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Epstein-Barr Virus (EBV) DNA in Sera of Patients with Primary EBV Infection

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Detection of Epstein-Barr Virus (EBV) DNA by PCR in serum had a sensitivity of 80%, a specificity of 94%, and positive and negative predictive values of 95 and 79%, respectively, for the diagnosis of primary EBV infection. We suggest that this is a useful addition to the panel of tests used for this purpose.

The clinical features of acute Epstein-Barr virus (EBV) infection overlap those of a variety of other infectious and noninfectious diseases, and reliable laboratory tests are important to aid the differential diagnosis. Although primary EBV infection can be diagnosed by an assay for heterophile antibodies in adults, this assay's sensitivity is low for children (1). The detection of immunoglobulin M (IgM) antibody to the virus capsid antigen (VCA) in the absence of antibody to EB nuclear antigen (EBNA) is regarded as suggestive of acute primary EBV infection because EBNA antibodies develop only in late convalescence (8). However, false-negative results may occur due to the transient nature of the VCA IgM response. Conversely, false-positive IgM reactions occur due to autoantibodies or other serum factors and due to anamnestic reactions or cross-reactions to other recent infections (10). The alternative approach of relying on the absence of EBNA antibody in the presence of VCA IgG may also lead to false-positive or false-negative conclusions. In primary infection of children (2) or immunocompromised patients, the appearance of EBNA antibodies may be unusually delayed, resulting in a false diagnosis of recent EBV infection. Furthermore, passive maternal antibody may confound the diagnosis in infants. Tests of the affinity of IgG antibody have recently been reported to be useful in differentiating recent from past infections (6).

EBV DNA was found in blood lymphocytes from 54 to 94% of EBV-seropositive healthy subjects (15). Detection and quantification of virus DNA in blood by PCR of mononuclear cells are useful for diagnosing and monitoring lymphoproliferative diseases (11, 13, 14). Viral DNA in cell-free serum or plasma of patients is useful for diagnosing primary human herpesvirus 6 infection (3). However, a previous study on PCR detection of EBV DNA in serum for the diagnosis of primary EBV infection found poor sensitivity and specificity (5).

To evaluate the diagnostic utility of detecting EBV DNA in serum, we investigated 81 serum samples submitted to our laboratory from patients investigated for suspected primary EBV infection and 40 negative control serum samples from EBV-seropositive blood donors (made anonymous and provided by the Hong Kong Red Cross Blood Transfusion Service). All serum samples were tested for EBV-specific serology, including VCA IgM, VCA IgG, early antigen (EA) IgG, and EBNA antibody (1, 9, 12). The avidity of VCA IgG antibody was determined as described previously (4).

The interpretation of the standard serological profiles used is shown in Table 1 (8). Antibody avidity test results were added to provide an expanded serological profile in which patients with low antibody affinity were defined as having primary infections while those with high antibody affinity were regarded as having past infections (Table 1). Using the expanded serological profile, those patients with detectable VCA IgG of low affinity in the absence of EBNA antibodies were categorized as having early (if IgM positive) or recent primary infections.

EBV DNA was extracted from 200 μl of serum using the QIAamp blood kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. Five microliters of the DNA eluate was used for a first PCR amplification with a 0.05 μM concentration of each sense and antisense primer (5′-GC CAGAGGTAAGTGGACTTT-3′ and 5′-TGGAGAGGTCA GGTTACTTA-3′, respectively) corresponding to the internal repeat *Bam*HI W fragment of EBV strain B95-8 to detect EBV DNA. The PCR mixture (50 μl) contained PCR buffer (10 mM Tris-HCl, 3 mM $MgCl_2$, 50 mM KCl, 0.1% gelatin [pH 8.3], 100 μM deoxynucleoside triphosphates, and 0.375 U of *Taq* polymerase (Boehringer Mannheim, Indianapolis, Ind.). Samples were then subjected to 20 cycles of amplification (1 min each at 94, 55, and 72°C) in a thermal cycler (Perkin-Elmer Cetus, Gouda, The Netherlands) followed by extension at 72°C for 10 min. Subsequently, a 5-μl aliquot of the first PCR product was transferred to a second PCR tube for nested PCR using two inner primers (5′-TTCTGCTAAGCCCAACACTC-3′ and 5′-CTGAAGGTGAACCGCTTA-3′), each at a 0.5 μM concentration. The PCR mixture was similar to that of the first PCR except that 2 mM $MgCl_2$ and 2.5 U of *Taq* polymerase were used. The reaction mixture was subjected to a further 30 cycles of amplification using the thermal cycling profile described above. PCR products were analyzed by agarose gel electrophoresis. The size of the nested-PCR product was 192 bp.

