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Clinical Reliability of IgG, IgA, and IgM Antibodies in Detecting Epstein-Barr Virus at Different Stages of Infection With a Commercial Nonrecombinant Polyantigenic ELISA

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We studied the diagnostic reliability of a modification of the Enzygnost EBV test (Behringwerke, Germany) for the detection of IgG, IgA, and IgM antibodies (Abs) in the diagnosis of Epstein-Barr virus (EBV) disease. One hundred and twenty-three serum samples were studied: 14 asymptomatic subjects without EBV infection, 48 patients with primary infection, 46 subjects with past EBV infection (11 patients with other acute infections), 8 patients without EBV infection but with other viral infection, and 7 patients with probable acute clonal stimulation of B lymphocytes caused by different microorganisms. Enzygnost EBV is based on an ELISA test with a pool of viral antigens. In our series the reliability of IgM for the diagnosis of recent primary EBV infection was: sensitivity 100%, specificity 95%, positive predictive value 90.5%, and negative predictive value 100%. The IgG detection with Enzygnost was: sensitivity 98%, specificity 100%, positive predictive value 100%, and negative predictive value 91.7%. Only two subjects had positive IgA. The Enzygnost test is an efficient method for the diagnosis of EBV infection although a few IgM false positives can occur. J. Clin. Lab. Anal. 13:65–68, 1999. © 1999 Wiley-Liss, Inc.

Key words: Epstein-Barr virus; ELISA; antibodies

INTRODUCTION

Epstein-Barr virus (EBV) infection is usually diagnosed by serological methods. These included IM-specific guinea pig absorbed heterophil agglutins or EBV-specific antibodies to VCA, EBNA, EA-D, EA-R, and MA. In primary infection, IgM to VCA, EBNA, EA, and heterophilic antibodies, and their corresponding IgA and IgG, can be detected, the latter persisting for many years (1–5). However, indirect diagnosis does not detect all actual illnesses because the antibodies may be absent, or may occur in situations of the clonal stimulation of B lymphocytes (due to a present infection by another pathogenic agent) (6–11). Different authors (12–15) have tried to solve some of these diagnostic problems by detecting sera IgG without avidity for the antigen (Ag), which is present in primary infections, although the absence of IgG and the rapid increase in its affinity for the antigen reduce the diagnostic reliability of this test.

Recently, most laboratories investigate antibody titers by ELISA rather than by IFA because of the former's techniques, greater objectivity, standardization, and automation (16,17). The use of a pool of antigens in IFA (Virgo, Switzerland), ELISA (Behringwerke), and Western blot (DPC, Germany), has been proposed to reduce the number of tests needed to diagnose the infection. These methods reduce time and costs and simplify the interpretation of the results. However, their reliability has not yet been demostrated.

We investigated the diagnostic reliability of a modification of the Enzygnost EBV test (Behringwerke), which uses the ELISA test with a pool of antigens for the detection of antibodies to diagnose the infection.

MATERIALS AND METHODS

One hundred and twenty-three serum samples were collected from patients at the St. Cecil University Hospital between 1991 and 1996 to investigate IgG, IgA, and IgM with Enzygnost EBV. The sera were divided into five panels according to the EBV infection and Lennette criteria (4):

Panel 1: Fourteen asymptomatic subjects without EBV infection defined by the absence of anti-VCA IgG, anti-VCA IgM, anti-EBNA IgG (ETI Sorin, Italy), and heterophilic antibodies (Biokit, Spain).

Panel 2: Forty-eight patients with primary infection (mean age of 5 ± 2 years) defined by the presence of anti-VCA IgG + IgM and the absence of IgG anti-EBNA 15–25 days after the onset of clinical symptoms, without IgM to other human herpes viruses (except human herpes virus-7 and

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-8, which were not tested). The presence of heterophilic antibodies was variable. The patients had fever or infectious mononucleosis (lymphocytosis, adenopharyngitis, and fever).

Panel 3: Forty-six subjects with past EBV infection, with (11 patients, panel 3a) or without (35 subjects, panel 3b) other viral infection (mean age of 25 ± 5 years). The patients had anti-VCA IgG and anti-EBNA IgG but not anti-VCA IgM. Panel 3a: 11 cases of acute or subacute infections with HAV (1), HBV (2), HIV (1), CMV (2), *Rickettsia conorii* (1), and Varicella-Zoster (3).

Panel 4: Eight patients without EBV infection but with other viral infection, defined by the absence of anti-VCA IgG, anti-EBNA IgG, and anti-VCA IgM. The diseases were: 1 acute measles virus infection (defined by specific signs and positive IgM, indirect ELISA with anti-IgG, [Behringwerke]), 1 acute influenza virus infection (defined by flu symptoms, CF titer > 1:64 to influenza virus type A without antibodies to other respiratory virus, [Behringwerke]), 2 chronic-HBV infections, two acute primary VZV infections, one acute adenovirus infection (defined by flu symptoms, CF titer > 1:64 to adenovirus without antibodies to other respiratory virus, [Behringwerke]) and 1 acute rubellavirus infection (defined by typical signs, positive specific IgM, capture ELISA [DiaSorin]).

