediatric PTLD patients.²³¹ Low numbers of EBV-specific CD4⁺ and CD8⁺ T cells measured by IFN- γ secretion after stimulation with autologous B-LCLs were detected at diagnosis, which increased significantly after reduction of immunosuppression and treatment with rituximab. A third study of 6 pediatric liver transplant patients with PTLD demonstrated reduced T cell IFN- γ secretion compared to healthy controls, and a correlation of low EBV-specific T cells with disease recurrence was observed after rituximab treatment.²³² In another pediatric study 44 asymptomatic thoracic organ recipients were examined for EB VL and EBV-specific CMI using IFN- γ secretion and detailed immunophenotyping.¹⁷⁵ This study revealed differences in cell phenotype correlated with VL with those patients displaying low VL showing a moderately activated T-cell status while patients with chronic high VL showing an exhausted EBV-specific T-cell phenotype. In addition, data from adult patients suggest that EBV-specific T cells from patients with PTLD are low in number²³³ and exhibit reduced IFN- γ secretion after EBV peptide stimulation, especially when stimulated with latent antigens compared to healthy controls.²³⁴ Using the non-functional multimer analysis Falco et al. assessed the frequency of CD8⁺ T cells against BZLF1, EBNA3A, and BMLF1 in the circulation

of 24 pediatric liver or kidney recipients.²³⁵ The vast majority of individuals had detectable T cells with BZLF1-specific T cells most common. T cells specific for EBV could be measured within 4 weeks in seronegative individuals receiving an organ from a seropositive donor. The proportion of EBV-specific T cells was similar to the levels seen in other studies with non-transplanted, healthy subjects, and none of the patients developed PTLD. A Japanese study confirmed comparable levels of EBV-specific T cells in chronic high EBV VL carriers and controls.⁵³ Similar results were obtained in a study in adults.²³⁴ Another study detected EBV-specific T cells in PTLD patients by multimer technology and found no alterations in specificity and numbers compared to non-PTLD subjects, which did not change during treatment for PTLD.²³³ Combining both phenotypic and functional analysis of CD8⁺ T cells in pediatric heart transplant recipients, differences were detected in patients carrying high EBV VL with cells displaying an exhausted phenotype (programmed death 1(+)/CD127(-)) accompanied by a decline in IFN- γ release.¹⁷⁵ This indicates that future studies may have to look beyond mere enumeration of EBV-CMI towards characterization of phenotypes and function.

Altogether, these data are somewhat conflicting and not sufficient to recommend routine surveillance of EBV-specific CMI in pediatric SOT patients. The utility of EBV-specific CMI assays may also vary by clinical context and differ in the setting of acute primary infection, patients EBV seropositive before transplant and those with persistent elevation in VL late after transplant. However, data to date are promising and require further validation in future prospective clinical trials using methods that involve more detailed phenotypic studies and assessment of functional responses to both lytic and latent EBV antigens.

Research priorities for knowledge gaps

- We recommend further research be focused on measurements of functional EBV-specific CMI responses in addition to phenotypic status.
- Assessment of EBV-specific CMI should be included into prospective clinical trials.

3.9 | Question 9: Are there other biomarkers that predict PTLD development?

3.9.1 | Question 9A: Can PTLD surveillance strategies be directed based on current literature indicating HLA type and/or mismatch-based PTLD risk?

Recommendations

- We do not recommend stratifying clinical surveillance strategies based on specific recipient or donor HLA-types or HLA mismatches.

Evidence Grade: Weak recommendation/low-quality evidence.

Supporting evidence/information

Association of EBV-positive PTLD with the HLA-type of the organ recipient as well as the degree of HLA-matching has been studied by multiple investigators mainly in the kidney transplant setting. Most studies are based on adult patients, however, a few included only pediatric patients^{236,237} or a cohort of children and adults.²³⁸ In contrast to EBV-positive classic Hodgkin lymphoma, in which protection by certain HLA-class I alleles (HLA-A2) is well accepted, an HLA effect could not be confirmed for EBV-positive PTLD in a large population-based study in Australia.²³⁹ In a large international pediatric cohort, recipient HLA-B40 was associated with a 2-fold increased risk of EBV+ PTLD development after kidney transplantation,²³⁷ which was confirmed by a longitudinal single center study in adults for EBV-seronegative recipients.²⁴⁰ In all other studies associations of certain HLA class I or class II alleles with increased risk of or protection from PTLD have only been discovered by single groups and lack confirmation by an independent cohort.^{238,240-244} There is evidence, that a higher degree of HLA-mismatching confers an increased risk of developing PTLD^{118,236} potentially due to increased immunosuppressive requirements. However, no specific recommendation for screening of HLA type to determine the risk of PTLD can be given based on individual HLA profiles.

3.9.2 | Question 9B: Can PTLD surveillance strategies be targeted based on recipient race or ethnicity or on host genetic polymorphisms?

Recommendations

- We do not recommend stratifying clinical surveillance strategies based on specific recipient race or ethnicity nor on recipient cytokine gene polymorphisms.
Evidence Grade: Weak recommendation/low-quality evidence.

Supporting evidence/information

This question has been addressed by expert literature review. Data are very scarce in the pediatric population.

A higher incidence of PTLD in Caucasian children compared to African American or Hispanic children has been described in pediatric renal transplant recipients.²⁴⁵ These results were corroborated by several subsequent analyses in adult SOT recipients: A higher incidence of PTLD was noted in non-African American renal transplant recipients²⁴⁶ as well as pancreas transplant recipients.²⁴⁷ Most recently, these results have been confirmed by a large single institutional analysis demonstrating a relative protection from PTLD in adult African American recipients of solid organ transplantation.²⁴⁰ In an Asian cohort of liver transplant recipients Chinese ethnicity showed a trend towards higher PTLD incidence compared to other south-east Asian ethnicities.²⁴⁸

Thus, four registry or single institutional studies report higher risk of PTLD in patients of Caucasian compared to African American ethnicity. However, there are no studies evaluating different monitoring or screening practices based on recipient ethnicity.

As variations in host immune responses to EBV are very likely to play a role in the establishment of persistent infection, occurrence of EBV disease, and/or progression to EBV-PTLD, several studies have investigated cytokine expression and/or cytokine gene polymorphisms aiming at improved risk-stratification and/or diagnosis of PTLD. In particular, interleukin 6 (IL-6) is a pro-inflammatory cytokine that impacts both innate and adaptive immunity. IL-6 expression is modulated by EBV infection and potentially related to EBV pathogenesis. Therefore, the prognostic/diagnostic role of IL-6 in EBV+ PTLD has been evaluated in several studies demonstrating an elevation of IL-6 at diagnosis of PTLD.²⁴⁹⁻²⁵¹ This effect, however, is unspecific and often relates to infection or general activation of the immune system. IL-10 has also been shown to act as an autocrine growth factor for EBV-positive B cells isolated from patients with PTLD.²⁵²

Genetic polymorphisms associated with a low expression of IFN- γ were found in 67% of a pediatric cohort containing 59 liver recipients with PTLD compared to 33% in those without PTLD.²⁵³ Utilizing both EBV VL and IFN- γ polymorphism detection increased specificity and positive predictive value in this patient cohort. No association was found with polymorphisms in the genes for TGF- β , TNF- α , IL-6, IL-10, and IL-2 in this study. A study in children and adults detected a possible association of TNF- α with PTLD, both on the cytokine level as well as on genetic analysis of variant alleles in the TNF- α promoter and TNF-R1 promoter regions.²⁵⁴ So far results have not been reproduced in a second cohort, and thus we cannot recommend cytokine gene polymorphism analysis as a basis for individually tailored PTLD-screening strategies.

3.9.3 | Question 9C: Can other biomarkers reliably be used to predict or allow for early detection of PTLD in SOT patients?

Recommendations

- We do not recommend use of the biomarkers evaluated (cytokine levels, Ig light chain assessment, soluble CD30, CXCL13 or other chemokines) for routine clinical use to predict or early detection of PTLD.
Evidence Grade: Weak recommendation/low-quality evidence.

Supporting evidence/information

This question has been addressed by expert literature review. In a series of pediatric SOT recipients at risk for and ultimately diagnosed with PTLD, serial serum samples were measured for IL-6 and IgE at baseline and every 3 months thereafter for up to 24 months post-transplant.²⁵⁰ Median serum IL-6 levels were significantly increased

in PTLD cases compared to non-PTLD cases at both 3–6 months and >6 months post-transplant. Univariate analysis showed that IL-6 levels at >6 months post-transplant correlated with both VL and with occurrence of PTLD. Combining elevated IL-6 with VL increased PPV. In contrast, plasma concentration of IL-10 did not differ between 11 chronic high EBV VL pediatric liver transplant recipients compared to 20 control pediatric liver transplant recipients.⁵³ Data on cytokine levels in adults are conflicting. Measurements of pro-inflammatory vs anti-inflammatory cytokine release in two independent cohorts of transplant patients identified impaired levels of IL-10 and IL-6. Interestingly, IL-6 levels, but not IL-10, significantly correlated with PTLD progression, highlighting the role of IL-6 as a B-cell growth factor to enhance B-cell proliferation, a phenomenon seen with PTLD.^{249,255} In a different study, IL-10 levels were correlated with EBV load but not PTLD course while IL-6 was not elevated.²⁵¹

Several markers have been proposed on the basis of EBV and/or lymphoma pathogenesis to be predictive of future PTLD development. In vitro infection of B cells by EBV induces expression of CD30, a tumor necrosis factor family member, on infected B cells,²⁵⁶ and subsequent shedding of soluble CD30 (sCD30) may lead to elevated levels in patient plasma. sCD30 was found to be elevated in adult transplant patients compared to healthy controls whether or not they had EBV-related disease.²⁵⁷ A significant elevation of sCD30 in adult and pediatric transplant patients with PTLD compared to healthy controls or patients without PTLD was also detected. However, elevated sCD30 also correlated with other infection (bacteria²⁵⁸; CMV²⁵⁹) as well as graft dysfunction²⁶⁰ indicating that this marker is not specific for PTLD.

