Epstein-Barr Virus

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ABSTRACT This review covers relevant clinical and laboratory information relating to Epstein-Barr virus (EBV) infections in immunocompromised hosts. It describes the epidemiology and clinical manifestations with a primary focus on disease in solid organ and stem cell transplant recipients. The review pays particular attention to diagnostic approaches, including serologic testing and imaging, with an expanded discussion on the role of measuring the EBV load in peripheral blood, identifying both strengths and limitations of this assay. Additional attention is paid to potential additional strategies of immunologic monitoring that may enhance the performance of EBV load monitoring.

EBV INFECTION IN THE IMMUNOCOMPROMISED HOST

Background and Clinical Information

Epstein-Barr virus (EBV), a gamma herpesvirus, is a ubiquitous cause of infection in humans worldwide (1). Evidence of prior infection is present in adults throughout the world, with over 90% showing a serologic response. Exposure typically occurs early in life, with the majority of children in developing countries becoming seropositive by age 5. While onset of infection is delayed in areas with greater socioeconomic development, adults are almost uniformly positive. EBV is most commonly transmitted by contact with respiratory secretions, which promotes access and entry into the reticuloendothelial cells of the upper respiratory tree. While the primary target cell of EBV is the B lymphocyte, infection of a wider range of cell types can occur in immunocompromised hosts, particularly in those of epithelial lineage. Pharyngeal infection is followed by dissemination of virus throughout the body, with B lymphocytes as the primary target. The immune response to infection mounts steadily, with expansion of EBV-specific cytolytic T-cell clones eventually recognizing and controlling the primary infection. Control of EBV proliferation is signaled by a shift from lytic viral activity (marked by lytic proteins associated with cell destruction, such as BZLF1 and BRLF1) to a latent phenotype in an immortalized B lymphocyte pool, which provides a lifelong source of low-grade reactivation.

Development of a serological response, with initial IgM and IgG to viral capsid antigen, followed by antibody to the EBV nuclear antigen developing months after infection, provides reliable markers for acute and chronic infection in immunocompetent hosts.

Symptoms of EBV infection vary widely based on the age and immune status of the patient. The majority of infections in younger children are benign and are often subclinical (1). In this group, the most common acute presentation of EBV infection is of a febrile viral upper respiratory illness, which is not distinguishable from illness associated with other common viral pathogens. Young adults who undergo primary infection are more likely to present with classic findings of infectious mononucleosis. This cardinal symptom complex of fever, pharyngitis, adenopathy, hepatosplenomegaly, and fatigue accompanied by laboratory evidence of hepatitis is not absolutely specific to EBV, but in young adults between 15 and 24 years of age, it represents the virtually pathognomonic findings of
EBV. In young solid-organ recipients, the early acquisition of infection is a key determinant of the predominance of primary EBV infection after transplant (2) and is associated with an increased risk for more severe outcomes.

While immunocompromised patients may manifest typical findings of EBV infection, they are at greater risk for severe complications of disease. Asymptomatic infection is common in all categories of patient and can occur at any age and in a diverse variety of clinical situations. As described below, the routine use of EBV nucleic acid detection in patients at risk has allowed recognition of asymptomatic infection as a frequent event. Even immunocompromised hosts frequently experience infection in the absence of clinical symptoms. Smets and colleagues noted that only 15% of a panel of pediatric liver transplant recipients developed symptoms with primary infection (3). Fever without a source is also a common disease presentation; therefore, EBV must be considered in the differential diagnosis of immunocompromised patients with unexplained fever. Patients may also present with a classic infectious mononucleosis syndrome, including hepatitis, adenopathy, and organomegaly.

Of note, disease presentations that are not widely observed in immune-competent individuals are especially important in a range of hosts with compromised immunity. EBV is capable of in vitro and in vivo transformation of host cells and associates with specific tumors (Hodgkin’s and non-Hodgkin’s lymphomas, nasopharyngeal carcinoma, and Burkitt’s lymphomas) in immunocompetent populations (4). This neoplastic potential is intensified in immunocompromised hosts.