Panel 5: Seven patients with probable acute clonal stimulation of B lymphocytes due to different microorganisms at EBV and false positive antibodies to EBV for acute infection (positive anti-VCA IgM, anti-VCA IgG, and anti-EBNA IgG; without current or recent EBV disease). The acute infections were: 7 infections by HAV, 1 mumps virus (MV) infection (defined by typical signs, positive specific IgM, indirect ELISA with anti-IgG, [Behringwerke]), 1 chronic HBV infection, and 2 primary herpes simplex virus (HSV) infections. The latter patients had fever and erythematous vesiculopapules on the mucous membrane, presence of antigen (capture ELISA, [Murex, England]) and specific IgM with anti-IgG (indirect ELISA, [Behringwerke]).

Subjects who had underlying diseases known to be associated with immunological diseases other than HIV infection were excluded from the study. The ELISA or CF for HVB, HAV, HIV, adenovirus, and influenzavirus were done at the Immunoserology Unit of the Microbiology Department (St. Cecil University Hospital).

Study of Heterophilic Antibodies (Monolatex)

Monolatex is a latex test used to detect IM-specific heterophilic antibodies with bovine erythrocyte antigens.

Study of IgG, IgA, and IgM to EBV

ETI Sorin

1. Anti-EBNA IgG: indirect ELISA that uses a synthetic peptide of the EBNA-1 protein.
2. Anti-VCA IgG: indirect ELISA that uses a synthetic peptide of VCA (p18).
3. Anti-VCA IgM: capture ELISA with the p18 antigen without removal of the IgG.

Serum samples were diluted to 1:100 and the manufacturer's instructions were followed for test validation. The results were expressed in arbitrary units per ml; values lower than 10, 0.69, and 5 arbitrary units (AU) of anti-VCA IgG, anti-VCA IgM, and anti-EBNA IgG, respectively were considered negatives. The upper limits for VCA IgG and EBNA IgG were 170 and 200 AU, respectively.

Enzygnost

Tests were carried out with an automated system (Behringwerke ELISA Processor III) which uses an indirect ELISA with peroxidase-conjugated caprine antiserum. The antigens used in this test were previously published (18) and contain an extract of EBV-transformed cells and induced to express VCA, EBNA-1, and EA-D. Sera used at a dilution of 1:231 for IgG and 1:42 for IgM and IgA (the latter with progressive dilution until a negative result was obtained). Samples for IgA and IgM were preabsorbed using ovine anti-IgG antibodies during 18 h at 4°C ([Behringwerke], the original test uses 30 min at room temperature). For precise evaluation of the IgG we used the α-method calculation system (17). The results were expressed in IU/ml using the Medical Research Council Research Standard A, 66/235 for infectious mononucleosis serum obtained from the WHO National Institute for Biological Standards and Controls (UK). To determine the cut-off point we followed the manufacturer's instructions for IgG, modifying the instructions for IgM (with a cut-off optical density > 0.2; the original test yields equivocal results if a value is repeated between 0.1 and 0.2). We followed the criteria of Dopatka and Schuy (18) for IgA (cut-off optical density of 0.6) the diagnosis of positive result and reactivated illness.

The sensitivity, specificity, and positive and negative predictive values were determinated for the Enzygnost IgG and IgM to compare the groups of primary infection or past infection. We used the value of IgM for the diagnosis of recent acute infection.

RESULTS

The results in Table 1 show a good correlation between the results obtained with Enzygnost and EBV status in patients from panels 1 through 4. In panel 5, Enzygnost detected IgM antibodies to EBV in 3 acute HAV infections, 1 acute MV

TABLE 1. Relationship Between the Panels of Samples and Results With the Enzygnost EBV System for Detecting IgG, IgA, and IgM to EVB

	Enzygnost: Positive (%)		
	IgG	IgA	IgM
Panel 1 (n:14)	0	0	0
Panel 2 (n:48)	96	0	100
Panel 3a (n:11)	100	9.1	0
Panel 3b (n:35)	100	0	0
Panel 4 (n:8)	0	0	0
Panel 5 (n:7)	100	14.3	71.4

Panel 1: Asymptomatic subjects without EBV infection; Panel 2: Patients with primary EBV infection and no other infection; Panel 3a: Patients with past EBV infection and other viral infection; Panel 3b: Subjects with past EBV infection and no other viral infection; Panel 4: Patients without EBV infection but with other viral infection; Panel 5: Patients with clonal stimulation of B lymphocytes and IgM to EBV.

infection, and 1 chronic HBV infection. In our series the reliability of IgM for the diagnosis of recent acute EBV infection was: sensitivity 100%, specificity 95%, positive predictive value 90.5%, and negative predictive value 100%. The IgG detection with Enzygnost was: sensitivity 98%, specificity 100%, positive predictive value 100%, and negative predictive value 91.7%. Only two subjects, with past EBV infection and other viral infection (panel 3a) and clonal stimulation of B lymphocytes and IgM to EBV (panel 5), respectively, had positive IgA.