B cells, including EBV+ B cells in PTLD lesions, express immunoglobulin (Ig) B cell receptor, composed of pairings of heavy and light chains, on the cell surface. In the setting of transplantation B cells and EBV-transformed B cells also secrete Ig and may release free light chain into the circulation. Elevated light chain levels or altered kappa/lambda ratios may be an indication of abnormal B cell activation or expansion. Free light chains are frequently elevated in adult SOT recipients with and without PTLD.^{261–263} One study suggests a correlation of free light chain levels with PTLD activity.²⁶² However, data are conflicting and do not support routine use of this form of monitoring in SOT recipients.

CXCL13 is a B cell homeostatic chemokine frequently elevated in non-immunocompromised lymphoma patients. A single institution study detected significantly elevated levels of sCXCL13 in a subset of pediatric PTLD patients compared to SOT recipients with EBV reactivation.²⁶⁴ Serum levels of CXCL13 started to increase up to 2 years before PLTD and correlated with disease course. However, these data have not been confirmed in prospective studies. Furthermore, sCXCL13 was also elevated in patients with acute renal allograft rejection,²⁶⁵ which may limit diagnostic significance.

Research priorities for knowledge gaps

- Although some candidates seem promising, biomarker studies for prediction of PTLD are currently limited due to their retrospective

nature, inconsistent findings, and absence of large-scale prospective cohort studies across different populations of patients and organ transplants. To increase diagnostic power, we recommend prospective multicenter studies of biomarkers (CXCL13, sCD30, IL-6) alone or in combination with other biomarkers and / or EBV VL as a predictor of EBV+PTLD, as a tool to follow response to treatment or as a tool to estimate the risk of PTLD recurrence. Investigators are encouraged to develop standardized tests for potentially useful candidates (sCD30, CXCL13).

AUTHOR CONTRIBUTIONS

All authors contributed to the original study idea and provided intellectual insight. J Preiksaitis, U Allen, CM Bollard, VR Dharnidharka, D Dulek, M Green, OM Martinez, D Metes, MG Michaels, F Smets, B Maecker-Kolhoff extracted data from the literature and drafted summaries. J Preiksaitis and B Maecker-Kolhoff wrote the first draft, found, imported, formatted, and inserted citations. U Allen, CM Bollard, VR Dharnidharka, D Dulek, M Green, OM Martinez, D Metes, MG Michaels, F Smets, RE Chinnock, P Comoli, L Danziger-Isakov, AI Dipchand, CO Esquivel, JA Ferry, TG Gross, RJ Hayashi, B Höcker, AG L'Huillier, SD Marks, GV Mazariegos, J Squires, SH Swerdlow, RU Trappe, G Visner, SA Webber, JD Wilkinson edited multiple versions of the manuscript. All the authors had full access to all data, contributed to and approved the final manuscript and accept responsibility for its submission for publication.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

- Allen UD, Preiksaitis JK, Practice ASTIDCo. 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.
- Humar A, Michaels M, Monitoring AIWGoID. American Society of Transplantation recommendations for screening, monitoring and reporting of infectious complications in immunosuppression trials in recipients of organ transplantation. *Am J Transplant*. 2006;6(2):262-274.
- Ahya VN, Douglas LP, Andreadis C, et al. Association between elevated whole blood Epstein-Barr virus (EBV)-encoded RNA EBV polymerase chain reaction and reduced incidence of acute lung allograft rejection. *J Heart Lung Transplant*. 2007;26(8):839-844.
- Bakker NA, Verschuuren EA, Erasmus ME, et al. Epstein-Barr virus-DNA load monitoring late after lung transplantation: a surrogate marker of the degree of immunosuppression and a safe guide to reduce immunosuppression. *Transplantation*. 2007;83(4):433-438.
- Shannon-Lowe C, Rickinson A. The global landscape of EBV-associated tumors. *Front Oncol*. 2019;9:713.
- Thorley-Lawson DA. EBV persistence—Introducing the virus. *Curr Top Microbiol Immunol*. 2015;390(Pt 1):151-209.
- Gullett JC, Nolte FS. Quantitative nucleic acid amplification methods for viral infections. *Clin Chem*. 2015;61(1):72-78.
- Fryer JF, Heath AB, Wilkinson DE, Minor PD, Collaborative Study G. A collaborative study to establish the 1st WHO International Standard for Epstein-Barr virus for nucleic acid amplification techniques. *Biologicals*. 2016;44(5):423-433.
- Martinez OM. Biomarkers for PTLTD diagnosis and therapies. *Pediatr Nephrol*. 2021;35(7):1173-1181.
- Metes D. Immune Responses to EBV in the Immunocompromised Host. In: Dharnidharka VR, Green M, Webber S, Trappe RU, eds. *Post-transplant lymphoproliferative disorders*. 2nd ed. Springer; 2021:51-64.
- Andrews J, Guyatt G, Oxman AD, et al. GRADE guidelines: 14. Going from evidence to recommendations: the significance and presentation of recommendations. *J Clin Epidemiol*. 2013;66(7):719-725.
- Guyatt G, Oxman AD, Akl EA, et al. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol*. 2011;64(4):383-394.
- Guyatt GH, Oxman AD, Vist GE, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ*. 2008;336(7650):924-926.
- Schunemann HJ, Jaeschke R, Cook DJ, et al. An official ATS statement: grading the quality of evidence and strength of recommendations in ATS guidelines and recommendations. *Am J Respir Crit Care Med*. 2006;174(5):605-614.
- Preiksaitis JK, Pang XL, Fox JD, et al. Interlaboratory comparison of Epstein-Barr virus viral load assays. *Am J Transplant*. 2009;9(2):269-279.
- Le QT, Zhang Q, Cao H, et al. An international collaboration to harmonize the quantitative plasma Epstein-Barr virus DNA assay for future biomarker-guided trials in nasopharyngeal carcinoma. *Clin Cancer Res*. 2013;19(8):2208-2215.
- Perandin F, Cariani E, Pollara CP, Manca N. Comparison of commercial and in-house Real-time PCR assays for quantification of Epstein-Barr virus (EBV) DNA in plasma. *BMC Microbiol*. 2007;7:22.
- Ruiz G, Pena P, de Ory F, Echevarria JE. Comparison of commercial real-time PCR assays for quantification of Epstein-Barr virus DNA. *J Clin Microbiol*. 2005;43(5):2053-2057.
- Ahsanuddin AN, Standish MC, Caliendo AM, Hill CE, Nolte FS. Validation of an Epstein-Barr viral load assay using the QIAAGEN Artus EBV TM PCR analyte-specific reagent. *Am J Clin Pathol*. 2008;130(6):865-869.
- Germi R, Lupo J, Semenova T, et al. Comparison of commercial extraction systems and PCR assays for quantification of Epstein-Barr virus DNA load in whole blood. *J Clin Microbiol*. 2012;50(4):1384-1389.
- Hayden RT, Hokanson KM, Pounds SB, et al. Multicenter comparison of different real-time PCR assays for quantitative detection of Epstein-Barr virus. *J Clin Microbiol*. 2008;46(1):157-163.
- Ito Y, Takakura S, Ichiyama S, et al. Multicenter evaluation of prototype real-time PCR assays for Epstein-Barr virus and cytomegalovirus DNA in whole blood samples from transplant recipients. *Microbiol Immunol*. 2010;54(9):516-522.
- Hayden RT, Preiksaitis J, Tong Y, et al. Commutability of the First World Health Organization International Standard for human cytomegalovirus. *J Clin Microbiol*. 2015;53(10):3325-3333.
- Preiksaitis JK, Hayden RT, Tong Y, et al. Are we there yet? impact of the first international standard for cytomegalovirus DNA on the harmonization of results reported on plasma samples. *Clin Infect Dis*. 2016;63(5):583-589.
- Abbate I, Piralla A, Calvario A, et al. Nation-wide measure of variability in HCMV, EBV and BKV DNA quantification among centers involved in monitoring transplanted patients. *J Clin Virol*. 2016;82:76-83.
- Hayden RT, Sun Y, Tang L, et al. Progress in quantitative viral load testing: variability and impact of the WHO quantitative international standards. *J Clin Microbiol*. 2017;55(2):423-430.
- Semenova T, Lupo J, Alain S, et al. Multicenter evaluation of whole-blood Epstein-Barr viral load standardization using the WHO international standard. *J Clin Microbiol*. 2016;54(7):1746-1750.
- Rychert J, Danziger-Isakov L, Yen-Lieberman B, et al. Multicenter comparison of laboratory performance in cytomegalovirus and Epstein-Barr virus viral load testing using international standards. *Clin Transplant*. 2014;28(12):1416-1423.
- Abeynayake J, Johnson R, Libiran P, et al. Commutability of the Epstein-Barr virus WHO international standard across two quantitative PCR methods. *J Clin Microbiol*. 2014;52(10):3802-3804.
- Buelow D, Sun Y, Tang L, Gu Z, Pounds S, Hayden R. Comparative evaluation of four real-time PCR methods for the quantitative detection of Epstein-Barr virus from whole blood specimens. *J Mol Diagn*. 2016;18(4):527-534.
- Tang L, Sun Y, Buelow D, et al. Quantitative assessment of commutability for clinical viral load testing using a digital pcr-based reference standard. *J Clin Microbiol*. 2016;54(6):1616-1623.
- Hubner M, Bozic M, Konrad PM, Grohs K, Santner BI, Kessler HH. Analytical and clinical performance of a new molecular assay for Epstein-Barr virus DNA quantitation. *J Virol Methods*. 2015;212:39-43.
- Vinuesa V, Solano C, Gimenez E, Navarro D. Comparison of the artus Epstein-Barr virus (EBV) PCR kit and the Abbott RealTime EBV assay for measuring plasma EBV DNA loads in allogeneic stem cell transplant recipients. *Diagn Microbiol Infect Dis*. 2017;88(1):36-38.
- Baron A, Gicquel A, Plantier JC, Gueudin M. Evaluation of four commercial extraction-quantification systems to monitor EBV or CMV viral load in whole blood. *J Clin Virol*. 2019;113:39-44.
- Hayden RT, Tang L, Su Y, et al. Impact of fragmentation on commutability of Epstein-Barr virus and cytomegalovirus quantitative standards. *J Clin Microbiol*. 2019;58(1):e00888-19.
- Preiksaitis J, Burton C. Technical aspects of Epstein-Barr viral load assays. In: Dharnidharka VR, Green M, Webber S, Trappe RU, eds. *Post-Transplant Lymphoproliferative Disorders*. 2nd ed. Springer; 2021:65-107.
- Yen-Lieberman B, Brambilla D, Jackson B, et al. Evaluation of a quality assurance program for quantitation of human immunodeficiency virus type 1 RNA in plasma by the AIDS

