Epstein-Barr virus: general factors, virus-related diseases and measurement of viral load after transplant

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The Epstein-Barr virus is responsible for infectious mononucleosis syndrome and is also closely associated to several types of cancer. The main complication involving Epstein-Barr virus infection, both in recipients of hematopoietic stem cells and solid organs, is post-transplant lymphoproliferative disease.

The importance of this disease has increased interest in the development of laboratory tools to improve post-transplant monitoring and to detect the disease before clinical evolution. Viral load analysis for Epstein-Barr virus through real-time polymerase chain reaction is, at present, the best tool to measure viral load. However, there is not a consensus on which sample type is the best for the test and what is its predictive value for therapeutic interventions.

Keywords: *Herpesvirus 4, human; Polymerase chain reaction; Lymphoproliferative disorders; Transplantation*

Introduction

Epstein-Barr virus (EBV) is classified in the family *Herpesviridae*, subfamily *Gamaherpesvirinae*, genus *Lymphocryptovirus* and species *Human herpesvirus* 4.[1,2] Sequencing-based studies revealed the existence of two types of EBV.[3] It is estimated that this ubiquitous virus has infected more than 90% of the world's population.[4-7] According to the Center for Disease Control and Prevention (Atlanta, USA), 95% of the adult population between the ages of 35 and 40 are carriers of EBV.[8]

EBV transmission occurs mainly through contact with oropharyngeal secretions containing the virus.[9,10] Nevertheless, it can also take place through blood and blood derivative transfusions and through organ and tissue transplantation.[11] Breast milk may also contain the virus, but this is an uncommon route of vertical transmission.[12] Additionally, EBV is also present in genital tract secretions.[13,14]

In developing countries, primary EBV infection usually happens during childhood in an asymptomatic or clinically non-specific manner.[15] In developed countries, however, it is more common for the primary infection to occur during adolescence or adulthood, and can result in the development of classical symptoms of infectious mononucleosis syndrome (IM).[13] Studies suggest that this may be explained by the amount of virus introduced in the body.[2]

In this review, the main diseases associated with EBV, the importance of measuring post-transplant viral load by polymerase chain reaction (PCR) and the differences found in the various transplantation types were assessed...in a concise manner.

EBV-related diseases

Infectious mononucleosis

IM usually occurs during adolescence or adulthood but can occasionally affect children and the elderly.[4] Also known as the "kissing disease",[16] it is commonly a self-limiting disease, which means it evolves to a cure without specific treatment. In 80% of the cases, there is the presence of heterophile antibodies.[9] Also, in the complete blood count, it is possible to see leukocytosis with high lymphocytosis and the presence of Downey cells (atypical lymphocytes). These lymphocytes have an enlarged cytoplasm and condensed nucleus. They are primarily T cells acting on the eradication of B cells infected by EBV.[17]

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Hodgkin's lymphoma

There is a strong association between Hodgkin's lymphoma (HL) and EBV infection, although its role in this disease's pathogenesis is not fully clarified. The virus has been found in around 40% of HL tumors.[18] Cases in children and the elderly are generally associated with EBV, while adults with HL are more frequently EBV-negative.[19]

The histological diagnosis differs from that of other lymphomas because it shows mononuclear Hodgkin's cells and their multinuclear variants known as Reed-Sternberg cells.[19] These two cell types are derived, in the majority of cases, from B cells.

Plasmatic viral load can be quantified in virtually all EBV-positive HL patients before treatment and the response to therapy is associated to the reduction in viral load.[18] These data suggest that the analysis of plasmatic DNA through real-time PCR is an excellent tool for the prognosis and monitoring of HL patients.[2]

Non-Hodgkin lymphoma

Although overall non-Hodgkin lymphoma (NHL) rates are high, there is a great variety of types of these lymphomas and a variation of incidence between countries. In general, only 5% of the tumors are EBV-positive. This ratio rises up to 40%, however, in AIDS-related cases.[20] Enlarged cervical, axillary and/or inguinal ganglia, excessive nocturnal sudoresis, fever, itchy skin and weight loss without apparent reason must be investigated by the physician.[21]

Burkitt's lymphoma

At present, Burkitt's lymphoma (BL) represents a subset of NHL and is a type of tumor composed of small malignant B lymphocytes. It can be classified in two types: endemic and sporadic. In the endemic type, there are three factors that contribute to its development: malaria, EBV, and the expression of the c-myc gene. The disease affects children in Equatorial Africa and New Guinea (malaria endemic zone), and frequently the tumor starts in the jaw. It is known that malaria causes T cell immunodeficiency, reducing the control over the proliferation of EBV-infected B cells. Over 95% of African patients with BL were previously infected by EBV.[19]