DISCUSSION

The diagnosis of EBV disease requires laboratory tests. Choice of such tests depends on the characteristics of the infectious process (19).

Value of IgM Titers With Enzygnost in the Diagnosis of Recent Acute Infection

Different options for the diagnosis of EBV are currently available. Gull and Ortho Diagnostic have ELISAs for detecting anti-VCA antibodies with the gp125 antigen, obtained from an infected lymphoblastoid cell line. This is the most important anti-VCA antibody, although it is not the only one detectable during primary infection (20). When VCA expressed by baculovirus (without seric reactivity shared with associated antigens) is used, high specificity was obtained, but so were many false negatives (21). Performance would probably be similar with other VCA peptides used separately (11). Four ELISA tests which detect anti-EA IgM, IgG, and IgA, and anti-EBNA IgG were designed by Biotest. These assays use EA-Ags (p54 and p138 peptides from EA diffuse component) obtained from recombinant *Escherichia coli*. An assay by Sigma Diagnostics for detecting anti-VCA and anti-EBNA IgG and IgM is also available. A test by DiaSorin for detecting anti-VCA and anti-EBNA is described previously in MATERIALS AND METHODS.

The Enzygnost EBV kit uses several lymphocyte-expressed antigens. In previous studies that compared Enzygnost with different methods (14,22), we found a good correlation with other ELISA or IFA methods that use only VCA. Enzygnost has optimal diagnostic reliability as long as patients with clonal stimulation of B cells are excluded. However, De Ory (23) recently found that in comparison with the IFA test, Enzygnost yielded a certain number of equivocal results. We also found serum samples that behaved equivocally when manufacturer´s instructions were used (unpublished results). Three methodological modifications improved the clinical correlation in our assay: 1) absorption with anti-IgG during 18 h, instead of 30 min as indicated by the manufacturer; 2) the positive control absorbance was used to obtain a correction factor that was calculated by dividing the nominal value from the positive control by mean optical density obtained in the assay. The optical density values of the samples were then corrected by multiplying them by this factor; and 3) we ignored samples with absorbance between 0.1 and 0.2. In our experience Enzygnost EBV for IgM showed adequate diagnostic reliability in the diagnosis of acute infection. In comparison with other systems that use recombinant antigens (12,24), it is the most reliable, although this may be due to the use of different antigenic bases. Generally, when separate antigens are used, false negatives can appear in the very early phase (without anti-VCA IgG or IgM), perhaps because of the rapid synthesis of anti-EBNA (with or without anti-EA). The inclusion of other antigens in the Enzygnost system such as EA-D, the second most important immunogen (18), ensures accurate diagnosis. Therefore this method, which uses several antigens, make it possible to detect recent infections in their most advanced phases (anti-VCA and anti-EBNA IgG positive, and anti-VCA IgM negative), although it remains affected by the biological phenomenon of the clonal stimulation of B lymphocytes not due to primary EBV infection (9). No single commercial system is able to distinguish this phenomenon. Furthermore, not all EBV infections cause clinical symptoms (18) and therefore virus-specific IgM can be also found in asymptomatic patients as a result of the remnants of IgM from a previous or reactivated infection. However, the equivocal diagnoses detected in our study suggest the need for additional research because of the individual variations in the biological response to the virus.

Proposed Utilization of the Enzygnost EBV Test

We recently demonstrated the diagnostic reliability of the Enzygnost EBV test in detecting IgG without avidity for the antigen to demonstrate recent primary EBV infections, even in the presence of IgM against microorganisms by clonal stimulation of B cells (14,15) (Spanish Patent #9500230). On the basis of these earlier results in conjunction with the present findings, we propose an application of the Enzygnost EBV reagent (Fig. 1) that can enhance the accuracy of the clinical diagnosis.

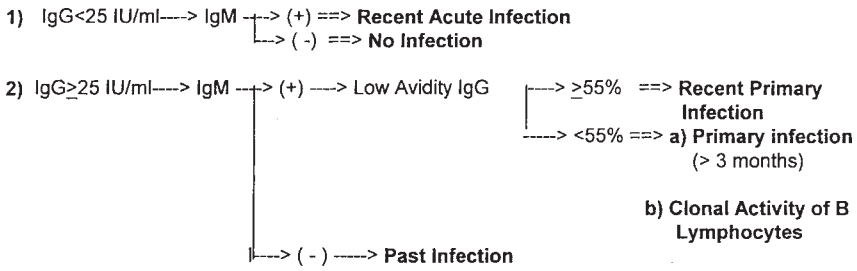

Fig. 1. Interpretation of serology in the EBV infection.

In conclusion, the Enzygnost system represents an efficient method for the detection of EBV infection, although a few false positives can appear. We propose the combined detection of IgG, IgM, and low-avidity IgG against the virus to determine the actual stage of infection.

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