The analytical sensitivity of the PCR assay was approximately 5 genome copies, using the plasmid vector pCRII-TOPO (Introgen, San Diego, Calif.) containing the *Bam*HI W fragment of the EBV genome as the reference standard. Using the standard serological profile as the reference, EBV DNA

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TABLE 1. Correlation of EBV serological profiles with viral DNA detection in serum by PCR

Diagnosis based on standard EBV seroprofile	No. of patients	VCA IgG avidity test results	Diagnosis based on expanded seroprofile[a]	EBV DNA in serum [no. of patients positive/no. tested (% positive)]	EA IgG antibody [no. of patients positive/no. tested (% positive)]
Early primary infection (VCA IgM^+ VCA IgG^+ $EBNA^-$)	31	Low (n = 30)	Primary infection	25/30 (83)	17/30 (57)
		High (n = 1)	Past infection	1/1	0/1 (0)
Recent primary infection (VCA IgM^- VCA IgG^+ $EBNA^-$)	27	Low (n = 15)	Primary infection	11/15 (73)	9/15 (60)
		High (n = 12)	Past infection	0/12 (0)	8/12 (67)
Equivocal (VCA IgM^+ VCA IgG^+ $EBNA^+$)	1	Low (n = 1)	Primary infection	1/1	0/1 (0)
		High (n = 0)	Not relevant	0/0	0/0 (0)
Past infection (VCA IgM^- VCA IgG^+ $EBNA^+$)	14	Low (n = 0)	Not relevant	0/0	0/0 (0)
		High (n = 14)	Past infection	1[b]/14 (7)	7/14 (50)
No infection VCA IgG^- $EBNA^-$)	8	NA[c]	No infection	0/8 (0)	0/8 (0)

[a] Includes VCA IgG and IgM, EBNA antibody, and avidity of VCA IgG (see text for details).
[b] This patient had EA IgG and weak EBNA antibodies. Clinical chart review was compatible with a diagnosis of infectious mononucleosis 3 weeks previously (see text).
[c] NA, not applicable.

was detectable in the serum in 26 (83.4%) of 31 patients diagnosed with early primary infections, 11 (41%) of 27 with recent primary infections, 1 (7%) of 14 with past infections, and none (0%) of those with no infection. The patient with an equivocal serological result was also EBV DNA positive. On this basis, EBV DNA detection in serum by PCR had a sensitivity of 63.8%, a specificity of 95.5%, a positive predictive value (PPV) of 97.4%, and a negative predictive value (NPV) of 50% for diagnosis of early or recent primary EBV infections. None of the 40 healthy EBV-seropositive blood donor controls had detectable EBV DNA in their sera.

The avidity of VCA IgG was examined in all 73 patients with detectable antibody in their sera as an additional serological marker of primary EBV infection. Of the 59 patients with early primary, recent primary, or equivocal serological profiles by the standard serological test panel, 46 had low-avidity antibody and were confirmed as having primary infections (Table 1). Of these, 37 of 46 (80%) had detectable EBV DNA in their sera while only 1 of 13 with high-avidity antibodies was viral DNA positive. Of 14 patients with a serological profile of past infection by the standard serological panel, none had low-avidity antibody and only 1 of these had detectable EBV DNA in serum (Table 1). By application of this expanded serological profile with antibody avidity included as the "gold standard," the sensitivity, specificity, PPV, and NPV of the test for EBV DNA in serum used for diagnosis of primary EBV infection were 80% (37 of 46 patients), 94% (33 of 35), 95% (37 of 39), and 79% (33 of 42), respectively.

The clinical charts of the patient with serologically presumed past infection but with detectable EBV DNA in the serum were reviewed by an independent pediatrician. This patient was a 2-year-old male with a mononucleosis-like illness who developed a macular-papular rash after treatment with antibiotics. The first serum sample for virological investigation was collected 3 weeks after the onset of the illness, and this may be the reason for the weakly positive EBNA antibodies and the high-avidity VCA IgG. Thus, this patient also had a probable recent primary EBV infection, and if so, the specificity and PPV of the EBV DNA test improve further to 97%. An alternative explanation is that this patient had disease associated with EBV reactivation.

When compared with the expanded serological profile, the test for EBV-EA IgG antibody had poor sensitivity (26 of 48 patients; 54%), specificity (20 of 35; 57%), and PPV (26 of 41; 63%) for the diagnosis of primary EBV infection. Our data confirm that EA IgG antibodies are not reliable options for the diagnosis of recent EBV infection.

There were a number of serum samples (15 of 46) with low-avidity VCA IgG and an absence of EBNA antibodies that were IgM negative (Table 1), confirming previous reports (7) that detection of VCA IgM is an insensitive test for diagnosing recent primary EBV infections. Although the specificity of the VCA IgM test seems to be high in the present study, we have previously found detectable EBV VCA IgM in one of four consecutive patients with parvovirus B19 infection (K. H. Chan and J. S. M. Peiris, unpublished data), illustrating the fact that false-positive IgM results do occur.

The presence of VCA IgG in the absence of EBNA antibody is also not always a reliable indicator of recent primary infection. In the present study, this profile did not agree with findings of low-avidity VCA IgG or EBV DNA in serum samples in 12 of 58 (21%) instances. This is not surprising because we have previously shown that EBNA antibodies may take longer than 8 months to appear in children (2).

The lack of detectable viral DNA in the sera of 40 seropositive healthy controls indicates that although most such individuals would be expected to be carrying EBV DNA in their lymphocytes (15), EBV DNA is not usually found in serum in the absence of active EBV disease. It is likely, however, that viral DNA in serum will be present in cases of EBV reactivation as well as in cases of primary infection, and tests for viral DNA will not discriminate between these two possibilities unless they are used in conjunction with serology. EBV reactivation is particularly relevant in the immunocompromised patient.

In conclusion, we find that neither a test of EBV VCA IgM

nor a test of the presence of VCA IgG in the absence of EBNA antibody is solely reliable for diagnosing primary EBV infection. PCR for EBV DNA in serum is a useful addition to the panel of tests available for this purpose, particularly if used as a confirmatory test in conjunction with serological tests.

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