- Clinical Trials Group virology laboratories. *J Clin Microbiol*. 1996;34(11):2695-2701.
38. Kuypers J, Jerome KR. Applications of digital PCR for clinical microbiology. *J Clin Microbiol*. 2017;55(6):1621-1628.
 39. Lam WKJ, Jiang P, Chan KCA, et al. Sequencing-based counting and size profiling of plasma Epstein-Barr virus DNA enhance population screening of nasopharyngeal carcinoma. *Proc Natl Acad Sci U S A*. 2018;115(22):E5115-E5124.
 40. Walton AH, Muenzer JT, Rasche D, et al. Reactivation of multiple viruses in patients with sepsis. *PLoS One*. 2014;9(2):e98819.
 41. Ryan JL, Fan H, Swinnen LJ, et al. Epstein-Barr Virus (EBV) DNA in plasma is not encapsidated in patients with EBV-related malignancies. *Diagn Mol Pathol*. 2004;13(2):61-68.
 42. Bauer CC, Aberle SW, Popow-Kraupp T, Kapitan M, Hofmann H, Puchhammer-Stockl E. Serum Epstein-Barr virus DNA load in primary Epstein-Barr virus infection. *J Med Virol*. 2005;75(1):54-58.
 43. Berger C, Day P, Meier G, Zingg W, Bossart W, Nadal D. Dynamics of Epstein-Barr virus DNA levels in serum during EBV-associated disease. *J Med Virol*. 2001;64(4):505-512.
 44. Cohen JL, Jaffe ES, Dale JK, et al. Characterization and treatment of chronic active Epstein-Barr virus disease: a 28-year experience in the United States. *Blood*. 2011;117(22):5835-5849.
 45. Burns DM, Tierney R, Shannon-Lowe C, et al. Memory B-cell reconstitution following allogeneic hematopoietic stem cell transplantation is an EBV-associated transformation event. *Blood*. 2015;126(25):2665-2675.
 46. Chan KC, Zhang J, Chan AT, et al. Molecular characterization of circulating EBV DNA in the plasma of nasopharyngeal carcinoma and lymphoma patients. *Cancer Res*. 2003;63(9):2028-2032.
 47. Miyake T, Iwatsuki K, Hirai Y, et al. The aim of the measurement of Epstein-Barr virus DNA in hydroa vacciniforme and hypersensitivity to mosquito bites. *J Med Virol*. 2020;92:3689-3696.
 48. To EW, Chan KC, Leung SF, et al. Rapid clearance of plasma Epstein-Barr virus DNA after surgical treatment of nasopharyngeal carcinoma. *Clin Cancer Res*. 2003;9(9):3254-3259.
 49. Fafi-Kremer S, Morand P, Brion JP, et al. Long-term shedding of infectious Epstein-Barr virus after infectious mononucleosis. *J Infect Dis*. 2005;191(6):985-989.
 50. Kimura H, Nishikawa K, Hoshino Y, Sofue A, Nishiyama Y, Morishima T. Monitoring of cell-free viral DNA in primary Epstein-Barr virus infection. *Med Microbiol Immunol*. 2000;188(4):197-202.
 51. Fafi-Kremer S, Brengel-Pesce K, Bargues G, et al. Assessment of automated DNA extraction coupled with real-time PCR for measuring Epstein-Barr virus load in whole blood, peripheral mononuclear cells and plasma. *J Clin Virol*. 2004;30(2):157-164.
 52. Kullberg-Lindh C, Saalman R, Olausson M, Herlenius G, Lindh M. Epstein-Barr virus DNA monitoring in serum and whole blood in pediatric liver transplant recipients who do or do not discontinue immunosuppressive therapy. *Pediatr Transplant*. 2017;21(5):e12875.
 53. Gotoh K, Ito Y, Ohta R, et al. Immunologic and virologic analyses in pediatric liver transplant recipients with chronic high Epstein-Barr virus loads. *J Infect Dis*. 2010;202(3):461-469.
 54. Greijer AE, Stevens SJ, Verkuijlen SA, et al. Variable EBV DNA load distributions and heterogeneous EBV mRNA expression patterns in the circulation of solid organ versus stem cell transplant recipients. *Clin Dev Immunol*. 2012;2012:543085.
 55. Rose C, Green M, Webber S, Ellis D, Reyes J, Rowe D. Pediatric solid-organ transplant recipients carry chronic loads of Epstein-Barr virus exclusively in the immunoglobulin D-negative B-cell compartment. *J Clin Microbiol*. 2001;39(4):1407-1415.
 56. Babcock GJ, Decker LL, Freeman RB, Thorley-Lawson DA. Epstein-Barr virus-infected resting memory B cells, not proliferating lymphoblasts, accumulate in the peripheral blood of immunosuppressed patients. *J Exp Med*. 1999;190(4):567-576.
 57. Schauer E, Webber S, Green M, Rowe D. Surface immunoglobulin-deficient Epstein-Barr virus-infected B cells in the peripheral blood of pediatric solid-organ transplant recipients. *J Clin Microbiol*. 2004;42(12):5802-5810.
 58. Fink S, Tsai MH, Schnitzler P, et al. The Epstein-Barr virus DNA load in the peripheral blood of transplant recipients does not accurately reflect the burden of infected cells. *Transpl Int*. 2017;30(1):57-67.
 59. Calattini S, Sereti I, Scheinberg P, Kimura H, Childs RW, Cohen JL. Detection of EBV genomes in plasmablasts/plasma cells and non-B cells in the blood of most patients with EBV lymphoproliferative disorders by using Immuno-FISH. *Blood*. 2010;116(22):4546-4559.
 60. Ito Y, Kawabe S, Kojima S, et al. Identification of Epstein-Barr virus-infected CD27+ memory B-cells in liver or stem cell transplant patients. *J Gen Virol*. 2011;92(Pt 11):2590-2595.
 61. Kimura H, Morita M, Yabuta Y, et al. Quantitative analysis of Epstein-Barr virus load by using a real-time PCR assay. *J Clin Microbiol*. 1999;37(1):132-136.
 62. Chaganti S, Heath EM, Bergler W, et al. Epstein-Barr virus colonization of tonsillar and peripheral blood B-cell subsets in primary infection and persistence. *Blood*. 2009;113(25):6372-6381.
 63. Coleman CB, Daud II, Ogolla SO, et al. Epstein-Barr virus type 2 infects T cells in healthy Kenyan children. *J Infect Dis*. 2017;216(6):670-677.
 64. Okuno Y, Murata T, Sato Y, et al. Defective Epstein-Barr virus in chronic active infection and haematological malignancy. *Nat Microbiol*. 2019;4(3):404-413.
 65. Bakker NA, Verschuuren EA, Veeger NJ, et al. Quantification of Epstein-Barr virus-DNA load in lung transplant recipients: a comparison of plasma versus whole blood. *J Heart Lung Transplant*. 2008;27(1):7-10.
 66. Bressollette-Bodin C, Coste-Burel M, Besse B, Andre-Garnier E, Ferre V, Imbert-Marcille BM. Cellular normalization of viral DNA loads on whole blood improves the clinical management of cytomegalovirus or Epstein Barr virus infections in the setting of pre-emptive therapy. *J Med Virol*. 2009;81(1):90-98.
 67. Hakim H, Gibson C, Pan J, et al. Comparison of various blood compartments and reporting units for the detection and quantification of Epstein-Barr virus in peripheral blood. *J Clin Microbiol*. 2007;45(7):2151-2155.
 68. Ishihara M, Tanaka E, Sato T, et al. Epstein-Barr virus load for early detection of lymphoproliferative disorder in pediatric renal transplant recipients. *Clin Nephrol*. 2011;76(1):40-48.
 69. Kanakry JA, Hegde AM, Durand CM, et al. The clinical significance of EBV DNA in the plasma and peripheral blood mononuclear cells of patients with or without EBV diseases. *Blood*. 2016;127(16):2007-2017.
 70. Kinch A, Oberg G, Arvidson J, Falk KI, Linde A, Pauksens K. Post-transplant lymphoproliferative disease and other Epstein-Barr virus diseases in allogeneic haematopoietic stem cell transplantation after introduction of monitoring of viral load by polymerase chain reaction. *Scand J Infect Dis*. 2007;39(3):235-244.
 71. Kullberg-Lindh C, Olofsson S, Brune M, Lindh M. Comparison of serum and whole blood levels of cytomegalovirus and Epstein-Barr virus DNA. *Transpl Infect Dis*. 2008;10(5):308-315.
 72. Limaye AP, Huang ML, Atienza EE, Ferrenberg JM, Corey L. Detection of Epstein-Barr virus DNA in sera from transplant recipients with lymphoproliferative disorders. *J Clin Microbiol*. 1999;37(4):1113-1116.
 73. Loginov R, Aalto S, Piiparinen H, et al. Monitoring of EBV-DNAemia by quantitative real-time PCR after adult liver transplantation. *J Clin Virol*. 2006;37(2):104-108.
 74. Ruf S, Behnke-Hall K, Gruhn B, et al. Comparison of six different specimen types for Epstein-Barr viral load quantification in peripheral blood of pediatric patients after heart transplantation or after allogeneic hematopoietic stem cell transplantation. *J Clin Virol*. 2012;53(3):186-194.

