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Epstein-Barr Virus (EBV)

EBV is a lytic, double stranded DNA virus belonging to the Gammaherpesvirinae subgroup of the Herpesviridae family. It infects predominantly B cells and in common with other Herpesviridae viruses, persists for life in a latent state following primary infection. Over 90% of adults worldwide are seropositive for EBV and there are commonly two peaks of primary infection. The first peak occurs in early childhood between the ages of 1-6 years old and infection at this stage is typically subclinical. A second peak of infection is observed in adolescents / young adults between the ages of 14-25 years old and it is within this group that clinical manifestations are most common, normally presenting as infectious mononucleosis.

The most common form of transmission is orally, as EBV is excreted in the saliva of most seropositive individuals. In early childhood this may occur through contact with oral secretions on shared items such as toys and bottles. In adolescents and young adults, it is thought that intimate oral contact is the most common route to infection. Other methods of transmission include through blood transfusion and organ transplantation in seronegative patients and possibly sexually.

In addition to being the major causative agent of infectious mononucleosis, EBV is associated as a cofactor for a number of other diseases including Burkitt's lymphoma, nasopharyngeal carcinoma, lymphoproliferative disease and lymphoma in immunosuppressed patients.

Infectious mononucleosis (IM)

Infectious mononucleosis is so called due to the 'atypical' mononuclear cells observed, the majority of which are activated CD8 T lymphocytes and many of the symptoms are as a result of the substantial T cell response that occurs to EBV infected B cells. Common symptoms in young adults include sore throat, swelling of the neck due to cervical lymph node enlargement, fever, sweating, chills and a general fatigue and malaise. These last approximately 10 days with the exception of cervical lymph node enlargement and fatigue, which can persist for at least 3 weeks. However, in some patients EBV may become chronic with symptoms persisting for over a year. Other complications are rare

but can include splenic rupture, hepatitis, airway obstruction, meningoencephalitis, haemolytic anaemia and thrombocytopenia.

EBV in immunocompromised patients

In organ transplant patients, EBV is typically asymptomatic due to the lack of immune response. This immunosuppression results in an increased risk of EBV-driven B cell proliferation and progression to lymphoproliferative disease (PTLD). Similarly, patients with X-linked lymphoproliferative syndrome are unable to produce an effective immune response to primary EBV, resulting in B cell lymphoproliferation. HIV-infected patients are another group at an increased risk of developing lymphomas, with 25% of these presenting as Burkitt's lymphomas, where there is a strong association with EBV.

Diagnostic tests and their interpretation

Serological testing

Diagnostic testing typically involves either detecting an immune response to the virus (serological tests), or the detection of the virus itself (PCR). In immunocompetent individuals, serological testing can be used to determine if a patient has a primary EBV infection or if they have been infected in the past. The heterophile antibody test may be the initial assay performed to determine if EBV is the cause of infectious mononucleosis in a patient. Heterophile antibodies are not EBV specific but are produced by the immune system as part of the response to EBV and are cross-species reactive. The cross-species reactivity is the basis of the assay principle, which uses mammalian red blood cells from various species to detect any heterophile IgM antibodies present. Drawbacks of this assay include that its lack of specificity may produce false positive and negative results. Additionally, children under 4 years old do not always develop heterophile antibodies following primary infection, giving a false negative result.

More specific antibody tests can be performed to help diagnose and stage EBV infection. The most common antibody assays for this purpose are viral capsid antigen IgG (VCA IgG), VCA IgM and EBV nuclear antigen – 1 IgG (EBNA – 1 IgG). Typically, VCA IgG and VCA IgM are the first antibodies to appear and are often present at the time of the onset of symptoms. Whilst VCA IgM antibodies disappear within 4 – 6 weeks, VCA IgG antibodies remain present for life. The presence of both VCA IgG and VCA IgM antibodies is therefore a good indicator of acute infection. In contrast to the VCA antibodies, EBNA – 1 IgG antibodies are undetectable during the first 3 – 4 weeks after the onset of symptoms and so their presence in conjunction with VCA IgG is suggestive of past infection. Some patients may not always produce these antibodies however, leading to indeterminate results. This is particularly relevant in immunocompromised individuals and further testing, typically by PCR may be required.

EBV DNA detection

By detecting EBV DNA, molecular amplification allows for the presence of the virus itself to be established and, with real-time PCR, quantified. This is of particular use in monitoring transplant patients at risk of acquiring EBV-induced post transplantation lymphoproliferative disorder (PTLD), with increasing viral loads indicating an increased possibility of PTLD development. As such, this can allow for pre-emptive intervention to be undertaken before PTLD progression.

At Micropathology Ltd, an in-house PCR assay is performed which utilises hydrolysis probes. This enables qualitative and quantitative detection of EBV DNA. Please refer to our User Handbook (available on our website) for further information and details of acceptable sample types. The assay is subject to rigorous quality controls and the laboratory subscribes to relevant external quality assurance schemes.

UKAS accredited sample types for EBV in the Screen 2 assay include: CSF, plasma, serum, and EDTA whole blood for quantitative detection. If samples are not homogenous, for example cell clumps in whole blood, stochastic differences in the input material during DNA extraction might lead to differences in quantitative results, especially in low viral loads. EBV has been successful detected in other sample types, such as biopsies, non-saliva swabs, aqueous humours, and tissues, however, validation of these sample types has not been performed. However, processing of non-UKAS-accredited sample types can still be performed, but will contain the caveat: “Assay for this organism is not UKAS accredited for this sample type.”