The most important manifestation is posttransplant lymphoproliferative disorder (PTLD), a complication of both solid organ transplant (SOT) and hematologic stem cell transplant (HSCT) patients.

PTLD is classified by pathologic appearance, ranging from infectious mononucleosis-like lesions, to polymorphic collections of EBV-infected B cells, to monomorphic collections and frank lymphoma (3). The development of PTLD is most likely to occur in EBV-seronegative hosts who experience infection after solid organ transplant, explaining the higher incidence in pediatric recipients. Conversely, development of PTLD in HSCT patients is most strongly associated with EBV-infected donors (6, 7).

The risks and specific associations of lymphoproliferation correlate with the specific categories of immune defects when considering EBV-associated tumor development. The highest risk profile for PTLD in SOT recipients is for EBV-seronegative recipients of seropositive donors. The low prevalence of EBV infection in children thus predicts much higher rates of EBV-driven malignancies in younger recipients. Rates of PTLD are also associated with specific organ types (Table 1), with pediatric recipients demonstrating rates of PTLD that are 4- to 10-fold greater than similar organs in adult recipients. Lung and intestinal transplants demonstrate the highest rates of PTLD in pediatric recipients, with estimates of 4% in lungs and 14% incidence in intestinal transplantation (8, 9). This association is maintained even in high-risk situations associated with chronic EBV carriage; prior studies of high viral load liver transplant recipients demonstrated rates of PTLD of 2.7%, versus 11% in intestinal transplant recipients (10, 11). Rates of PTLD and EBV infection vary for HSCT recipients based on transplant type—allogeneic HSCT rates often run greater than 10%, while cord blood transplant incidence is reported as high as 30% (6, 12, 13). In contrast to these patient groups, patients with primary immune-deficiency present with diverse manifestations of EBV infection. Patients with X-linked lymphoproliferative disorder present with uncontrolled EBV proliferation, which may progress to fulminant hemophagocytic lymphohistiocytosis (14). In contrast, HIV-infected patients often tolerate EBV infection initially, but later are at higher risk for EBV-driven malignancies such as lymphoma (15). Because of the strong association between SOT and HSCT and EBV-driven complications, the remainder of this review will focus on the role of EBV in these disorders. While other manifestations of EBV-driven disease such as encephalitis, pneumonitis, and hepatitis can occur, the impact of posttransplant lymphoproliferative disorder and the challenges of its diagnosis are the central theme of our discussion.

**Table 1** Cumulative 1- and 5-year incidence of PTLD in pediatric and adult SOT recipients by transplanted organ as reported in the 2010 Organ Procurement and Transplantation Network/Scientific Registry of Transplant Recipients (OPTN/SRTR) Annual Report (9)

<table>
<thead>
<tr>
<th>Organ</th>
<th>Pediatric</th>
<th>Adult</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 year</td>
<td>5 year</td>
</tr>
<tr>
<td>Lung/heart—lung</td>
<td>4.0</td>
<td>16%</td>
</tr>
<tr>
<td>Liver</td>
<td>2.1%</td>
<td>4.7%</td>
</tr>
<tr>
<td>Pancreas (isolated)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Heart</td>
<td>1.6%</td>
<td>5.7%</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.3%</td>
<td>2.4%</td>
</tr>
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</table>

*Data for intestinal transplant recipients not broken down by pediatric versus adult and therefore not included.*
Diagnostic Approaches

Serologic testing

The diagnosis of EBV infection and disease in immunocompromised hosts is more complicated than in immune-competent individuals, largely due to the fact that serologic diagnostic testing is confounded by the patient’s potential inability to mount appropriate antibody responses as well as the frequent presence of passively acquired antibody from blood products in many of these patients. Accordingly, while serologic studies may be obtained in some immunocompromised hosts with suspected EBV infection, the resulting interpretation must take these factors into consideration. Serologic testing for EBV infection should be avoided in patients with congenital or acquired antibody deficiencies as well as in patients with known exposure to passive antibody in the last 6 to 12 months. This approach would include all recipients of solid organ and hematopoietic stem cell transplants for at least the first 1 to 2 years following transplantation. In accordance with this principle, EBV serologic status should be documented prior to transplant on donors and recipients as this information can be used in assessing risk and guiding the need for potential preventive interventions.