In the sporadic variant of BL, although histologically similar to the endemic form, only between 20 and 30% of tumor cells carry the EBV genome.[22] It is important to note that, in the AIDS-related sporadic variant, 30-40% of the tumors are EBV-positive.[23]

Post-transplant lymphoproliferative disease

Post-transplant lymphoproliferative disease (PTLD) occurs due to the iatrogenic suppression of T cell functions[24] and is characterized by an abnormal proliferation of B cells.[5] The main risk factors are post-transplant primary infection, previous splenectomy, second transplant, patient age, acute or chronic graft-versus-host disease (GvHD), co-infection by cytomegalovirus or other viruses, regimen and intensity of immunosuppression therapy (use of anti-thymocyte globulin - ATG), T cell depletion,[25] transplants with HLA disparity[26] and EBV-negative recipient with EBV-positive donor.[7]

The use of umbilical cord blood as a source of cells for allogeneic transplants also increases the risk of PTLD. Explanations include the low quality of T cells from cord blood, donor-recipient HLA incompatibility and/or partial compatibility and the use of ATG.[27]

PTLD pathogenesis differs between hematopoietic stem cell transplantation (HSCT) and solid-organ transplantation (SOT). As immunosuppression in SOT has to be maintained throughout the patient's life, there is a constant risk of PTLD. The incidence varies according to the transplanted organ. The following rates are reported: intestines and multiple organs - 11 to 13%; lungs - 2 to 9%; heart - 2 to 6%; liver - 1 to 3%; and kidney - 1%.[28,29] One explanation may be related to the type and intensity of the immunosuppressive regime.[7]

A multicentric study of 26,901 patients submitted to HSCT suggested a high rate of PTDL in over 50-year-old individuals and also in patients submitted to a second transplant. Other risk factors were also taken into consideration, such as T cell depletion, partial HLA combination, and acute or chronic GvHD. The study in question divided the patients in four groups and calculated the disease rates: individuals without important risk factors, patients with one, two, or more risk factors. The incidences of PTLD were 0.2, 1.1, 3.6 and 8.1%, respectively.[30]

The highest frequency of this complication occurs in children and it is often reported during the first year post-transplant with a high incidence during the first six months. It can also happen later, however, as late as ten years after the transplant.[6,25,31] The disease encompasses a great diversity of pathological conditions that often makes the development of a standard classification system difficult.[7] There can be a predominance of monomorphic or polymorphic T and B cells. It can present itself as a self-limited lympho-proliferation or as a fulminant disease, and be localized or of ample dissemination.[32] Despite the variants, the World Health Organization (WHO) recommends its classification in four categories: (a) initial lesions; (b) polymorphic PTLD; (c) monomorphic (lymphomatous) PTLD; (d) classical Hodgkin's lymphoma-type PTLD.[5,7,28]

There are several forms of treatment: interventions can be preventive, or start at the moment of identification of a probable or proven EBV-caused disease.[6] Treatment is based on the reduction or removal of the immunosuppressive therapy, the use of anti-CD20 antibody (rituximab), antiviral drugs and, when necessary, conventional antineoplastic therapy.[25]

A recent review showed that a single dose of rituximab was efficient to prevent PTLD.[24] Although many studies certify the efficiency and safe administration of this therapy,[33] it is important to say that one of the limitations of anti-CD20 is its specificity for B cells (infected or not), causing a systemic reduction in this cell population[5] and hypogammaglobulinemia.[34]

Prophylactic use of antiviral agents has been discouraged due to the lack of efficiency.[35] Ganciclovir and aciclovir can reduce EBV replication, but these are not active in PTLD.[24] The role of passive immunization with the intravenous anti-CMV antibody remains uncertain.[7] In a study involving renal transplant patients, the use of anti-CMV IV was beneficial in the first year after transplant.[36]

A promising treatment is the use of EBV-specific cytotoxic T lymphocyte infusions cultivated from samples of the recipient in SOT[23,33] and from the donor in HSCT[37] to prevent and treat PTLD.[37,38]

The confirmation of the disease requires biopsy tests and histological analysis.[24] Notwithstanding, viral load analysis is paramount for post-transplant monitoring. Besides the measurement of virus load, the demand for alternative assays to evaluate PTLD risk increases every year. Tests to quantify EBV-specific T lymphocytes are proving promising.[5]

It is important to remember, however, that there are EBV-negative PTLD cases. These are rare and common in late onset cases (50 months post-transplant on average). In such situations, viral load assays for EBV will not detect PTLD.[39] According to Muti et al.[40] around 30% of late onset cases present a pattern of EBV-negative lymphoma.