75. Stevens SJ, Pronk I, Middeldorp JM. Toward standardization of Epstein-Barr virus DNA load monitoring: unfractionated whole blood as preferred clinical specimen. *J Clin Microbiol.* 2001;39(4):1211-1216.
76. Stevens SJ, Verschuuren EA, Pronk I, et al. Frequent monitoring of Epstein-Barr virus DNA load in unfractionated whole blood is essential for early detection of posttransplant lymphoproliferative disease in high-risk patients. *Blood.* 2001;97(5):1165-1171.
77. Tsai DE, Douglas L, Andreadis C, et al. EBV PCR in the diagnosis and monitoring of posttransplant lymphoproliferative disorder: results of a two-arm prospective trial. *Am J Transplant.* 2008;8(5):1016-1024.
78. Tsai DE, Nearey M, Hardy CL, et al. Use of EBV PCR for the diagnosis and monitoring of post-transplant lymphoproliferative disorder in adult solid organ transplant patients. *Am J Transplant.* 2002;2(10):946-954.
79. Wada K, Kubota N, Ito Y, et al. Simultaneous quantification of Epstein-Barr virus, cytomegalovirus, and human herpesvirus 6 DNA in samples from transplant recipients by multiplex real-time PCR assay. *J Clin Microbiol.* 2007;45(5):1426-1432.
80. Wadowsky RM, Laus S, Green M, Webber SA, Rowe D. Measurement of Epstein-Barr virus DNA loads in whole blood and plasma by TaqMan PCR and in peripheral blood lymphocytes by competitive PCR. *J Clin Microbiol.* 2003;41(11):5245-5249.
81. Wagner HJ, Fischer L, Jabs WJ, Holbe M, Pethig K, Bucszy P. Longitudinal analysis of Epstein-Barr viral load in plasma and peripheral blood mononuclear cells of transplanted patients by real-time polymerase chain reaction. *Transplantation.* 2002;74(5):656-664.
82. Wagner HJ, Wessel M, Jabs W, et al. Patients at risk for development of posttransplant lymphoproliferative disorder: plasma versus peripheral blood mononuclear cells as material for quantification of Epstein-Barr viral load by using real-time quantitative polymerase chain reaction. *Transplantation.* 2001;72(6):1012-1019.
83. Fafi-Kremer S, Morand P, Barranger C, et al. Evaluation of the Epstein-Barr virus R-gene quantification kit in whole blood with different extraction methods and PCR platforms. *J Mol Diagn.* 2008;10(1):78-84.
84. Wagner HJ, Jabs W, Smets F, et al. Real-time polymerase chain reaction (RQ-PCR) for the monitoring of Epstein-Barr virus (EBV) load in peripheral blood mononuclear cells. *Klin Padiatr.* 2000;212(4):206-210.
85. Jabs WJ, Hennig H, Kittel M, et al. Normalized quantification by real-time PCR of Epstein-Barr virus load in patients at risk for posttransplant lymphoproliferative disorders. *J Clin Microbiol.* 2001;39(2):564-569.
86. Lee TH, Montalvo L, Chrebtow V, Busch MP. Quantitation of genomic DNA in plasma and serum samples: higher concentrations of genomic DNA found in serum than in plasma. *Transfusion.* 2001;41(2):276-282.
87. Lazzarotto T, Chierighin A, Piralla A, et al. Cytomegalovirus and Epstein-Barr virus DNA kinetics in whole blood and plasma of allogeneic hematopoietic stem cell transplantation recipients. *Biol Blood Marrow Transplant.* 2018;24(8):1699-1706.
88. Hocker B, Fickenscher H, Delecluse HJ, et al. Epidemiology and morbidity of Epstein-Barr virus infection in pediatric renal transplant recipients: a multicenter, prospective study. *Clin Infect Dis.* 2013;56(1):84-92.
89. Morton M, Coupes B, Roberts SA, et al. Epstein-Barr virus infection in adult renal transplant recipients. *Am J Transplant.* 2014;14(7):1619-1629.
90. Kanda T, Yajima M, Ikuta K. Epstein-Barr virus strain variation and cancer. *Cancer Sci.* 2019;110(4):1132-1139.
91. Maloney EM, Busque VA, Hui ST, et al. Genomic variations in EBNA3C of EBV associate with posttransplant lymphoproliferative disorder. *JCI Insight.* 2020;5(6):e131644.
92. Correia S, Bridges R, Wegner F, et al. Sequence variation of Epstein-Barr virus: viral types, geography, codon usage, and diseases. *J Virol.* 2018;92(22):e01132-18.
93. Neves M, Marinho-Dias J, Ribeiro J, Sousa H. Epstein-Barr virus strains and variations: geographic or disease-specific variants? *J Med Virol.* 2017;89(3):373-387.
94. Palser AL, Grayson NE, White RE, et al. Genome diversity of Epstein-Barr virus from multiple tumor types and normal infection. *J Virol.* 2015;89(10):5222-5237.
95. Santpere G, Darre F, Blanco S, et al. Genome-wide analysis of wild-type Epstein-Barr virus genomes derived from healthy individuals of the 1,000 Genomes Project. *Genome Biol Evol.* 2014;6(4):846-860.
96. Vaysberg M, Hatton O, Lambert SL, et al. Tumor-derived variants of Epstein-Barr virus latent membrane protein 1 induce sustained Erk activation and c-Fos. *J Biol Chem.* 2008;283(52):36573-36585.
97. Hopwood PA, Brooks L, Parratt R, et al. Persistent Epstein-Barr virus infection: unrestricted latent and lytic viral gene expression in healthy immunosuppressed transplant recipients. *Transplantation.* 2002;74(2):194-202.
98. Kasztelewicz B, Jankowska I, Pawlowska J, Teisseyre J, Dzierzanowska-Fangrat K. Epstein-Barr virus gene expression and latent membrane protein 1 gene polymorphism in pediatric liver transplant recipients. *J Med Virol.* 2011;83(12):2182-2190.
99. Kroll J, Li S, Levi M, Weinberg A. Lytic and latent EBV gene expression in transplant recipients with and without post-transplant lymphoproliferative disorder. *J Clin Virol.* 2011;52(3):231-235.
100. Moran J, Carr M, Waters A, et al. Epstein-Barr virus gene expression, human leukocyte antigen alleles and chronic high viral loads in pediatric renal transplant patients. *Transplantation.* 2011;92(3):328-333.
101. Qu L, Green M, Webber S, Reyes J, Ellis D, Rowe D. Epstein-Barr virus gene expression in the peripheral blood of transplant recipients with persistent circulating virus loads. *J Infect Dis.* 2000;182(4):1013-1021.
102. Ruf S, Behnke-Hall K, Gruhn B, Reiter A, Wagner HJ. EBV load in whole blood correlates with LMP2 gene expression after pediatric heart transplantation or allogeneic hematopoietic stem cell transplantation. *Transplantation.* 2014;97(9):958-964.
103. Zawilinska B, Kosinska A, Lenart M, et al. Detection of specific lytic and latent transcripts can help to predict the status of Epstein-Barr virus infection in transplant recipients with high virus load. *Acta Biochim Pol.* 2008;55(4):693-699.
104. Munz C. Latency and lytic replication in Epstein-Barr virus-associated oncogenesis. *Nat Rev Microbiol.* 2019;17(11):691-700.
105. Bergallo M, Gambarino S, Pinon M, et al. EBV-encoded microRNAs profile evaluation in pediatric liver transplant recipients. *J Clin Virol.* 2017;91:36-41.
106. Hassan J, Dean J, De Gascun CF, et al. Plasma EBV microRNAs in paediatric renal transplant recipients. *J Nephrol.* 2018;31(3):445-451.
107. Carvalho-Queiroz C, Johansson MA, Persson JO, et al. Associations between EBV and CMV seropositivity, early exposures, and gut microbiota in a prospective birth cohort: a 10-year follow-up. *Front Pediatr.* 2016;4:93.
108. Condon LM, Cederberg LE, Rabinovitch MD, et al. Age-specific prevalence of Epstein-Barr virus infection among Minnesota children: effects of race/ethnicity and family environment. *Clin Infect Dis.* 2014;59(4):501-508.
109. Balfour HH Jr, Sifakis F, Sliman JA, Knight JA, Schmeling DO, Thomas W. Age-specific prevalence of Epstein-Barr virus infection among individuals aged 6-19 years in the United States and factors affecting its acquisition. *J Infect Dis.* 2013;208(8):1286-1293.
110. Biggar RJ, Henle G, Bocker J, Lennette ET, Fleisher G, Henle W. Primary Epstein-Barr virus infections in African infants. II. Clinical

