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Reliability of Four Methods for the Diagnosis of Acute Infection by Epstein-Barr Virus

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We studied the reliability of new indirect tests in the diagnosis of acute infection by Epstein-Barr virus (EBV). Studied for all samples were: method 1, the heterophil antibodies (Abs) (Monolatex, Biokit, Germany); method 2, the IgM Abs to EBV with ELISA tests (antigen pools, Enzygnost, Behringwerke, Germany); method 3, EA (Biotest Diagnostics, Germany); and method 4, the IgG avidity test. The reliability of the four tests for the detection of primary infection by EBV was: sensitivity (method 1: 89.1%; method 2: 100%; method 3: 79.7%; method 4: 99%); specificity (method 1: 98%; method 2: 100%; method 3: 84%; method 4: 100%); positive predictive value (method 1: 97.6%; method 2: 100%; method 3: 73.6%; method 4: 100%), and negative predictive value (method 1: 90.7%; method 2: 100%; method 3:84%; method 4: 99%). The IgG avidity test (method 4) is simple and automated in the laboratory and is very useful for ascertaining, from a single sample, the time since infection. It is criteria of recent primoinfection higher levels than 55% of IgG with low avidity for the antigen. The investigation of the Abs to antigen pools (method 2) by ELISA of virus had a high reliability, but the investigation of heterophil Abs by latex (method 1) and the Abs IgM to EA (method 2) were lacking in sensibility regarding their use in the diagnosis of the primoinfection. J. Clin. Lab. Anal. 11:78–81.

Key words: Epstein-Barr virus; cytomegalovirus; herpes simple virus; IgG; IgM

INTRODUCTION

Laboratory diagnosis of the infection made active by the Epstein Barr virus (EBV) is of great importance to establish the diagnostic differential with other processes and possibly to plan a treatment [1,2]. During infection by EBV, IgM antibodies (Abs) do not act in a sufficiently consistent way for use in the diagnosis of primary infection, reinfection, or reactivation, whether symptomatic or not. These Abs can give false negative results, because they are not synthetized, or because the quantity that is synthetized is difficult to detect [3]. In addition, they can yield false positive results, because of their persistence and their presence in processes unrelated to this infection [4], because they may be detected in reactivations or reinfections [5–8], because of heterotypical reactions between those viruses in circulation in the white blood cells and the main target cell (CMV, EBV, and HHV-6) [9,10]. The heterophil Abs go undetected in > 50% of children < 7 years old [3]. Diagnosis of active infection based on detection of a fourfold increase in the IgG titer is conflicting. The increase may not be detected, since IgGs usually appear very soon after onset of symptoms [11].

For these reasons, indirect microbiological diagnosis is difficult, and there are other new commercial tests to resolve technical problems. In this report, we study the reliability of new indirect tests in the diagnosis of acute infection by EBV.

MATERIALS AND METHODS

The samples studied and their clustering into panels are shown in Table 1. They were analysed retrospectively, after being taken 15–25 days subsequent to the onset of clinical symptoms. Primary infection and reactivation were defined by the presence of IgM and specific IgG and the absence of IgM to the other human herpes viruses (except human herpesvirus-7, which was not tested) (Enzygnost EIA, Behringwerke, Germany) and at least the presence of consistent clinical findings in the first instance. Past infection was defined by the absence of symptoms and IgM specific, with IgG. Acute infection by EBV was present with infectious mononucleosis (fever, lymphadenopathy, atypical lymphocytosis, and liver and spleen increased in size) and IgM anti-VCA (75% of patients) (EBV, IFA, Gull, USA) or IgG anti-VCA (EBV, IFA, Gull) without Abs anti-EBNA (25% of patients) (EBV, IFA, Gull) and IgM negative to the other human herpes viruses. Past infection was defined by the presence of IgG anti-VCA and Abs anti-EBNA without symptoms. The mean age of the patients was 29.3 years, with a range of

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TABLE 1. Serum Sample Panels Studied and Their Relationship to Clinical Picture

Infection	Clinical phase	Panel	Total Patients
HSV	Primary infection	1a	31
	Reactivation	1b	6
	Past infection	1c	50
CMV	Primary infection	2a	75
	Reactivation	2b	3
	Past infection	2c	50
EBV	Primary infection	3a	100[a]
	Past infection	3b	50

[a]25 patients ≤ 7 years old; 75 patients > 7 years old.

2–84 years. All specimens were either tested immediately, stored at 4°C and tested within 48 hr, or frozen at –20°C and subsequently tested.

Studied in all samples were the heterophil Abs (method 1; Monolatex, Biokit, Germany) and the IgM Abs to EBV with indirect ELISA tests (method 2, Enzygnost, Behringwerke, Germany; method 3, Biotest Diagnostics, Germany) using anti-IgG (RF absorbent, Behringwerke). The IgG avidity test for the EBV (method 4) was calculated in group 3.

Monolatex is a latex test that studied the heterophil Abs with bovine erithocyte antigens. Method 2 is an indirect ELISA with a pool of EBNA, EA-D, VCA, and complete virus. The antigen is derived from permanently EBV-infected lymphoblastoid cells in which viral synthesis has been stimulated. Method 3 is an indirect ELISA with recombinant antigens p54 and p138 (fusion protein) from EA, expressed in *Escherichia coli.* In method 4, we used an automated and modified system in house from Behringwerke (Bhering ELISA Processor III) with a commercial test (indirect ELISA), carried out once without denaturing agent and once with 8-molar urea, added to the test for 10 min after incubation of the samples. Exact evaluation of the Abs was performed by the Alpha method calculation system (Behringwerke) [12]. The use of reagents with a specific quantitative composition makes it possible to adjust the test absorbance to a standard curve that correlates international units with optical density. To determine the incidence of low avidity IgG Abs, we took the difference between the values obtained from the two test runs (with and without urea) and the result was expressed as a percentage of the total IgG Abs. The test was repeated twice, the mean value obtained being used, provided dispersion did not exceed 10%. We considered levels of low avidity Abs above 55% to be significant and those between 50% and 55% to be questionable.

