DIAGNOSTIC IMPORTANCE OF HETEROPHILE ANTIBODIES AND IMMUNOGLOBULINS IgA, IgE, IgM AND LOW-AVIDITY IgG AGAINST EPSTEIN-BARR VIRUS CAPSID ANTIGEN IN CHILDREN

M. VOTAVA[1], D. BARTOŠOVÁ[2], A. KRCHŇÁKOVÁ[1], K. CRHOVÁ[2], L. KUBINOVÁ[1]

[1]Department of Microbiology, Masaryk University School of Medicine and St. Anna Hospital, Pekařská 53, 656 91 Brno; [2]Department of Children's Infectious Diseases, J.G. Mendel Faculty Children's Hospital, Brno, Czech Republic

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Summary. – The use of several serological markers in diagnostics of acute Epstein-Barr virus (EBV) infection in children and adolescents is recommended. We investigated the sera of 299 individuals with clinically suspect infectious mononucleosis for heterophile antibodies and EBV viral capsid antigen (VCA)-specific immunoglobulins IgA, IgE, IgM and low-avidity IgG. Heterophile antibodies were positive in 26%, VCA-specific IgA in 30%, IgE in 35%, IgM in 32% and low-avidity IgG in 37% of cases. The acute EBV infection defined as a case having either positive IgM or heterophile antibodies was present in 40% of persons. Compared with regard to this criterion the sensitivity, specificity, and positive and negative predictive values of individual tests were as follows: heterophile antibodies – 66, 100, 100 and 82%; IgA – 53, 84, 69 and 73%; IgE – 54, 78, 62 and 72%; IgM – 81, 100, 100 and 89%; low-avidity IgG – 66, 82, 71 and 78%. All markers except heterophile antibodies were positive even in some infants aged below 2 years. We consider the detection of low-avidity IgG and VCA-specific IgE an useful adjunct for the diagnostics of acute EBV infection in children.

Key words: Epstein-Barr virus; avidity; immunoglobulins; children

Introduction

The diagnostics of EBV infection in children is not easy. In infancy the clinical symptomes as well as the results of laboratory tests are much less clear-cut so that the standard laboratory methods seem to be less sensitive. To enhance the reliability of a positive result in children the use of several diagnostic tests is needed (Sumaya and Ench, 1985). A standard procedure is represented by the detection of VCA-specific IgM and IgG, and of antibodies against EBV nuclear antigen (EBNA), the latter being usually absent in acute EBV infection. Earlier, the detection of VCA-specific IgA was recommended (Evans and Niederman, 1982). Recently, the avidity of VCA-specific IgG was employed for the diagnostics of acute primary EBV infection (Andersson *et al.*, 1994; de Ory *et al.*, 1993; Gray, 1995; Vetter *et al.*, 1994). The detection of specific IgE for the diagnostics of an acute infection was described in toxoplasmosis (Wong, 1993; Pinon, 1990), but never in viral infections. We have attempted to compare the usefulness of detection of heterophile antibodies and VCA-specific IgA, IgE, IgM and low-avidity IgG for the diagnostics of acute EBV infection in children and adolescents.

Abbreviations: EBV = Epstein-Barr virus; EBNA = EBV nuclear antigen; ELISA = enzyme-linked immunosorbent assay; Ig = immunoglobulins; IF = immunofluorescence; PBS = phosphate-buffered saline; VCA = viral capsid antigen

Materials and Methods

Sera were taken from 299 children and young persons of 1 to 18 years of age during hospitalization with an illness resembling infectious mononucleosis, two samples spaced at least 10 days apart. They were inactivated at 56°C for 30 mins and kept frozen at -20°C. Paired sera from one person were tested simultaneously. An acute infection case was taken for positive when either the test for IgM antibody or

both tests for heterophile antibodies were positive. There were altogether 119 (40%) such cases in the studied group. When the test for VCA-specific IgG was negative even with the reconvalescent sample, an acute EBV infection was excluded (considered negative).

Heterophile antibodies were assayed by micromodifications of standard ox cell haemolysis and horse erythrocytes agglutination tests (Votava, 1992). For the diagnosis of acute EBV infection the positivity of both tests was required.

VCA-specific IgA, IgE and IgM were determined by indirect immunofluorescence (IF) with 4 hrs-incubation of examined serum diluted 1:10 with phosphate-buffered saline (PBS) (Schmitz and Scherer, 1972) using appropriate rabbit fluorescein-isothiocyanate conjugates against different classes of human Ig (Sevac). Antigen preparations were made from P3HR-1 cells grown in RPMI-1640 medium with 20% bovine foetal serum.

Sensitivity, specificity, and positive and negative predictive values of tests for different types of antibodies were calculated in relation to the proved acute EBV infection according to Sackett (1992).

Determination of avidity. Sera were diluted 1:10 and tested by indirect IF on two parallel sets of acetone-fixed smears of P3HR-1 cells. After 30 mins at 37°C one slide was rinsed with PBS and incubated 5 mins in PBS containing 8 mol/l urea (de Ory *et al.*, 1993), while another slide was treated with PBS only. The slides were washed 3 times for 5 mins with PBS, rinsed with distilled water and dried. Then the rabbit conjugate against human IgG was added. A reduction of the fluorescence brightness in urea-treated specimen at least by two crosses in comparison with the control was considered the evidence of low-avidity IgG in the examined serum.

Results

Results of our study summarized in Table 1 show that an acute EBV infection was proved in 119 out of 299 (40%) cases, mostly by the positivity of VCA-specific IgM (96 cases, 32%). Never a positive result of any test was present in cases negative for VCA-specific IgG in the second sample.

