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Original article

USE OF IMMUNOBLOT IgM IN PATIENTS WITH SEROLOGICAL AND CLINICAL EVIDENCE OF PRIMARY EBV INFECTION AND REACTIVATION

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ABSTRACT

Purpose: Anti-VCA IgM is a marker for establishing primary infection with Epstein-Barr Virus (EBV), it usually appears in combination with anti-VCA IgG. It has been shown that there is a risk of non-specific IgM reactivity due to cross-reactions, interference with rheumatoid factor or autoantibodies. These antibodies may also occur during reactivation. In these cases, Immunoblot based tests may be useful to confirm the ELISA result. We compared the results of anti-VCA IgM in ELISA and Immunoblot IgM in patients with evidence of primary EBV infection (infectious mononucleosis, IM) and/or reactivation/reinfection.

Materials/Methods: We examined 32 serum samples with commercial immunoblot (Euroline Anti-EBV Profile 2 (IgM), Euroimmun, Germany). Samples were tested primarily for anti-VCA IgM/IgG in ELISA. Patients with IM were 11, and those with probable reactivation/reinfection - 21.

Results: We found positive results at 31.3% (95% CI: 16.1% -50.0 %, n = 10) of all subjects. Patients with IM and isolated anti-VCA IgM in ELISA (81.8%) were negative in Immunoblot IgM. Positive in Immunoblot IgM was 38.1% (n = 8) of the patients with suspected reactivation. We confirmed a primary infection in three of them due to the low avidity of anti-VCA IgG and missing anti-EBNA1 IgG. In five of the patients, the presence of anti-VCA IgM may be interpreted as reactivation/reinfection.

Conclusion: Patients with IM and isolated anti-VCA IgM models in ELISA were not confirmed in the Immunoblot test. Approximately 43% of patients of possible reactivation was also negative in the test.

Keywords: Epstein-Barr virus, anti-VCA IgM, Immunoblot IgM, Infectious mononucleosis, EBV reactivation,

INTRODUCTION

EBV is a wide-spread and ubiquitous gamma-herpesvirus [1]. In Bulgaria, the average seropositivity is 83%, and it is age-dependent. After 26 years more than 90% of the population is infected with the virus. In the same study, we found a bimodal model of primary infection with infection most often between 1-5 years and 16-20 years [2]. ELISA is the preferred method of EBV diagnosis. Anti-VCA IgM is a marker for establishing primary infection with Epstein-Barr virus (EBV), and it usually appears in combination with anti-VCA IgG. In cases of primary infection, the absence of anti-EBNA1 IgG is also detected [3–5]. In the serological diagnosis of EBV, different serological profiles exist due to the variability in antibody formation against the viral antigens, and this creates difficulty in interpreting the stage of infection. A problem in the diagnosis of primary EBV infection is isolated anti-VCA IgM. This is considered a very early infection, but it should be confirmed by other tests to reject non-specific reactivity due to cross-reactions, interference with rheumatoid factor or autoantibodies [3,5]. Another problem in the diagnosis of EBV remains the detection of viral reactivation. According to some authors, one of the markers may be re-emerging anti-VCA IgM [6].

Immunoblot tests are considered highly informative because of the possibility of proving antibodies simultaneously against several antigens, with good sensitivity and with the highest complexity of the information received. They are relatively easy to perform and are capable of recording on an apparatus, which reduces the risk of subjective assessment [7].

We applied the Immunoblot IgM analysis to two groups of patients primarily tested in ELISA. In the first group, we aimed to confirm a primary infection for which clinical data and predominantly isolated anti-VCA IgM

models in ELISA were available. In the second group, we tried to confirm cases of reactivation in patients with diagnoses other than IM and at age, where fewer primary infections were expected based on the previous seroepidemiological analysis.

MATERIALS AND METHODS

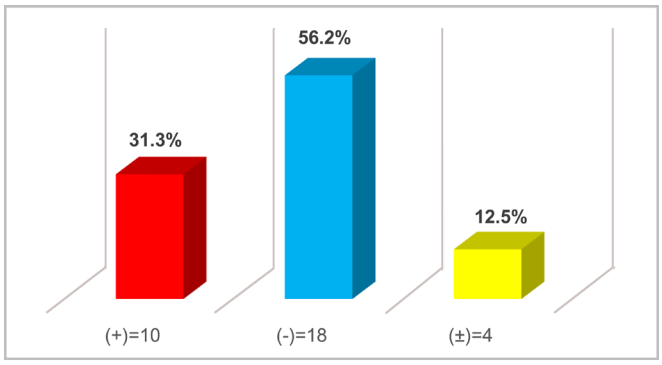
We examined 32 serum samples (all positive in the ELISA anti-VCA IgM assay) in commercial immunoblot IgM (Euroline Anti-EBV Profile 2 (IgM), Euroimmun, Germany). This is a quality assay and serves for detecting IgM class antibodies to 5 different EBV antigens - VCA gp125, VCA p19, EBNA-1, p22 and EA -D in serum or plasma. Antigens are recombinant or native (VCA gp125), highly purified to differentiate acute and past infection with the virus in one sample. We followed the standard test procedure of the manufacturer. The EUROLineScan software was used with EBV2.1 EL IgM. Positive samples have at least one VCA band with moderate or intense color intensity. Negative are samples where both VCA bands are negative. Samples with low intensity of coloration were interpreted as undefined. SPSS v.23 statistical software was used for data analyzes. Quantitative variables were reported as mean, and standard deviation (mean±SD) and the qualitative variables were reported as a number and a proportion (%). We used Fisher's test to evaluate dependencies.