- and serological observations during seroconversion. *Int J Cancer*. 1978;22(3):244-250.
111. Chan KH, Tam JS, Peiris JS, Seto WH, Ng MH. Epstein-Barr virus (EBV) infection in infancy. *J Clin Virol*. 2001;21(1):57-62.
 112. Reynaldi A, Schlub TE, Piriou E, et al. Modeling of EBV infection and antibody responses in Kenyan infants with different levels of malaria exposure shows maternal antibody decay is a major determinant of early EBV infection. *J Infect Dis*. 2016;214(9):1390-1398.
 113. Burton C, Mabilangan C, Preiksaitis J. Incidence of Epstein Barr virus (EBV) DNAemia in EBV seronegative solid organ transplant (SOT) recipients with EBV seropositive donors. *Am J Transplant*. 2018;18(suppl 4):756.
 114. Chinnock R, Webber SA, Dipchand AI, et al. A 16-year multi-institutional study of the role of age and EBV status on PTLD incidence among pediatric heart transplant recipients. *Am J Transplant*. 2012;12(11):3061-3068.
 115. Yanik EL, Nogueira LM, Koch L, et al. Comparison of cancer diagnoses between the US solid organ transplant registry and linked central cancer registries. *Am J Transplant*. 2016;16(10):2986-2993.
 116. Opelz G, Daniel V, Naujokat C, Dohler B. Epidemiology of pre-transplant EBV and CMV serostatus in relation to posttransplant non-Hodgkin lymphoma. *Transplantation*. 2009;88(8):962-967.
 117. vanLeeuwen MT, Grulich AE, Webster AC, et al. Immunosuppression and other risk factors for early and late non-Hodgkin lymphoma after kidney transplantation. *Blood*. 2009;114(3):630-637.
 118. Caillard S, Lamy FX, Quelen C, et al. Epidemiology of posttransplant lymphoproliferative disorders in adult kidney and kidney pancreas recipients: report of the French registry and analysis of subgroups of lymphomas. *Am J Transplant*. 2012;12(3):682-693.
 119. Dharnidharka VR, Lamb KE, Gregg JA, Meier-Kriesche HU. Associations between EBV serostatus and organ transplant type in PTLD risk: an analysis of the SRTR National Registry Data in the United States. *Am J Transplant*. 2012;12(4):976-983.
 120. Sampaio MS, Cho YW, Shah T, Bunnapradist S, Hutchinson IV. Impact of Epstein-Barr virus donor and recipient serostatus on the incidence of post-transplant lymphoproliferative disorder in kidney transplant recipients. *Nephrol Dial Transplant*. 2012;27(7):2971-2979.
 121. McDonald RA, Smith JM, Ho M, et al. Incidence of PTLD in pediatric renal transplant recipients receiving basiliximab, calcineurin inhibitor, sirolimus and steroids. *Am J Transplant*. 2008;8(5):984-989.
 122. Narkewicz MR, Green M, Dunn S, et al. Decreasing incidence of symptomatic Epstein-Barr virus disease and posttransplant lymphoproliferative disorder in pediatric liver transplant recipients: report of the studies of pediatric liver transplantation experience. *Liver Transpl*. 2013;19(7):730-740.
 123. Peters AC, Akinwumi MS, Cervera C, 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.
 124. Quinlan SC, Pfeiffer RM, Morton LM, Engels EA. Risk factors for early-onset and late-onset post-transplant lymphoproliferative disorder in kidney recipients in the United States. *Am J Hematol*. 2011;86(2):206-209.
 125. Schober T, Framke T, Kreipe H, et al. Characteristics of early and late PTLD development in pediatric solid organ transplant recipients. *Transplantation*. 2013;95(1):240-246.
 126. West SC, Friedland-Little JM, Schowengerdt KO Jr, et al. Characteristics, risks, and outcomes of post-transplant lymphoproliferative disease >3 years after pediatric heart transplant: a multicenter analysis. *Clin Transplant*. 2019;33(5):e13521.
 127. L'Huillier AG, Dipchand AI, Ng VL, et al. Posttransplant lymphoproliferative disorder in pediatric patients: characteristics of disease in EBV-seropositive recipients. *Transplantation*. 2019;103(11):e369-e374.
 128. Nassif S, Kaufman S, Vahdat S, et al. Clinicopathologic features of post-transplant lymphoproliferative disorders arising after pediatric small bowel transplant. *Pediatr Transplant*. 2013;17(8):765-773.
 129. Wozniak LJ, Mauer TL, Venick RS, et al. Clinical characteristics and outcomes of PTLD following intestinal transplantation. *Clin Transplant*. 2018;32(8):e13313.
 130. Colombini E, Guzzo I, Morolli F, et al. Viral load of EBV DNAemia is a predictor of EBV-related post-transplant lymphoproliferative disorders in pediatric renal transplant recipients. *Pediatr Nephrol*. 2017;32(8):1433-1442.
 131. Abbott RJ, Pachnio A, Pedroza-Pacheco I, et al. Asymptomatic primary infection with Epstein-Barr virus: observations on young adult cases. *J Virol*. 2017;91(21):e00382-17.
 132. Hadinoto V, Shapiro M, Greenough TC, Sullivan JL, Luzuriaga K, Thorley-Lawson DA. On the dynamics of acute EBV infection and the pathogenesis of infectious mononucleosis. *Blood*. 2008;111(3):1420-1427.
 133. Hochberg D, Souza T, Catalina M, Sullivan JL, Luzuriaga K, Thorley-Lawson DA. Acute infection with Epstein-Barr virus targets and overwhelms the peripheral memory B-cell compartment with resting, latently infected cells. *J Virol*. 2004;78(10):5194-5204.
 134. Klutts JS, Ford BA, Perez NR, Gronowski AM. Evidence-based approach for interpretation of Epstein-Barr virus serological patterns. *J Clin Microbiol*. 2009;47(10):3204-3210.
 135. Shi T, Huang L, Luo L, Yu Q, Tian J. Diagnostic value of serological and molecular biological tests for infectious mononucleosis by EBV in different age stages and course of the disease. *J Med Virol*. 2021;93(6):3824-3834.
 136. Davis JE, Sheritt MA, Bharadwaj M, et al. Determining virological, serological and immunological parameters of EBV infection in the development of PTLD. *Int Immunol*. 2004;16(7):983-989.
 137. Laurent A, Klich A, Roy P, et al. Pediatric renal transplantation: a retrospective single-center study on epidemiology and morbidity due to EBV. *Pediatr Transplant*. 2018;22(3):e13151.
 138. Sato T, Fujieda M, Tanaka E, et al. Monitoring of Epstein-Barr virus load and antibody in pediatric renal transplant patients. *Pediatr Int*. 2008;50(4):454-458.
 139. Suzuki T, Ikezumi Y, Okubo S, et al. Epstein-Barr virus DNA load and seroconversion in pediatric renal transplantation with tacrolimus immunosuppression. *Pediatr Transplant*. 2007;11(7):749-754.
 140. Preiksaitis JK, Diaz-Mitoma F, Mirzayans F, Roberts S, Tyrrell DL. Quantitative oropharyngeal Epstein-Barr virus shedding in renal and cardiac transplant recipients: relationship to immunosuppressive therapy, serologic responses, and the risk of posttransplant lymphoproliferative disorder. *J Infect Dis*. 1992;166(5):986-994.
 141. Toyoda M, Moudgil A, Warady BA, Puliya DP, Jordan SC. Clinical significance of peripheral blood Epstein-Barr viral load monitoring using polymerase chain reaction in renal transplant recipients. *Pediatr Transplant*. 2008;12(7):778-784.
 142. Carpentier L, Tapiero B, Alvarez F, Viau C, Alfieri C. Epstein-Barr virus (EBV) early-antigen serologic testing in conjunction with peripheral blood EBV DNA load as a marker for risk of posttransplantation lymphoproliferative disease. *J Infect Dis*. 2003;188(12):1853-1864.
 143. Riddler SA, Breinig MC, McKnight JL. Increased levels of circulating Epstein-Barr virus (EBV)-infected lymphocytes and decreased EBV nuclear antigen antibody responses are associated with the development of posttransplant lymphoproliferative disease in solid-organ transplant recipients. *Blood*. 1994;84(3):972-984.
 144. Green M, Squires JE, Chinnock RE, et al. The IPTA Nashville consensus conference on Post-Transplant Lymphoproliferative disorders after solid organ transplantation in children: II-consensus guidelines for prevention. *Pediatr Transplant*. 2022;11:e14350.