Table 1. Serological markers of acute EBV infection

Age (years)	No. of cases	No. of cases of acute EBV infection		Positive tests				
		Proved	Excluded	HAb	IgA	IgE	IgM	LAG
1-5	70	19 (27%)	12 (17%)	7 (10%)	17 (24%)	19 (27%)	16 (23%)	15 (21%)
6-10	87	33 (38%)	9 (10%)	23 (26%)	23 (26%)	25 (29%)	27 (31%)	33 (38%)
11-15	103	47 (46%)	13 (13%)	34 (33%)	34 (33%)	39 (38%)	36 (35%)	42 (41%)
≥16	39	20 (51%)	1 (3%)	15 (38%)	17 (44%)	21 (54%)	17 (44%)	20 (51%)
Total	299	119 (40%)	35 (12%)	79 (26%)	91 (30%)	104 (35%)	96 (32%)	110 (37%)

HAb = heterophile antibodies; LAG = low-avidity IgG.

Most frequently found antibodies were low-avidity IgG (110 cases, 37%) and IgE (104 cases, 35%). On the other hand, heterophile antibodies were detected in 79 cases (26%) only.

The sensitivity, specificity, and positive and negative predictive values of tests for different types of antibodies were as follows: heterophile antibodies – 66, 100, 100 and 82%; IgA – 53, 84, 69 and 73%; IgE – 54, 78, 62 and 72%; IgM – 81, 100, 100 and 89%; low-avidity IgG – 66, 82, 71 and 78%.

The results showed some age differences. An acute EBV infection could be proved – on the basis of the presence of either IgM or heterophile antibodies – most easily (in 51% of cases) in the oldest age group, but with difficulties in infants (in 27% of cases only). On the other hand, on the basis of negative VCA-specific IgG in the second sample, an acute EBV infection could be excluded in 12 (17%) infants but only in 1 person (3%) in the oldest age group. The positive findings of remaining antibodies followed the same age pattern as IgM or heterophile antibodies, the latter being rarely found in infants (in 10% of cases only).

There were 18 infants aged 2 years or less in the first age group. In 4 (22%) of them an acute infection was proven according to positive IgM, while in 5 (28%) it was excluded. In this group, VCA-specific IgA were found in 4 cases, IgE in 6, low-avidity IgG in 4 and heterophile antibodies in none.

Discussion

Usefulness of standard serologic signs of acute EBV infection was repeatedly called in question. VCA-specific IgM are sometimes absent in acute infection, especially in children (Sumaya and Ench, 1985), or present in other situations (Schillinger *et al.*, 1993). EBNA antibodies appear sometimes too early for their absence during the onset to be a reliable sign of acute infection. Since EBNA-2 antibodies seem to be a culprit, the detection of EBNA-1 antibodies only by a special procedure has been recommended (Andersson *et al.*, 1994). Since we could not afford to maintain all necessary EBV-infected cell lines in our laboratory we had to concentrate on anti-VCA antibodies only.

Our serologic markers of acute EBV infection, i.e. positive IgM and heterophile antibodies, are little sensitive. If we take them as a standard for evaluation of another test, its sensitivity and specificity seems to be low. We believe that in children it is better first to compare just the positivity of different tests.

Low-avidity IgG were found most frequently, in 21% of children aged 1-5 years and in about a half of adolescents, on average in 37% of cases. DeOry *et al.* (1993) have found low-avidity IgG in 95% of selected 94 patients with serologically and clinically proven acute EBV infection. Nei-

ther Andersson *et al.* (1994) nor Gray (1995) have observed analogous values. Vetter *et al.* (1994) have reported low-avidity positive cases in 87 of 100 patients with clinically positive acute EBV infection and positive VCA-specific IgM. Low values observed by us can be explained by the fact that our patients were mostly children.

VCA-specific IgE were found on average in 35% of cases, in the youngest age group most often of all markers, namely in 19 cases of 58 (27%). So far we are not aware of any report on the diagnostic use of virus-specific IgE. In toxoplasmosis, both specific IgE and IgA have been recetly considered very promising markers of active infection because of their better correlation with the seroconversion in comparison with specific IgM. Both were never detected in serum specimens from otherwise seronegative individuals or from patients with chronic toxoplasmosis (Wong *et al.*, 1993; Pinon *et al.*, 1990). Also we have never detected them in serum specimens negative for VCA-IgG. As important we regard the detection of VCA-IgE in 6 of 18 infants aged less than 2 years.

VCA-IgM were detected in 32% of our patients. It only confirms the relatively low sensitivity of this standard marker of acute EBV infection in children (Sumaya and Ench, 1985).

Elevated VCA-IgA titers is an useful serologic marker of nasopharyngeal carcinoma (Henle and Henle, 1976). The use of this antibody as a marker of acute EBV infection was recommended by Evans and Niederman (1982) and confirmed in our Department as a useful adjunct of EBV serology in children (Chalupa *et al.*, 1992). We found it in 24% of children less than 5 years old.

Heterophile antibodies are very often absent in acute EBV infection in infants (Sumaya and Ench, 1985). In our study, they were present in 10% only in the youngest group and in none of 18 infants aged less than 2 years.

It is difficult to evaluate the specificity of acute EBV infection markers. Its low values in our study are proportional to the strictness of our definition of acute infection. The specificity of VCA-IgM was challenged e.g. by Schillinger (1993). The dynamics of appearance of different markers may influence their specificity in relation to the chosen standard. In adults, low-avidity IgG are usually present in the first week after the onset of infection and disappear at the end of the second month (Andersson *et al.*, 1993).

Different classes of VCA-specific antibodies as well as low-avidity IgG can be detected by one type of serological reaction, either indirect IF or enzyme-linked immunosorbent assay (ELISA, Gray, 1995), and in indirect IF using one cell culture type only, which is in favour of small laboratories.

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