RESULTS

Patients with first-line clinical data we considered to be the first group – they represented 34.4% (n = 11) and had a mean age of 2.6 years (SD ± 2.0). Male predominated - 81.8% (n = 9). The serological profile of the group was as follows: 9/11 (81.8%) were anti-VCA IgM positive/anti-VCA IgG negative and 2/11 (18.2%) – anti-VCA IgM positive/anti-VCA IgG positive. Patients with putative reactivation formed the second group. They represented 65.6% (n = 21), with an average age of 35.6 years (SD ± 22.2), again with male prevalence – 57.1% (n = 12).

We found positive results at 31.3% (95% CI: 16.1% -50.0%, n=10) of all subjects. Patients with negative results had a higher proportion - 56.2% (95% CI: 37.7% - 73.6%, n=18) (Figure 1).

Fig. 1. Immunoblot IgM assay results



The mean age of patients with a positive Immunoblot was 28.6 (SD ± 24.3). All were anti-VCA IgM positive/anti-VCA IgG positive in ELISA. Depending on the clinical diagnosis, the distribution was as follows: 2 - with IM and 8 with other diagnoses. Half of these patients were over 28 years of age. The proportion of positive women - 36.4% (95% CI: 10.9% - 69.2%, n = 4) was higher than that of men - 28.6% (95% CI: 11.3% - 52.2%, n = 6). In 6 of the patients, a band was found only in the VCA p19 and one in the VCA gp125 region. In three patients a combination of antibodies against the two antigens was visualized.

In the group of patients with a negative test score, the mean age was lower - 17.3 (SD ± 22.4). Half of the cases were with clinical diagnosis IM, and all had a serological profile of anti-VCA IgM positive/anti-VCA IgG negative in ELISA.

We found higher positivity in patients with a possible reactivation. Only in this group, we received indeterminate results. The distribution of positive and negative patients in Immunoblot analysis by target groups is presented in Table 1. We did not detect a statistically significant difference with the Fisher's test when comparing the two groups (p> 0.05).

Table 1. Number and proportion of the tested patients with Immunoblot IgM

Results	NI	Proportion (%, 95%CI)	NII	Proportion (%, 95%CI)
Positive	2	18.2% (2.3%-51.8%)	8	38.1% (18.1%-61.6%)
Negative	9	81.8% (48.2%-97.7%)	9	42.8% (21.8%-66.0%)
undefined	0	0	4	19.0% (5.4%-41.9%)
All	11	100%	21	100%

NI- patients with acute EBVinfection; NII-patients with reactivation probably;

DISCUSSION

Only 14 patients diagnosed with infectious mononucleosis in the first 6 months of 2017 showed positive anti-VCA IgM without anti-VCA IgG in ELISA. Nine of them were included in the study. In this group, we included two patients also positive for anti-VCA IgG. As a serological indicator of primary infection in combination with antibodies against VCA, many authors recommend using tests to prove heterophile antibodies or anti-EA (D) [3,5]. We found an isolated IgM immune response predominantly in patients under 5 years of age, which questions the use of tests to prove heterophile antibodies given their undetect-

ability in this age group. Therefore, we studied patients with Immunoblot IgM.

We obtained predominantly negative results - 81.8%. These were all patients with isolated ELISA anti-VCA IgM responses. In these cases, the possibility of taking a second sample should be considered, and due to the risk of false reactivity other agents such as human cytomegalic virus due to similar transmission mechanisms should be sought. Primary EBV infection in Immunoblot IgM assay was confirmed in 18.2% of those who tested positive for both classes of antibody against VCA in ELISA. Therefore, for the more accurate serological diagnosis of infectious mononucleosis in ELISA, it is important to detect both classes of antibodies against VCA.

Following infection with EBV, the virus persists in B cells [1]. The infection may be periodically reactivated in both immunocompromised and immunocompetent subjects. There is a problem with reactivation in immunocompromised patients as it leads to serious complications [4,8]. For the detection of EBV reactivation, the best method is PCR [9], but the first step is to use serological methods as cheaper and more affordable. There is still no consensus on the issue of the best serological marker. Various authors report the appearance of anti-VCA IgM or anti-EA (D) [4,6]. We selected 21 serum samples with anti-VCA IgM in ELISA and a non-IM diagnosis, mostly in adults where the primary infection is not a common event. In this group, 8 out of 21 patients (38.1%) were positive in the Immunoblot analysis, 5 of whom were over 26 years of age. Analyzing the results and the rest of the applied tests in 5 of the cases we suspect reactivation/reinfection due to the presence of anti-EBNA1 IgG and high IgG avidity (results not shown). We do not exclude the possibility of primary infection with prolonged IgM persistence. On the other hand, false positivity was found in 75% of the cases in the IgM Immunoblot test in comparison to immunofluorescence assay (IFA). This is particularly true in the case of a positive band only in the area of VCA p19 [10]. The same authors show that with the increase in band intensities (1+ to 3+), the frequency of IFA reactivity to demonstrate anti-VCA IgM increased from 9.9% to 29.5% for the p19 positive group and 24% to 85.7 % for the gp125 positive group. In our study done with the same tests, we have established a predominant positivity in the VCA p19. We believe that, given the subjectivity of IFA reporting, a better comparison can be made with PCR.