145. Allen UD, Esquivel CO, L'Huillier AG, et al. The IPTA Nashville consensus conference on Post-Transplant lymphoproliferative disorders after solid organ transplantation in children: management of post-transplant lymphoproliferative disorder in children and adolescents. *Pediatr Transplant*. 2022:e14333.
146. Dunmire SK, Verghese PS, Balfour HH Jr. Primary Epstein-Barr virus infection. *J Clin Virol*. 2018;102:84-92.
147. Grimm JM, Schmeling DO, Dunmire SK, et al. Prospective studies of infectious mononucleosis in university students. *Clin Transl Immunol*. 2016;5(8):e94.
148. Njie R, Bell AI, Jia H, et al. The effects of acute malaria on Epstein-Barr virus (EBV) load and EBV-specific T cell immunity in Gambian children. *J Infect Dis*. 2009;199(1):31-38.
149. Balfour HH Jr, Odumade OA, Schmeling DO, et al. Behavioral, virologic, and immunologic factors associated with acquisition and severity of primary Epstein-Barr virus infection in university students. *J Infect Dis*. 2013;207(1):80-88.
150. Jayasooriya S, de Silva TI, Njie-jobe J, et al. Early virological and immunological events in asymptomatic Epstein-Barr virus infection in African children. *PLoS Pathog*. 2015;11(3):e1004746.
151. Piriou E, Asito AS, Sumba PO, et al. Early age at time of primary Epstein-Barr virus infection results in poorly controlled viral infection in infants from Western Kenya: clues to the etiology of endemic Burkitt lymphoma. *J Infect Dis*. 2012;205(6):906-913.
152. Hoagland RJ. The transmission of infectious mononucleosis. *Am J Med Sci*. 1955;229(3):262-272.
153. Svedmyr E, Ernberg I, Seeley J, et al. Virologic, immunologic, and clinical observations on a patient during the incubation, acute, and convalescent phases of infectious mononucleosis. *Clin Immunol Immunopathol*. 1984;30(3):437-450.
154. Ville S, Imbert-Marcille BM, Coste-Burel M, et al. Impact of antiviral prophylaxis in adults Epstein-Barr Virus-seronegative kidney recipients on early and late post-transplantation lymphoproliferative disorder onset: a retrospective cohort study. *Transpl Int*. 2018;31(5):484-494.
155. Yamada M, Nguyen C, Fadakar P, et al. Epidemiology and outcome of chronic high Epstein-Barr viral load carriage in pediatric kidney transplant recipients. *Pediatr Transplant*. 2018;22(3):e13147.
156. Dunmire SK, Grimm JM, Schmeling DO, Balfour HH Jr, Hogquist KA. The incubation period of primary Epstein-Barr virus infection: viral dynamics and immunologic events. *PLoS Pathog*. 2015;11(12):e1005286.
157. Cho YU, Chi HS, Jang S, Park SH, Park CJ. Pattern analysis of Epstein-Barr virus viremia and its significance in the evaluation of organ transplant patients suspected of having post-transplant lymphoproliferative disorders. *Am J Clin Pathol*. 2014;141(2):268-274.
158. Reynaldi A, Schlub TE, Chelimo K, et al. Impact of Plasmodium falciparum coinfection on longitudinal Epstein-Barr virus kinetics in Kenyan children. *J Infect Dis*. 2016;213(6):985-991.
159. Niesters HG, van Esser J, Fries E, Wolthers KC, Cornelissen J, Osterhaus AD. Development of a real-time quantitative assay for detection of Epstein-Barr virus. *J Clin Microbiol*. 2000;38(2):712-715.
160. Kenagy DN, Schlesinger Y, Weck K, Ritter JH, Gaudreault-Keener MM, Storch GA. Epstein-Barr virus DNA in peripheral blood leukocytes of patients with posttransplant lymphoproliferative disease. *Transplantation*. 1995;60(6):547-554.
161. Silins SL, Sherritt MA, Silleri JM, et al. Asymptomatic primary Epstein-Barr virus infection occurs in the absence of blood T-cell repertoire perturbations despite high levels of systemic viral load. *Blood*. 2001;98(13):3739-3744.
162. Preiksaitis J. Epstein-Barr viral load testing: role in the prevention, diagnosis and management of posttransplant lymphoproliferative disorders. In: Dharnidharka VR, Green MSW, eds. *Post-Transplant Lymphoproliferative Disorders*. Springer; 2010:45-67.
163. Manlhiot C, Pollock-Barziv SM, Holmes C, et al. Post-transplant lymphoproliferative disorder in pediatric heart transplant recipients. *J Heart Lung Transplant*. 2010;29(6):648-657.
164. Hoshino Y, Nishikawa K, Ito Y, Kuzushima K, Kimura H. Kinetics of Epstein-Barr virus load and virus-specific CD8+ T cells in acute infectious mononucleosis. *J Clin Virol*. 2011;50(3):244-246.
165. Nakai H, Kawamura Y, Sugata K, et al. Host factors associated with the kinetics of Epstein-Barr virus DNA load in patients with primary Epstein-Barr virus infection. *Microbiol Immunol*. 2012;56(2):93-98.
166. Slyker JA, Casper C, Tapia K, et al. Clinical and virologic manifestations of primary Epstein-Barr virus (EBV) infection in Kenyan infants born to HIV-infected women. *J Infect Dis*. 2013;207(12):1798-1806.
167. Green M, Cacciarelli TV, Mazariegos GV, et al. Serial measurement of Epstein-Barr viral load in peripheral blood in pediatric liver transplant recipients during treatment for posttransplant lymphoproliferative disease. *Transplantation*. 1998;66(12):1641-1644.
168. Kumar D, Patil N, Husain S, et al. Clinical and virologic outcomes in high-risk adult Epstein-Barr virus mismatched organ transplant recipients. *Clin Transplant*. 2017;31(7):e13000.
169. Hadinoto V, Shapiro M, Sun CC, Thorley-Lawson DA. The dynamics of EBV shedding implicate a central role for epithelial cells in amplifying viral output. *PLoS Pathog*. 2009;5(7):e1000496.
170. Bingler MA, Feingold B, Miller SA, et al. Chronic high Epstein-Barr viral load state and risk for late-onset posttransplant lymphoproliferative disease/lymphoma in children. *Am J Transplant*. 2008;8(2):442-445.
171. Das B, Morrow R, Huang R, Fixler D. Persistent Epstein-Barr viral load in Epstein-Barr viral naive pediatric heart transplant recipients: Risk of late-onset post-transplant lymphoproliferative disease. *World J Transplant*. 2016;6(4):729-735.
172. Green M, Soltys K, Rowe DT, Webber SA, Mazareigos G. Chronic high Epstein-Barr viral load carriage in pediatric liver transplant recipients. *Pediatr Transplant*. 2009;13(3):319-323.
173. Kamei H, Ito Y, Kawada J, et al. Risk factors and long-term outcomes of pediatric liver transplant recipients with chronic high Epstein-Barr virus loads. *Transpl Infect Dis*. 2018;20(4):e12911.
174. Lau AH, Soltys K, Sindhi RK, Bond G, Mazariegos GV, Green M. Chronic high Epstein-Barr viral load carriage in pediatric small bowel transplant recipients. *Pediatr Transplant*. 2010;14(4):549-553.
175. Macedo C, Webber SA, Donnenberg AD, et al. EBV-specific CD8+ T cells from asymptomatic pediatric thoracic transplant patients carrying chronic high EBV loads display contrasting features: activated phenotype and exhausted function. *J Immunol*. 2011;186(10):5854-5862.
176. Moran J, Dean J, De Oliveira A, et al. Increased levels of PD-1 expression on CD8 T cells in patients post-renal transplant irrespective of chronic high EBV viral load. *Pediatr Transplant*. 2013;17(8):806-814.
177. Moormann AM, Chelimo K, Sumba OP, et al. Exposure to holoendemic malaria results in elevated Epstein-Barr virus loads in children. *J Infect Dis*. 2005;191(8):1233-1238.
178. Piriou E, van Dort K, Otto S, van Oers MH, van Baarle D. Tight regulation of the Epstein-Barr virus setpoint: interindividual differences in Epstein-Barr virus DNA load are conserved after HIV infection. *Clin Infect Dis*. 2008;46(2):313-316.
179. Piriou E, Jansen CA, van Dort K, et al. Reconstitution of EBV latent but not lytic antigen-specific CD4+ and CD8+ T cells after HIV treatment with highly active antiretroviral therapy. *J Immunol*. 2005;175(3):2010-2017.
180. Piriou ER, van Dort K, Nanlohy NM, Miedema F, van Oers MH, van Baarle D. Altered EBV viral load setpoint after HIV seroconversion is in accordance with lack of predictive value of EBV load for the