RESULTS

The percentages of positive results about tests 1–4 are shown in Table 2. The reliability of the four tests for the detection of primary infection by EBV are shown in Table 3.

DISCUSSION

Several workers have shown the avidity of IgG Abs changes with the time since infection, whether viral (5,13–17) or bacterial [18–22], and suggest that when the percentage of IgG Abs with a low avidity for the antigen is equal to or higher than 50%, we are dealing with a recent primary infection. We found that, using breakpoints of 50%, the study of IgG class Ab avidity can help to distinguish a short-term from a long-term acute infection, since the yield of this assay is close to 100% for all parameters. The fact that it is not 100% may be due to various causes: persistence of immature (or not very protective) Abs in patients with a past infection or reactivation; rapid maturation of Ab avidity in patients with a primary infection; problems in the commercial antigen used, which would mean that it is not sufficiently sensitive to detect Abs in a particular virus subtype; or the need to use different denaturing agents that would make it possible to distinguish the presence of low-avidity Abs with greater sensitivity. Eight-molar urea may be less sensitive than diethylamine here, because urea may not be able to detect low-avidity Abs more than 3 months after infection, as in the case of reported rubella [10]. However, the use of one or other denatur-

TABLE 2. Percent of Positive Results Obtained in Panels 1, 2, and 3 for Methods 1–4.

				Method 4	
Panel	Method 1	Method 2	Method 3	46–55% low-avidity	>55% low-avidity
1a	0	0	0	–	
1b	0	0	0	–	
1c	0	0	0	–	
2a	0	0	0	–	
2b	0	0	0	–	
2c	0	0	0	–	
3a	17.8[a] 66.8[b]	100	79.7	1	99
3b	2	0	16	6	0

[a]Patients ≤7 years old.
[b]Patients > 7 years old.

TABLE 3. Diagnostic Yield of Methods 1–4

	Method 1			Method 4	
	>7 years old	Method 2	Method 3	50%	55%
Sensitivity	89.1%	100%	79.7%	100%	99%
Specificity	98%	100%	84%	98%	100%
Positive predictive value	97.6%	100%	73.6%	98.6%	100%
Negative predictive value	90.7%	100%	84%	100%	99%

ing agent is affected by the type of organism inducing the Ab response and the strength of the denaturing effect caused and must be investigated for each case. The breakpoints revised in the literature for considering the presence of low-avidity Abs vary from 70–75% [23–36] and 50% [16,27], depending on the denaturing agent employed, the type of organism in question, and the requirements of the individual author. The former authors who argue in favour of higher breakpoints use 6-molar urea or diethylamine as a denaturing agent. We initially used a 50% breakpoint and later 55%, discarding samples with values between 50% and 55%, and found that the yield improved slightly where the breakpoint was not set in the same way. In this manner, we obtained a diagnostic yield of 100%, similar to the results (94.7%) obtained by De Ory [5] for the sensitivity, and higher for the specificity, detected in 27 cases of past infections and two cases of Abs IgG of low avidity.

If we take into account that the diagnostic yield of IgM Abs study in acute infection is not 100%, the combination of an IgG avidity result with an IgM study could resolve conflicting cases. Thus there are IgM-negative primary infections with low-avidity IgG Abs and IgM-positive primary infections with IgG Abs of normal avidity, which would indicate a nonrecent infection. Using ELISA specific for viral capsid antigen or early antigen, others authors [28] have obtained similar results. Nevertheless, our system uses a pool of antigens, and it is simpler and automated. Taking into account the avidity behavior of IgG Abs in recent primary infections, the study of this parameter may be useful for ascertaining the etiology of an infection when there are various IgM Abs by cross-reactivity between antigens (between HSV and VZV, or between CMV and HHV-6), or because of heterotypical reactions between infections by CMV, EBV, or HHV-6.

The reliability of IgM Abs to EA is confusing. For some authors they are highly specific [29], different from the De Ory studies [30], which obtained an important cross-reaction with herpesvirus. We obtained a sensibility of 79.7% and a specificity of 84%, figures inferior to the obtained results with the avidity test, but similar to those obtained by Linde [31]. Perhaps the characteristic of the test (the antigen is EA) and fusion protein present (p138) lowered the specificity of the test.

Concerning the heterophil Abs, the results are similar to Gray's [11] in patients >7 years old (sensibility 87%; specificity 94.8%) with other tests for heterophils Abs.

Finally, there was good agreement between ELISA from Behring (method 2) and IFA from Gull. That is possibly because both use VCA and EBNA, but we cannot exclude a higher sensibility for method 2 that includes other different antigens. Previously, we (32) and other authors (33) realized a comparative study between the ELISA from Behring and other ELISA's tests for IgM Abs to VCA and obtained good agreement.

In conclusion, the IgG avidity test is simple and automated in the laboratory (differing with other commercial disposable tests). Techniques quantifying the avidity of specific IgG Abs together with traditional serological methods (tests for IgG and IgM) are very useful for ascertaining, from a single sample, the time since infection. It is criteria of recent primoinfection higher levels than 55% of IgG with low avidity for the antigen. The investigation of the Abs to antigen pools of virus had high reliability. The investigation of heterophil Abs by latex and the Abs IgM to EA were lacking in sensibility to use in the diagnosis of the primoinfection.

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