EBV load measurement by nucleic acid amplification testing

Over the last 20 years, the measurement of EBV loads in the peripheral blood using nucleic acid amplification testing (e.g., PCR) has become an essential tool in the diagnosis and monitoring of EBV infection and disease in immunocompromised hosts. Measurement of EBV viral loads is widely recommended to aid in the diagnosis and management of EBV disease in solid organ recipients (16), stem cell transplant recipients (6), and other immunocompromised hosts (17). Quantitative EBV load measurement has also been proposed as a guide to the initiation of preemptive interventions against the development of EBV disease (5, 18, 19). Despite this long experience, a number of limitations in the ability to interpret results of EBV load measurement in the peripheral blood continue to exist. The most important limitation is that data defining clinically relevant breakpoints and performance specifications of these assays applicable to all centers are lacking. This is particularly true in defining a universally applicable level of EBV load that accurately predicts the presence of EBV disease including PTLD in solid organ and stem cell transplant recipients. In general, populations of immunocompromised hosts with active EBV disease usually have markedly elevated loads in comparison to those with evidence of EBV infection that do not develop disease. The major difficulty in defining the relationship between level of load and the likelihood that EBV disease is present in these patients is that EBV load monitoring has not been standardized between laboratories. Thus, while serial results of EBV load monitoring have been shown to be consistent within a single laboratory, substantial variability exists in the results of EBV load measurement between different laboratories (up to a 3.30-log difference on the same samples in a recent study (20), limiting the ability to extrapolate published single-center data to generate reliable EBV viral load thresholds which should trigger diagnostic and therapeutic interventions. Accordingly, individual centers need to review and correlate results of their EBV load assays to clinically relevant events to determine appropriate thresholds for their patients. Because of inconsistencies in results between labs, patients undergoing serial monitoring should have viral load measurements performed by the same laboratory even when they leave their hospital, to assure the reliability of following loads over time. It is hoped that the recently released 1st WHO International Standard for Epstein-Barr Virus for Nucleic Acid Amplification Techniques will allow for enhanced standardization which may overcome some of the current concerns noted above (21).

Additional concerns exist with the sensitivity of EBV loads for the diagnosis of EBV infection and disease. Although the majority of patients with proven EBV disease have been found to have markedly elevated EBV loads in their individual center, a small number of immunocompromised patients with proven EBV disease including PTLD have very low or even negative EBV loads in the peripheral blood. While most patients develop an elevated EBV load prior to onset of symptoms, the EBV load in patients with rapidly progressive disease (particularly stem cell transplant recipients) may present with clinical symptoms so quickly that measurement of the load at the onset of clinical symptoms might underestimate the likelihood of the presence of EBV disease and PTLD. For such patients, the analytical sensitivity of EBV load measurement will likely be enhanced by repeat measurement of the EBV load 4 to 7 days later. Additionally, some solid organ transplant recipients with proven EBV disease including PTLD have had persistent low or nondetectable EBV loads in the peripheral blood (2). Some lung transplant recipients with proven EBV disease and low loads or nondetectable loads in the peripheral blood had elevated EBV loads in bronchoalveolar lavage fluid alone (22).
Problems also exist with the specificity of EBV assays. While it is true that the majority of patients with proven EBV disease will have a load that exceeds local thresholds for disease, many organ transplant recipients have high viral loads in the absence of clinical disease. While some of these patients may eventually progress and develop disease, some patients will not progress even when high loads persist over long periods of time (10, 11, 23). While some experts have suggested that use of plasma or serum specimens may improve the specificity of EBV load assays, comparative data confirming this are lacking, and reports of patients having EBV disease in the presence of a positive whole blood or PBL assay and negative plasma or serum result have been observed.