- occurrence of AIDS-related non-Hodgkin lymphoma. *J Immunol.* 2004;172(11):6931-6937.
181. Hoshino Y, Katano H, Zou P, et al. Long-term administration of valacyclovir reduces the number of Epstein-Barr virus (EBV)-infected B cells but not the number of EBV DNA copies per B cell in healthy volunteers. *J Virol.* 2009;83(22):11857-11861.
 182. Halliday N, Smith C, Atkinson C, et al. Characteristics of Epstein-Barr viraemia in adult liver transplant patients: a retrospective cohort study. *Transpl Int.* 2014;27(8):838-846.
 183. Schaffer K, Hassan J, Staines A, et al. Surveillance of Epstein-Barr virus loads in adult liver transplantation: associations with age, sex, posttransplant times, and transplant indications. *Liver Transpl.* 2011;17(12):1420-1426.
 184. Bamoulid J, Courivaud C, Coaquette A, et al. Subclinical Epstein-Barr virus viremia among adult renal transplant recipients: incidence and consequences. *Am J Transplant.* 2013;13(3):656-662.
 185. Lazzarotto T, Chiereghin A, Piralla A, et al. Kinetics of cytomegalovirus and Epstein-Barr virus DNA in whole blood and plasma of kidney transplant recipients: implications on management strategies. *PLoS One.* 2020;15(8):e0238062.
 186. Cook L, Starr K, Boonyaratankornkit J, Hayden R, Sam SS, Caliendo AM. Does size matter? comparison of extraction yields for different-sized dna fragments by seven different routine and four new circulating cell-free extraction methods. *J Clin Microbiol.* 2018;56(12):e01061.
 187. Green M, Bueno J, Rowe D, et al. Predictive negative value of persistent low Epstein-Barr virus viral load after intestinal transplantation in children. *Transplantation.* 2000;70(4):593-596.
 188. Allen U, Hebert D, Petric M, et al. Utility of semiquantitative polymerase chain reaction for Epstein-Barr virus to measure virus load in pediatric organ transplant recipients with and without posttransplant lymphoproliferative disease. *Clin Infect Dis.* 2001;33(2):145-150.
 189. Schubert S, Renner C, Hammer M, et al. Relationship of immunosuppression to Epstein-Barr viral load and lymphoproliferative disease in pediatric heart transplant patients. *J Heart Lung Transplant.* 2008;27(1):100-105.
 190. Michelson P, Watkins B, Webber SA, Wadowsky R, Michaels MG. Screening for PTLD in lung and heart-lung transplant recipients by measuring EBV DNA load in bronchoalveolar lavage fluid using real time PCR. *Pediatr Transplant.* 2008;12(4):464-468.
 191. Luskin MR, Heil DS, Tan KS, et al. The impact of EBV status on characteristics and outcomes of posttransplantation lymphoproliferative disorder. *Am J Transplant.* 2015;15(10):2665-2673.
 192. Kalra A, Roessner C, Jupp J, et al. Epstein-Barr virus DNAemia monitoring for the management of post-transplant lymphoproliferative disorder. *Cytotherapy.* 2018;20(5):706-714.
 193. Styczynski J, Gil L, Tridello G, et al. Response to rituximab-based therapy and risk factor analysis in Epstein Barr virus-related lymphoproliferative disorder after hematopoietic stem cell transplant in children and adults: a study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. *Clin Infect Dis.* 2013;57(6):794-802.
 194. Oertel S, Trappe RU, Zeidler K, et al. Epstein-Barr viral load in whole blood of adults with posttransplant lymphoproliferative disorder after solid organ transplantation does not correlate with clinical course. *Ann Hematol.* 2006;85(7):478-484.
 195. van Esser JW, Niesters HG, Thijssen SF, et al. Molecular quantification of viral load in plasma allows for fast and accurate prediction of response to therapy of Epstein-Barr virus-associated lymphoproliferative disease after allogeneic stem cell transplantation. *Br J Haematol.* 2001;113(3):814-821.
 196. Yang J, Tao Q, Flinn IW, et al. Characterization of Epstein-Barr virus-infected B cells in patients with posttransplantation lymphoproliferative disease: disappearance after rituximab therapy does not predict clinical response. *Blood.* 2000;96(13):4055-4063.
 197. Corcoran C, Rebe K, van der Plas H, Myer L, Hardie DR. The predictive value of cerebrospinal fluid Epstein-Barr viral load as a marker of primary central nervous system lymphoma in HIV-infected persons. *J Clin Virol.* 2008;42(4):433-436.
 198. Ivers LC, Kim AY, Sax PE. Predictive value of polymerase chain reaction of cerebrospinal fluid for detection of Epstein-Barr virus to establish the diagnosis of HIV-related primary central nervous system lymphoma. *Clin Infect Dis.* 2004;38(11):1629-1632.
 199. Yanagisawa K, Tanuma J, Hagiwara S, Gatanaga H, Kikuchi Y, Oka S. Epstein-Barr viral load in cerebrospinal fluid as a diagnostic marker of central nervous system involvement of AIDS-related lymphoma. *Intern Med.* 2013;52(9):955-959.
 200. Bossolasco S, Cinque P, Ponzoni M, et al. Epstein-Barr virus DNA load in cerebrospinal fluid and plasma of patients with AIDS-related lymphoma. *J Neurovirol.* 2002;8(5):432-438.
 201. Weinberg A, Li S, Palmer M, Tyler KL. Quantitative CSF PCR in Epstein-Barr virus infections of the central nervous system. *Ann Neurol.* 2002;52(5):543-548.
 202. Liu QF, Ling YW, Fan ZP, et al. Epstein-Barr virus (EBV) load in cerebrospinal fluid and peripheral blood of patients with EBV-associated central nervous system diseases after allogeneic hematopoietic stem cell transplantation. *Transpl Infect Dis.* 2013;15(4):379-392.
 203. Cavaliere R, Petroni G, Lopes MB, Schiff D, International Primary Central Nervous System Lymphoma Collaborative G. Primary central nervous system post-transplantation lymphoproliferative disorder: an International Primary Central Nervous System Lymphoma Collaborative Group Report. *Cancer.* 2010;116(4):863-870.
 204. Evens AM, Choquet S, Kroll-Desrosiers AR, et al. Primary CNS posttransplant lymphoproliferative disease (PTLD): an international report of 84 cases in the modern era. *Am J Transplant.* 2013;13(6):1512-1522.
 205. Paranjothi S, Yusen RD, Kraus MD, Lynch JP, Patterson GA, Trulock EP. Lymphoproliferative disease after lung transplantation: comparison of presentation and outcome of early and late cases. *J Heart Lung Transplant.* 2001;20(10):1054-1063.
 206. Parrish A, Fenchel M, Storch GA, et al. Epstein-Barr viral loads do not predict post-transplant lymphoproliferative disorder in pediatric lung transplant recipients: a multicenter prospective cohort study. *Pediatr Transplant.* 2017;21(6):e13011.
 207. Bauer CC, Jaksch P, Aberle SW, et al. Relationship between cytomegalovirus DNA load in epithelial lining fluid and plasma of lung transplant recipients and analysis of coinfection with Epstein-Barr virus and human herpesvirus 6 in the lung compartment. *J Clin Microbiol.* 2007;45(2):324-328.
 208. Costa C, Elia M, Astegiano S, et al. Quantitative detection of Epstein-Barr virus in bronchoalveolar lavage from transplant and nontransplant patients. *Transplantation.* 2008;86(10):1389-1394.
 209. Balfour HH Jr, Hokanson KM, Schacherer RM, et al. A virologic pilot study of valacyclovir in infectious mononucleosis. *J Clin Virol.* 2007;39(1):16-21.
 210. Haque T, Crawford DH. PCR amplification is more sensitive than tissue culture methods for Epstein-Barr virus detection in clinical material. *J Gen Virol.* 1997;78(Pt 12):3357-3360.
 211. Ikuta K, Satoh Y, Hoshikawa Y, Sairenji T. Detection of Epstein-Barr virus in salivas and throat washings in healthy adults and children. *Microbes Infect.* 2000;2(2):115-120.
 212. Johnson KH, Webb CH, Schmeling DO, Brundage RC, Balfour HH Jr. Epstein-Barr virus dynamics in asymptomatic immunocompetent adults: an intensive 6-month study. *Clin Transl Immunol.* 2016;5(5):e81.
 213. Ling PD, Lednicki JA, Keitel WA, et al. The dynamics of herpesvirus and polyomavirus reactivation and shedding in healthy adults: a 14-month longitudinal study. *J Infect Dis.* 2003;187(10):1571-1580.