Nasopharyngeal carcinoma

This disease has its highest incidence in the south of China, where it represents around 20% of all cancer cases in adults.[41] It is also found in northeast Africa and among the indigenous peoples of Alaska. In Europe and North America incidence rates are lower than 1/100,000 inhabitants.[42]

Over 99% of the cases are EBV-related and the viral genome is found in modified epithelial cells.[43] Tests on DNA extracted from these cells revealed that all the cases in areas of high, intermediate and low incidence were consistently EBV-positive.[44]

The varied percentages of EBV-positive cases in some associated pathologies are listed in Table 1.

Table 1 - Pathologies and percentages of Epstein-Barr virus-positive cases[20]

Disease	Percentage
Infectious mononucleosis	> 99%
Oral hairy leukoplakia	> 95%
Hodgkin's disease (all subtypes)	40%
Hodgkin's disease (AIDS-related)	> 95%
Non-Hodgkin lymphoma (all subtypes)	5%
Non-Hodgkin lymphoma (AIDS-related)	40%
Burkitt's lymphoma (Africa)	> 95%
Burkitt's lymphoma (North America)	20%
Burkitt's lymphoma (AIDS-related)	30%
Nasopharyngeal carcinoma (Asia)	> 99%
Nasopharyngeal carcinoma (North America)	75%

Post-transplant Epstein-Barr virus viral load measurement

Qualitative and quantitative PCR methods are precise, highly sensitive and specific techniques. However, the simple detection of EBV does not offer the clinician an overview of the patient's real condition.[2] The technique of quantitative real-time PCR for EBV is becoming an essential part of monitoring protocols of transplant patients, as well as a predictive parameter for PTLD.[45,46] Moreover, the relation between viral load increase and PTLD has not been properly elucidated.[1]

In high-risk patients submitted to HSCT and SOT, it is recommended to quantify EBV once a week for at least three months after the transplant. After this period, it is suggested a follow-up only for patients with reactivation or diseases related to EBV.[6]

There is no consensus on the best sample type, extraction method, viral target gene, qPCR platforms and tools and measurement units to evaluate EBV-related complications. In fact, there is not yet a standard method (commercial or in-house) of EBV detection.[6]

Standards of preventive interventions for PTLD have been defined in transplant centers (HSCT and SOT) according to the population studied and local experience. It is important to say, however, that these cut-off levels have not been validated by multicentric studies.[47] A recent review confirms that there is not yet an indicative limit for the beginning of preventive interventions.[48]

Kimura et al.[2] in another recent review affirm that whole blood (WB) is the best option to test for PTLD although the authors believe that further studies are necessary to clarify the question. Another study compared EBV viral load measurement using WB, peripheral blood mononuclear cells (PBMC) and plasma. The sensitivity of qPCR was similar between WB and PBMC but lower in plasma.[49]

A multicentric comparison opted for WB sampling, based on studies that showed better sensitivity using this material and also because this option was more commonly used amongst participating laboratories.[49-51]

Gulley et al.[25] argue that it is not appropriate to affirm that WB sampling for PTLD is more informative than plasma. In this disease, there can be relatively few circulating cells and other models of latency, lytic replication and virion production may occur. These factors, without doubt, lead to

different EBV concentrations in the cell fraction and plasma.[25,52,53]

Two studies comparing detection in WB and plasma concluded that plasma was the best option.[54,55] Niesters et al.[56] verified, in their study, that there is a relationship between DNA present in plasma and EBV-related diseases.

Some researchers prefer to work with blood or plasma because mononuclear cells may be difficult to obtain in patients with leukopenia during the first months after transplant.[48,57] Furthermore, in patients that were administered rituximab, the destruction of B cells can affect the number of copies in peripheral lymphocytes.[24]

In 24 review studies,[1,27,33,38,45,46,53,54,56-71] the most widely used extraction method was the commercially available QIAamp® DNA Blood Mini Kit (QIAGEN, Hilden, Germany) and the choice real-time quantification system was by hydrolysis probes (TaqMan® probes). Only one of the cited studies did not apply a real-time method.[65]

In eight studies, the sample material of choice was WB,[46,61,64,66-70] PBMC was used in five studies[1,45,60,63,65] and plasma or serum in eight.[27,31,53,56-59,62] Two comparative studies were performed,[38,54] one of which between PBMC and plasma - showing higher sensitivity in plasma - and the other between WB and PBMC, showing similar results using both materials. The experiment described by Lay et al.[71] used plasma, WB and PBMC, but without the purpose of comparing sample sensitivity.

Final considerations

The role of EBV in all of its associated diseases is not yet fully known. Diagnosis and monitoring of these pathologies are of crucial importance for clinical management. Post-transplant EBV monitoring is essential, especially in patients with considerable risk factors. Increased viral load may be an indication of imminent PTLD and prophylactic interventions can prevent or hinder this disease's progression.

Due to the diversity of methodologies used in each institution, it is difficult to recommend a universal predictive value for PTLD. Ideally, a multicentric study to standardize a qPCR technique and to establish a single predictive value should be developed.[11,17]

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