Concerns over specificity highlight a final important controversy in the measurement of EBV load in the peripheral blood by nucleic acid amplification tests and may be another explanation for why universally applicable clinically relevant breakpoints have not been defined. Debate over what is the optimal compartment of peripheral blood to test has not been fully defined, with conflicting results for assays using peripheral blood lymphocytes, whole blood, or plasma (16, 24–27). Peripheral blood lymphocytes (PBL) and peripheral blood mononuclear cells contain EBV within infected B cells. Conversely, serum and plasma sampling measure the presence of viral DNA, either contained in mature virions or as fragments, which are more common in acute infection or EBV-driven malignancies (24). Whole blood sampling has also been used to minimize sample preparation; EBV load measurements from whole blood correlate well with PBL/peripheral blood mononuclear cell levels but not with plasma/serum loads (27). As a consequence of these differences in compartments, some experts feel that measurement of EBV load in serum or plasma may provide greater specificity compared to measurements derived from PBL or whole blood samples. However, experience at multiple centers suggests a decrease in sensitivity using this approach (2). A large, comparative trial would be required to fully define the performance of testing from different compartments.

Additional diagnostic modalities
Radiographic testing
In addition to measurement of EBV load in peripheral blood, patients with suspected EBV disease should undergo radiographic evaluation using computed tomography of neck, chest and abdomen to identify lesions not apparent from symptoms or examination (7, 16). These imaging studies should also be performed when PTLD is confirmed to allow for staging. Magnetic resonance imaging of the brain is paramount if there are any central nervous system symptoms such as headache, focal neurologic findings, or visual changes. Some experts advocate routine magnetic resonance imaging or computed tomography of the head in all patients at the time of initial imaging, particularly in children, to identify asymptomatic lesions (16). Increasing interest has also focused on the use of positron emission tomography scanning in the evaluation for EBV disease including PTLD (28). Experience to date has not defined its exact role, including whether all immunocompromised patients with proven or suspected EBV disease should undergo one or serial positron emission tomography scan evaluations.

Histopathology
The definitive diagnosis of EBV disease (including PTLD) is made by biopsy of lesions or affected tissue. In addition to confirming the presence of EBV, results of the biopsy are frequently used to help categorize the EBV disease manifestation, which frequently helps guide therapeutic approaches in affected patients. The biopsy also serves the role of ruling out other opportunistic infections that might require alternate therapy or be present concurrently. Because the bowel can frequently be involved in EBV disease, early endoscopy and colonoscopy should be performed in patients with unexplained abdominal pain and diarrhea. In addition, recipients of intestinal transplants may manifest similar symptoms with rejection or infection with other pathogens. Biopsy specimens should be evaluated by a pathologist familiar with EBV disease (including PTLD and other lymphoproliferative processes). For transplant recipients, histologic disease should be characterized according to WHO Consensus definitions (29). Specific assays should be performed to characterize the involved cell (T-cell versus B cell) with emphasis on evaluating cell markers such as CD20 which may influence therapeutic options and in situ hybridization for EBV-encoded RNA, a marker of EBV-infected cells (30).

Monitoring of EBV Infection
Careful attention for resolution of clinical signs, symptoms, and aberrant laboratory tests associated with the presence of EBV disease is the most reliable approach to assessing the clinical response of the patient with symptomatic EBV infection. In addition, many experts recommend serial measurement of the EBV load to follow clinical response to therapy in those being treated for EBV disease. Serial measurement of EBV load after
transplant has also been used to identify those at risk of developing EBV disease (as potential candidates for preemptive interventions). While there is consensus agreement for the role of EBV load monitoring for at least transplant recipients, concerns over the previously noted limitations with specificity of the assay limit our full understanding of the meaning of results in patients with elevated loads, particularly those that persist over time in the absence of clinical symptoms. The optimal frequency for assessing EBV load at specific time points post transplant for varying circumstances (e.g., surveillance, follow-up of elevated load, responses to treatment) remains center-specific, and a gold standard is not well defined.