The remaining three (19, 22 and 28 years old) patients were considered as a primary infection, combining the blot results with the anti-VCA IgG avidity and anti-EBNA 1 IgG.

In our study, the Immunoblot IgM analysis excludes reactivation of the infection in a significant percentage of the investigated - 42.8% (95% CI: 21.8% - 66.0%) and rather directed to non-specific reactivity. In a similar study, 43 patients positive for both types of antibodies against VCA and anti-EBNA1 IgG found that 21 of them had a previous infection. Using a blot test in 18 of these patients, IgM positivity was confirmed in 10, while in other cases they considered false IgM positivity in the ELISA. In three, they detected antibodies to the human cytomegalic virus, which explains anti-VCA IgM reactivity [11]. Blot tests can, therefore, be useful for refining false positive anti-VCA IgM results in ELISA.

CONCLUSION

Isolated anti-VCA IgM serological models in subjects with IM have not been confirmed in Immunoblot IgM, and they remain a serious diagnostic challenge. Studies of such PCR groups could provide information on the presence or absence of primary EBV infection. Although IM is a self-limiting disease, it should not be underestimated because of a higher risk of developing Hodgkin's lymphoma. Blot-based tests may be useful for detecting false reactivity and show better results in the case of detection of reactivation with the presence of anti-VCA IgM in ELISA. Interpretation of results based on serological research requires good qualifications and knowledge of all options, both with regard to the time of provision of the test sample and of the diagnostic tests.

REFERENCES:

1. Cohen JI. Epstein-Barr virus infection. *N Engl J Med.* 2000 Aug 17; 343(7):481–92. [PubMed] [Crossref]

2. Kostadinova T, Ivanova L, Raykov T, Stojkova Z, Tsankova G. Seroprevalence of Epstein-Barr Virus in North-Eastern Bulgaria. *ACTA Microbiol Bulg.* 2016 Sep;32(3):33-8.

3. De Paschale M, Clerici P. Serological diagnosis of Epstein-Barr virus infection: Problems and solutions. *World J Virol.* 2012 Feb 12;1(1):31-43. [PubMed] [Crossref]

4. Gärtner BC, Hess RD, Bandt D, Kruse A, Rethwilm A, Roemer K, et al. Evaluation of four commercially available Epstein-Barr virus enzyme immunoassays with an immunofluorescence assay as the reference method. *Clin Diagn Lab Immunol.* 2003 Jan;10(1): 78–82. [PubMed] [Crossref]

5. Hess RD. Routine Epstein-Barr virus diagnostics from the laboratory perspective: Still challenging after 35 years. *J Clin Microbiol.* 2004 Aug; 42(8):3381-7. [PubMed] [Crossref]

6. Obel N, Høier-Madsen M, Kangro H. Serological and clinical findings in patients with serological evidence of reactivated Epstein-Barr virus infection. *APMIS.* 1996 Jun; 104(6):424-8. [PubMed]

7. Bauer G. Simplicity through complexity: immunoblot with recombinant antigens as the new gold standard in Epstein-Barr virus serology. *Clin Lab.* 2001;47(5-6):223-30. [PubMed]

8. Adler B, Schaadt E, Kempkes B, Zimber-Strobl U, Baier B, Bornkamm GW. Control of Epstein-Barr virus reactivation by activated CD40 and viral latent membrane protein 1. *Proc Natl Acad Sci U S A.* 2002 Jan 8;99(1): 437-42. [PubMed] [Crossref]

9. Maurmann S, Fricke L, Wagner H-J, Schlenke P, Hennig H, Steinhoff J, et al. Molecular parameters for precise diagnosis of asymptomatic Epstein-Barr virus reactivation in healthy carriers. *J Clin Microbiol.* 2003 Dec;41(12):5419-28. [PubMed]

10. Altuplu I, Aksoy A, Zeytinoplu A, Orman M. [Evaluation of immunoblot-based assay for detecting Epstein-Barr virus viral capsid antibodies]. [in Turkish] *Mikrobiyol Bul.* 2010 Apr;44(2):231-6. [PubMed]

11. Nystad TW, Myrmel H. Prevalence of primary versus reactivated Epstein-Barr virus infection in patients with VCA IgG-, VCA IgM- and EBNA-1-antibodies and suspected infectious mononucleosis. *J Clin Virol.* 2007 Apr; 38(4):292-7. [PubMed] [Crossref]

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