EBV SEROLOGY AND NASOPHARYNGEAL CARCINOMA

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MARCH 28, 1989
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Introduction

Epstein Barr virus (EBV) is a DNA virus of the Herpes family. Infection in infancy and childhood is often asymptomatic. Primary infection in adolescence and adulthood is generally manifested as infectious mononucleosis. In addition to its involvement in acute viral syndromes, EBV has been associated with malignancy. It has been implicated in both Burkitt's Lymphoma and Nasopharyngeal Carcinoma (NPC). In vitro studies show the presence of viral DNA, as well as antigen expression, in lymphoid tissue and nasopharyngeal epithelium.

Nasopharyngeal carcinoma is an uncommon tumor in North America, but is one of the most common malignancies seen in some parts of China and Southeast Asia. There are three classifications of NPC: Squamous Cell Carcinoma, Nonkeratinizing Squamous Cell Carcinoma, and Undifferentiated Carcinoma. The last two groups are associated with EBV. Since these tumors often present at a late stage or as an occult primary, it would be useful to have a test to detect them. EBV serology has proved to be a useful diagnostic and prognostic test in some settings. In populations where there is a high prevalence of NPC, EBV serology has been used as a screening test.
Several different antibodies are measured in relationship to NPC. Both IgG and IgA to viral capsid antigen (VCA) and early antigen/diffuse (EA/D) are assayed. In an acute infection, IgM and IgG anti-VCA are produced. IgM disappears in about four weeks, but IgG persists at low levels. In NPC, IgG is present at much higher levels than with an acute infection. In addition, IgA anti-VCA, which is not seen with an acute infection, is present at high levels with NPC.

Antibodies to early antigen (EA) show both a diffuse (cytoplasmic and nuclear) and a restricted (cytoplasmic only) pattern. These are present transiently with acute infections and reactivations. High titers of anti-EA/R (restricted) have been associated with Burkitt's lymphoma. Anti-EA/D, both IgG and IgA, are associated with NPC.

Methodology

Anti-VCA

Smears are prepared with the P3-HR1 cell line, with 10-15% of the cells expressing VCA. A drop of a cell suspension is placed on a glass slide and fixed with acetone. Acetone fixed smears can be used to measure both IgG and IgA. Fourfold dilutions of the patients' serum are incubated with the fixed cells. Fluorescein isothiocyanate conjugated antiserum to human Ig subclasses is then added and the presence of fluorescence is measured.
Anti-EA/D

To express EA, the Raji cell line must be either superinfected with infectious virus from a P3-HR1 cell culture, or induced by treatment with a variety of agents. Once this is accomplished, cell suspensions are used to prepare acetone fixed smears. An indirect immunofluorescent assay similar to that done for anti-VCA is run. Acetone fixed smears can detect both diffuse (speckled staining seen throughout the cell) and restricted (staining of the cytoplasm only) patterns. Methanol fixed smears preferentially remove the EA/R.

Discussion

The association between NPC and EBV has been recognized for many years. NPC has a high prevalence in some parts of the world. It often presents at a late stage or as an occult primary. These facts make a readily available marker such as viral serology ideal for diagnosis and prognosis. In fact, numerous studies have shown that EBV serology is useful in regards to NPC.

Titers to VCA and to EA/D have proven to be the most useful in NPC. Nearly all patients with NPC have elevated VCA-IgG titers. However, this is not specific, since this antibody can persist following a primary infection. VCA-IgA is far more specific (9-18% of healthy patients are positive), but much less sensitive. The sensitivity of this test varies from 65-89% depending on the study and
classification of the NPC. It has the highest sensitivity for WHO type 2 and 3 (nonkeratinizing and undifferentiated) NPC. Titers to EA/D have similar profiles. 94-100% of patients have positive titers when both IgG and IgA are taken into account. IgA titers are most specific (~98%), but least sensitive, being present in 47% of patients. IgG titers are less specific (~65%), but more sensitive (present in 83-94% of patients).

Taken individually, no single titer would be useful for diagnosis. However, when used as a profile, elevated titers are highly suggestive of NPC. In particular, the presence of VCA-IgA, EA/D-IgG and EA/D-IgA together, makes the probability that NPC is present 12 times more likely than that of other regional malignancies. In North America, where the incidence of this malignancy is low (1-2/100,000), the diagnostic utility of these tests is most important in the patient presenting with cervical nodes and an unknown primary. However, in areas of high incidence, such as southern China (18/100,000), the sensitivity and specificity of VCA-IgA in detecting NPC becomes much higher (93-95% and 97%, respectively). In this situation the test can be used successfully as a screen.

EBV serology also appears to have prognostic value in NPC. Several studies have shown that the height of the VCA and EA/D titers correlate with the tumor burden. Several studies also indicate that following these titers may be useful in predicting relapse after treatment.
Antibodies to nuclear antigens (EBNA) are also present in NPC but, have not been as well characterized and do not appear to be as useful. A latent membrane protein antibody also appears to have some prognostic significance (its presence being correlated with a good prognosis). However, this is measured by an antibody dependent cellular toxicity assay which limits its utility.

In conclusion, EBV serology has diagnostic and prognostic utility in NPC. In areas where there is a high incidence of the disease, VCA-IgA assays are a useful screening test. In other areas, such as North America where the disease is uncommon, measuring a panel of VCA-IgA, EA/D-IgG and EA/D-IgA is a useful aid to diagnosis in the patient with a high index of suspicion for NPC. It also has utility in following the course of the disease.

References


