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Review

EBV viral load detection in clinical virology

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Abbreviations: QC, quantitative competitive PCR; SQ, semi-quantitative PCR; RT, real-time PCR; PBMC, peripheral blood mononuclear cells; WB, whole blood; N/A, not available; *n*, number of patients; Tx, transplant; P, for pre-emptive therapy; D, for PTLD diagnosis; Sen, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value; EBV, Epstein–Barr virus; CTL, cytotoxic T cells; NPC, nasopharyngeal carcinoma; IM, infectious mononucleosis; NK, cells natural killer cells; PTLD, post-transplant lymphoproliferative disorder; HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency; PBL, peripheral blood cells; CNS, central nervous system; CSF, cerebrospinal fluid; VL, viral load; SOT, solid organ transplant recipient; HSCT, hematopoetic stem cell transplant recipient; PCNSL, primary CNS lymphoma.

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1. Epstein–Barr virus (EBV)-associated diseases and therapeutic approaches

EBV infects virtually the entire human population and persists throughout the lifetime of its host. In lower socioeconomic strata and developing nations, infection is almost universally acquired in early childhood, and is usually subclinical. In industrialized nations, particularly among the upper socioeconomic strata, subjects are often infected in adolescence and early adult life when 23–74% of infections result in the infectious mononucleosis (IM) syndrome, an immunopathological disease where symptoms are the result of an exaggerated T cell response to a self-limited lymphoproliferative process. Unusual and severe complications in the form of hemophagocytic syndromes and chronic EBV infections occur rarely. Viral reactivation can occur in seropositive subjects. This does not cause symptoms in the immunocompetent patient but serious disease can occur in immunosuppressed hosts. Although its role in the pathogenesis is not yet clear, EBV may also play a role in autoimmune disease development.[1,2] EBV is strongly associated with the development of a wide variety of neoplasms occurring in a relatively small subset of EBV-infected individuals, including lymphoid tumours like Burkitt lymphoma, Hodgkin lymphoma, T and/or natural killer (NK) cell lymphoma, epithelial malignancies such as nasopharyngeal carcinoma and gastric carcinoma and a mesothelial tumour, leiomyosarcoma (reviewed by Gulley and Tang[3] EBV-associated lymphoproliferative disorders, sometimes indistinguishable from lymphomas found in immunocompetent subjects, are particularly prevalent in immunosuppressed individuals including AIDS patients, transplant recipients (post-transplant lymphoproliferative disorder, PTLD) and patients with congenital immunodeficiencies such as X-linked lymphoproliferative disorders.[3,4]

2. Technical aspects of EBV viral load measurement

Over the past decade EBV viral load (VL) assessments, most often in peripheral blood compartments have been extensively implemented in clinical practice, most often for the prevention, diagnosis and monitoring of EBV-associated malignancies and lymphoproliferative disorders. However, the use of this laboratory tool is limited by lack of standardization. In several settings, the preferred sample type-whole blood (WB), peripheral blood mononuclear cells (PBMC)/lymphocytes (PBL), or plasma/serum, reporting units, specific quantitative levels for use trigger points for intervention or disease diagnosis, optimal monitoring algorithms and the cost-effectiveness of EBV (VL) testing in populations at high and low risk of EBV disease remain unresolved issues (reviewed by Preiksaitis, de Paoli et al., Kimura, Gulley and Tang).[3,5–7] EBV VL is easy to measure, but results are often difficult to interpret.

Both DNA and RNA VL can be measured. EBV DNA is measured most commonly; RNA detection has only been demonstrated to be useful in the restricted clinical setting of nasopharyngeal carcinoma.[8] In general, quantitative VL assessment is superior to qualitative detection since, using assays commonly used in the clinical laboratory EBV DNA can sometimes be detected in immunocompetent individuals and often in immunosuppressed patients without symptoms or clinical sequelae.[9,10]

Although some commercial assays are available, many laboratories continue to use in house developed assays. Most assays use real-time nucleic acid testing (NAT), often employing the polymerase chain reaction (PCR) for amplification. Depending on the clinical need (high sensitivity, exact quantification) different regions of the EBV genome have been targeted. The BAMHI-W region (part of EBNA-1 gene) is present in multiple copies and therefore is an excellent target choice for a highly sensitive PCR but is found in different copy numbers in each individual EBV genome (7–11 copies per genome). When exact quantification is required single copy gene regions such as thymidine kinase or virus capsid antigen or other parts of the EBNA-1 gene are better targets. EBV DNA measured in plasma is often not encapsidated but free DNA and might be highly fragmented. In this setting small product sizes may be preferable.[11,12]

External standards are used in most real-time assays. For accurate quantitation, the standards should be extracted together with the clinical samples and in a matrix resembling the clinical sample (e.g. EBV-negative cells), not diluted in buffer. Although plasmids are often used as standards, others recommend the use of Namalwa cells that harbour two genomes per cell. Additional options include the use of Raji cells or viral particles counted by electron microscopy.

Currently EBV VL result reporting formats are variable and include reporting per unit volume (usually copies/ml when testing fluid samples like CSF, plasma or whole blood (WB)), in copies/μg DNA (when testing cellular specimens such as whole blood, peripheral blood mononuclear cells (PBMC) or tissue biopsies) or in copies or number of positive cells/number of isolated cells (when PBMC, PBL or leukocytes are isolated). In a recent study of EBV VL units reported in WB, a close correlation between copies/ml and copies/μg DNA was observed with similar dynamic trending in patients using both reporting formats suggesting normalization to cell number or genomic DNA in cellular specimens may not be necessary.[13]

Currently used EBV VL assays vary considerably in extraction methods, gene targets and reporting units. External quality assurance studies have demonstrated significant variability in both qualitative and quantitative EBV VL results reporting among centers, limiting the validity of inter-institutional result comparison.[5,14] Proficiency panel results in both Europe (QCMD) and North America (College of American pathologists) have illustrated that for analytes assayed using nucleic acid testing and reported quantitatively, inter-laboratory variability in result reporting is significantly reduced when an international reference standard is available and commercial assays calibrated to this standard are used (personal communication). A World Health Organization international reference standard for EBV DNA using an EBV-infected cell line as the source material is currently being produced by the National Institutes for Biologic Standards and Controls (NIBSC, UK) and is expected to be available by October 2010. It is hoped that the use of calibrators traceable to this standard in both commercial and in house developed assays will reduce variability in result reporting and allow the quality improvement process addressing variables other than calibrators to proceed more rapidly.

3. Interpretation

Interpretation of VL results is often difficult, particularly in immunosuppressed patients. Even, though EBV may play a major pathophysiologic role in a specific disease, the EBV infection that triggers oncogenesis may antecede disease diagnosis by many years and many other host and environmental cofactors may play a role in disease development. Moreover the nature of EBV DNA in different stages of disease and in different biologic compartments is heterogeneous.

3.1. What is being measured

Both immunocompetent and immunosuppressed subjects can be simultaneously infected with multiple EBV strains. Clear differences in the relative abundance of strains in distinct compartments, the oral cavity, leukocytes and plasma exist. The impact of these dif-