These concerns have prompted interest in adjunctive testing assays which might enhance the performance of the EBV load measurement. Since the development of EBV disease in immunocompromised patients represents an imbalance between the host’s immune response and viral-driven proliferation of immortalized B cells, attention has focused on measurement of EBV cytotoxic T lymphocyte (CTL) response. A provocative study in pediatric liver transplant recipients looked concurrently at EBV loads and EBV CTL activity using ELISPOT; the investigators found a 100% positive predictive value for the development of PTLD in recipients who experienced primary EBV infection without developing a significant EBV CTL response (31, 32). Not surprisingly, others have also noted reduced EBV CTL levels (using commercial measurement of CD3+ T-cell response to PHA) in PTLD patients when compared to asymptomatic reactivation of EBV (33). Other investigators characterized the level of CTL responses (low or high) combined with the presence of undetectable, low, or high EBV loads and found that those with persistently high EBV loads had a low CTL response on the basis of an “immune exhaustion” phenotype, which they felt predisposed these patients to PTLD (31).

The presence of EBV-specific CTLs has most commonly been identified using interferon gamma release assays or ELISPOTs. Additionally, the presence and functional capabilities of EBV-specific CTLs has also been assessed using flow-based (tetramer and multimer) assays. While measurement of EBV-specific CTL appears promising as a clinically helpful adjunct marker, no such assays are currently FDA-approved for clinical use.

A number of additional candidate markers have been considered as potential adjunct assays to the EBV load. While previous candidate markers (e.g., mRNA for LMP2a) have not been successfully validated, newer options including free light chains, sCD30, IL-6, CXCL13, and NK cells are of current interest. Future data will hopefully clarify which, if any, of these candidate markers might rise to the level of being of clinical value.

**Prognosis**

The outcome of EBV infection in immunocompromised hosts is variable and is influenced by a diverse array of clinical factors. Estimating risk for infection involves the correlation of a number of these individual data points; thus, a simple equation for risk estimation and outcome prediction is not feasible. Determining which infections are more likely to result in clearance and establishment of long-lasting immunity and which may progress to more serious manifestations such as PTLD requires risk stratification through examination of key underlying factors, which helps to rank the available therapeutic options. Key factors to consider include:

- **Primary versus reactivation/secondary infection.** Regardless of host or type of immunocompromised state, primary infection with EBV is associated with a greater level of risk in hosts with impaired immunity. The increased rates of PTLD in pediatric SOT recipients are strongly associated with the increased rate of seronegativity in pediatric patients, as noted above. While recurrence of EBV viremia may represent a risk for complications of EBV, the development of T-cell-specific immunity in all hosts is associated with clearance of primary infection and is a key factor in the protection against EBV progression to more serious disease.

- **Type of transplant.** Previous work in many centers has established a stratification of risk dependent on the specific transplant type. Among SOT, organs associated with a need for higher baseline levels of immune suppression (intestine, heart and lung) are associated with correspondingly higher rates of progression to PTLD (18). This risk can also influence outcomes even from reactivation, as indicated by low rates of PTLD development (2.7%) in pediatric liver recipients with high viral load carriage versus pediatric heart transplant patients with a 40% rate of progression to PTLD (16, 23).

   Recipients of HSCT are also varied in their risk for EBV infection and progression to PTLD based on the type of transplant (6). Because of the dependence of EBV risk on donor infection and immunity, recipients of CBT are naturally at higher risk due to their developing immune function and
lack of preexisting immunity to EBV. Second allogeneic HSCT also increase risk (23, 25). The nature of conditioning regimens has influence on risk, as T-cell depletion will limit transfer of immune cells critical to the effective defense against EBV infection and monitoring for PTLD development.

- **Level of immune suppression.** While the types of SOT and HSCT correlate with risk of EBV complications, increased immune suppression in a broad spectrum of recipients can be associated with PTLD. Increases in baseline immunosuppressants, such as tacrolimus, constitute a smaller risk, but addition of corticosteroids for the treatment of rejection can augment risk. The receipt of antilymphocyte therapy is a strong predictor of increased risk with primary or reactivated EBV infection (21). Whether polyclonal antithymocyte globulin or more targeted biologics such as alemtuzumab, these broad-spectrum cytolytic therapies are often associated with long-lasting suppression of cell-mediated immune function and increased risk for any EBV infection. Similarly, therapy for acute or chronic GVHD in HSCT augments immune suppression and increases risk for PTLD.