214. Yager JE, Magaret AS, Kuntz SR, et al. Valganciclovir for the suppression of Epstein-Barr virus replication. *J Infect Dis*. 2017;216(2):198-202.
215. Yao QY, Rickinson AB, Gaston JS, Epstein MA. In vitro analysis of the Epstein-Barr virus: host balance in long-term renal allograft recipients. *Int J Cancer*. 1985;35(1):43-49.
216. Verghese PS, Schmeling DO, Filtz EA, Grimm JM, Matas AJ, Balfour HH Jr. Transplantation of solid organ recipients shedding Epstein-Barr virus DNA pre-transplant: A prospective study. *Clin Transplant*. 2017;31(11):e13116.
217. Macedo C, Zeevi A, Bentlejewski C, et al. The impact of EBV load on T-cell immunity in pediatric thoracic transplant recipients. *Transplantation*. 2009;88(1):123-128.
218. Lee TC, Goss JA, Rooney CM, et al. Quantification of a low cellular immune response to aid in identification of pediatric liver transplant recipients at high-risk for EBV infection. *Clin Transplant*. 2006;20(6):689-694.
219. Rickinson AB, Long HM, Palendira U, Munz C, Hislop AD. Cellular immune controls over Epstein-Barr virus infection: new lessons from the clinic and the laboratory. *Trends Immunol*. 2014;35(4):159-169.
220. Wiesmayr S, Webber SA, Macedo C, et al. Decreased Nkp46 and NKG2D and elevated PD-1 are associated with altered NK-cell function in pediatric transplant patients with PTLT. *Eur J Immunol*. 2012;42(2):541-550.
221. LeVasseur R, Ganjoo J, Green M, et al. Lymphocyte subsets may discern treatment effects in children and young adults with post-transplant lymphoproliferative disorder. *Pediatr Transplant*. 2003;7(5):370-375.
222. Ekman-Joelsson BM, Wahlander H, Synnergren M, Sager M, Mellgren K. Post-transplant lymphoproliferative disease is associated with early sternotomy and left ventricular hypoplasia during infancy: a population-based retrospective review. *Cardiol Young*. 2017;27(9):1823-1831.
223. Offor UT, Bacon CM, Roberts J, et al. Transplantation for congenital heart disease is associated with an increased risk of Epstein-Barr virus-related post-transplant lymphoproliferative disorder in children. *J Heart Lung Transplant*. 2021;40(1):24-32.
224. Beland K, Dore-Nguyen M, Gagne MJ, et al. Torque Teno virus load as a biomarker of immunosuppression? New hopes and insights. *J Infect Dis*. 2014;210(4):668-670.
225. Dharnidharka VR, Ruzinova MB, Chen CC, et al. Metagenomic analysis of DNA viruses from posttransplant lymphoproliferative disorders. *Cancer Med*. 2019;8(3):1013-1023.
226. Martinez OM, Krams SM. The immune response to Epstein-Barr virus and implications for posttransplant lymphoproliferative disorder. *Transplantation*. 2017;101(9):2009-2016.
227. Taylor GS, Long HM, Brooks JM, Rickinson AB, Hislop AD. The immunology of Epstein-Barr virus-induced disease. *Annu Rev Immunol*. 2015;33:787-821.
228. Haque T, Wilkie GM, Jones MM, 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):1123-1131.
229. Prockop S, Doubrovina E, Suser S, 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.
230. Smets F, Latinne D, Bazin H, et al. Ratio between Epstein-Barr viral load and anti-Epstein-Barr virus specific T-cell response as a predictive marker of posttransplant lymphoproliferative disease. *Transplantation*. 2002;73(10):1603-1610.
231. Wilsdorf N, Eiz-Vesper B, Henke-Gendo C, et al. EBV-specific T-cell immunity in pediatric solid organ graft recipients with post-transplantation lymphoproliferative disease. *Transplantation*. 2013;95(1):247-255.
232. Savoldo B, Rooney CM, Quiros-Tejiera RE, et al. Cellular immunity to Epstein-Barr virus in liver transplant recipients treated with rituximab for post-transplant lymphoproliferative disease. *Am J Transplant*. 2005;5(3):566-572.
233. Sebelin-Wulf K, Nguyen TD, Oertel S, et al. Quantitative analysis of EBV-specific CD4/CD8 T cell numbers, absolute CD4/CD8 T cell numbers and EBV load in solid organ transplant recipients with PTLT. *Transpl Immunol*. 2007;17(3):203-210.
234. Macedo C, Donnenberg A, Popescu I, et al. EBV-specific memory CD8+ T cell phenotype and function in stable solid organ transplant patients. *Transpl Immunol*. 2005;14(2):109-116.
235. Falco DA, Nepomuceno RR, Krams SM, et al. Identification of Epstein-Barr virus-specific CD8+ T lymphocytes in the circulation of pediatric transplant recipients. *Transplantation*. 2002;74(4):501-510.
236. Opelz G, Dohler B. Impact of HLA mismatching on incidence of posttransplant non-hodgkin lymphoma after kidney transplantation. *Transplantation*. 2010;89(5):567-572.
237. Opelz G, Dohler B. Pediatric kidney transplantation: analysis of donor age, HLA match, and posttransplant non-Hodgkin lymphoma: a collaborative transplant study report. *Transplantation*. 2010;90(3):292-297.
238. Hussain SK, Makgoeng SB, Everly MJ, et al. HLA and risk of diffuse large B cell lymphoma after solid organ transplantation. *Transplantation*. 2016;100(11):2453-2460.
239. Jones K, Wockner L, Thornton A, et al. HLA class I associations with EBV+ post-transplant lymphoproliferative disorder. *Transpl Immunol*. 2015;32(2):126-130.
240. Lustberg ME, Pelletier RP, Porcu P, et al. Human leukocyte antigen type and posttransplant lymphoproliferative disorder. *Transplantation*. 2015;99(6):1220-1225.
241. Pourfarziani V, Einollahi B, Taheri S, Nemati E, Nafar M, Kalantar E. Associations of Human Leukocyte Antigen (HLA) haplotypes with risk of developing lymphoproliferative disorders after renal transplantation. *Ann Transplant*. 2007;12(4):16-22.
242. Reshef R, Luskin MR, Kamoun M, et al. Association of HLA polymorphisms with post-transplant lymphoproliferative disorder in solid-organ transplant recipients. *Am J Transplant*. 2011;11(4):817-825.
243. Subklewe M, Marquis R, Choquet S, et al. Association of human leukocyte antigen haplotypes with posttransplant lymphoproliferative disease after solid organ transplantation. *Transplantation*. 2006;82(8):1093-1100.
244. Wheless SA, Gulley ML, Raab-Traub N, et al. Post-transplantation lymphoproliferative disease: Epstein-Barr virus DNA levels, HLA-A3, and survival. *Am J Respir Crit Care Med*. 2008;178(10):1060-1065.
245. Dharnidharka VR, Sullivan EK, Stablein DM, Tejani AH, Harmon WE. Risk factors for posttransplant lymphoproliferative disorder (PTLD) in pediatric kidney transplantation: a report of the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS). *Transplantation*. 2001;71(8):1065-1068.
246. Nee R, Hurst FP, Dharnidharka VR, Jindal RM, Agodoa LY, Abbott KC. Racial variation in the development of posttransplant lymphoproliferative disorders after renal transplantation. *Transplantation*. 2011;92(2):190-195.
247. Jackson K, Ruppert K, Shapiro R. Post-transplant lymphoproliferative disorder after pancreas transplantation: a United Network for Organ Sharing database analysis. *Clin Transplant*. 2013;27(6):888-894.
248. 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):e12798. <https://doi.org/10.1111/tid.12798>

249. Hinrichs C, Wendland S, Zimmermann H, et al. IL-6 and IL-10 in post-transplant lymphoproliferative disorders development and maintenance: a longitudinal study of cytokine plasma levels and T-cell subsets in 38 patients undergoing treatment. *Transpl Int*. 2011;24(9):892-903.
250. Barton M, Wasfy S, Hebert D, et al. Exploring beyond viral load testing for EBV lymphoproliferation: role of serum IL-6 and IgE assays as adjunctive tests. *Pediatr Transplant*. 2010;14(7):852-858.
251. Baiocchi OC, Colleoni GW, Caballero OL, et al. Epstein-Barr viral load, interleukin-6 and interleukin-10 levels in post-transplant lymphoproliferative disease: a nested case-control study in a renal transplant cohort. *Leuk Lymphoma*. 2005;46(4):533-539.
252. Beatty PR, Krams SM, Martinez OM. Involvement of IL-10 in the autonomous growth of EBV-transformed B cell lines. *J Immunol*. 1997;158(9):4045-4051.
253. Lee TC, Savoldo B, Barshes NR, et al. Use of cytokine polymorphisms and Epstein-Barr virus viral load to predict development of post-transplant lymphoproliferative disorder in paediatric liver transplant recipients. *Clin Transplant*. 2006;20(3):389-393.
254. McAulay KA, Haque T, Crawford DH. Tumour necrosis factor gene polymorphism: a predictive factor for the development of post-transplant lymphoproliferative disease. *Br J Cancer*. 2009;101(6):1019-1027.
255. Tosato G, Jones K, Breinig MK, McWilliams HP, McKnight JL. Interleukin-6 production in posttransplant lymphoproliferative disease. *J Clin Invest*. 1993;91(6):2806-2814.
256. Gregory CD, Edwards CF, Milner A, et al. Isolation of a normal B cell subset with a Burkitt-like phenotype and transformation in vitro with Epstein-Barr virus. *Int J Cancer*. 1988;42(2):213-220.
257. Haque T, Chaggar T, Schafers J, Atkinson C, McAulay KA, Crawford DH. Soluble CD30: a serum marker for Epstein-Barr virus-associated lymphoproliferative diseases. *J Med Virol*. 2011;83(2):311-316.
258. Fernandez-Ruiz M, Parra P, Lopez-Medrano F, et al. Serum sCD30: a promising biomarker for predicting the risk of bacterial infection after kidney transplantation. *Transpl Infect Dis*. 2017;19(2):e12668. <https://doi.org/10.1111/tid.12668>
259. Grenzi PC, Campos EF, Silva HT Jr, et al. Post-transplant soluble CD30 levels are associated with early subclinical rejection in kidney transplantation. *Transpl Immunol*. 2015;32(2):61-65.
260. Amirzargar MA, Amirzargar A, Basiri A, et al. Early post-transplant immune monitoring can predict long-term kidney graft survival: soluble CD30 levels, anti-HLA antibodies and IgA-anti-Fab auto-antibodies. *Hum Immunol*. 2014;75(1):47-58.
261. Engels EA, Preiksaitis J, Zingone A, Landgren O. Circulating antibody free light chains and risk of posttransplant lymphoproliferative disorder. *Am J Transplant*. 2012;12(5):1268-1274.
262. Borrow R, Scheer A, Cockwell P, et al. Serum-free light chains adjusted for renal function are a potential biomarker for post-transplant lymphoproliferative disorders. *Ann Hematol*. 2019;98(3):625-632.
263. Engels EA, Jennings LW, Everly MJ, et al. Donor-specific antibodies, immunoglobulin-free light chains, and BAFF levels in relation to risk of late-onset PTLD in liver recipients. *Transplant Direct*. 2018;4(6):e353.
264. Schiffer L, Henke-Gendo C, Wilsdorf N, et al. CXCL13 as a novel marker for diagnosis and disease monitoring in pediatric PTLD. *Am J Transplant*. 2012;12(6):1610-1617.
265. Mao Y, Wang M, Zhou Q, et al. CXCL10 and CXCL13 Expression were highly up-regulated in peripheral blood mononuclear cells in acute rejection and poor response to anti-rejection therapy. *J Clin Immunol*. 2011;31(3):414-418.

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