- **Level of specific EBV immunity.** Data examining the specific level of EBV-specific T-cell activity suggest that this is an important correlate for the risk of progression to PTLD. An example is a 2002 study of pediatric liver transplant recipients, which demonstrated that the development of PTLD correlated both to elevated levels of EBV viremia and to depletion of EBV-specific cytotoxic T lymphocyte activity by ELISPOT. Similar data from single centers have suggested a similar relationship in heart transplant patients (23, 34). While limited by the availability of commercial standardized assays for the measurement of this activity, this clinical circumstance represents an important future field of study for the improvement of risk assessment and prognosis in EBV infection in the immunocompromised host.

**Therapeutic Approaches**

While antiviral agents play a key role in the therapy of many infections in immunocompromised hosts, EBV is notable for the limited role that antiviral therapy plays. Lytic virus does express drug targets such as virally encoded kinases, which are common to other herpesviridae and predict activity for agents such as ganciclovir. However, the important role of EBV-infected and immortalized B lymphocytes (which does not provide targets for ganciclovir activity) reduces its importance in therapy. Therapy thus focuses on alternatives, which balance stimulating immune responses to EBV infection and destruction of EBV-infected lymphocyte populations.

The primary therapeutic option with all manifestations of EBV infection is the reduction or cessation of immune suppression (2, 3, 35). In controlled trials examining this intervention in SOT patients, up to two-thirds of patients will show a clinical and virologic response, with reduction of circulating EBV viremia and in many cases regression of PTLD lesions. This approach is the first line of therapy for all management of EBV infection, but it may not be available in cases of concomitant rejection. With recipients of HSCT, ongoing GVHD, which requires continued immune suppression, also may compromise the ability to modulate steroid and calcineurin inhibitor regimens.

Failure of first-line therapy with reduced immune suppression leads to the use of second-line therapies, which continue to be studied. The use of rituximab for direct targeting of EBV-infected B lymphocytes has increased in frequency in both SOT and HSCT patients (2, 12, 36). A recent study of European SOT practices reported that 15% of programs used rituximab as preemptive treatment of EBV viremia to prevent PTLD, while 50% employed reduction of immune suppression (37). Surprisingly, the routine use of rituximab has not been accompanied by risk estimation of side effects such as hypogammaglobulinemia and opportunistic infections such as PML (2). This area of research is in need of well-designed trials to determine best practices for the management of EBV loads, which do not respond to reduction of immunosuppression. Progression to higher-grade PTLD lesions, including non-Hodgkin’s lymphoma, warrants the use of chemotherapy regimens studied for these clinical situations. A recent Children’s Oncology Group study of low-dose cyclophosphamide with prednisone and rituximab had a 69% response rate, and work continues to delineate the ideal therapeutic regimens for pediatric and adult populations (38).

A final area of development for therapies includes the use of adoptive immunotherapy for treatment of EBV infection and PTLD. While these strategies have good evidence for their use in HSCT (6), their use in SOT remains anecdotal (7). While early studies were encouraging in the observed responses to adoptive therapy (39–41), the slow development and lack of generalizability of these therapies has continued to limit their use. It seems clear that further work is warranted in these cases, as the transfer of effective cellular immunity...
would likely play a key role in combination with other therapies in the effective treatment of EBV complications.

Summary/Conclusion

EBV is associated with a range of clinical disease in the immunocompromised patient. Clinical syndromes vary from localized, benign manifestations (e.g., EBV hepatitis) to PTLD including true lymphoma. The diagnosis of EBV disease in this population is challenging, but measurement of EBV viral load has improved the detection and management of these syndromes. However, the lack of a common standard for EBV measurement as well as secondary markers which enhance the specificity of EBV loads remain a challenge. Because of this, biopsy and histologic evaluation remains the gold standard for defining EBV-associated disease in the immunocompromised patient. Future directions include comparative studies of EBV loads in different disease states utilizing the WHO standard and the incorporation of validated secondary markers to improve diagnosis and treatment of EBV disease.

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REFERENCES


