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Original article

THE ROLE OF anti-EBNA1 IgG DETERMINATION IN EBV DIAGNOSTICS

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ABSTRACT

Purpose: In Bulgaria, the diagnosis of Epstein-Barr virus (EBV) infection is performed via ELISA testing of IgM and IgG against viral capsid antigen (anti-VCA IgM and anti-VCA IgG). With the current study, we try to answer is there any benefit of determination of IgG against the nuclear antigen of EBV (anti-EBNA-1 IgG) in the laboratory practice.

Material/Methods: The prospective study included 82 serum/plasma samples tested for anti-VCA IgM, anti-VCA IgG, anti-EBNA1 IgG and anti-VCA IgG avidity in ELISA (Euroimmun, Luebeck, Germany). Quantitative variables were reported as mean, and standard deviation (mean ±SD) and the qualitative variables were reported as a number and a relative proportion (%).

Results: Anti-EBNA1 IgG positive patients were 74.4% (95% CI:63.6% - 83.4%) of all tested individuals. Their mean age was significantly higher (30.5;SD±20.5)of this of patients without anti-EBNA1 IgG (14.5; SD±14.1) ($p < 0.05$).The first group of patients (with infectious mononucleosis, anti-VCA IgM negative) had the highest number of anti-EBNA1 IgG negative results. Negative for anti-EBNA 1 IgG were 12% of patients with Hodgkin's lymphoma.

Conclusion: Determination of anti-EBNA1 IgG together with anti-VCA should be considered in the initial serological testing in EBV diagnostics. As different immune responses against the EBNA1 antigen exist, clinicians should interpret the results carefully with regard to the clinical symptoms, the immune status and the laboratory markers. We found anti-EBNA1 IgG ELISA tests exceptionally useful to distinguish primary and past infections in anti-VCA IgM(+)/anti-VCA IgG (+) patients.

Keywords: Epstein-Barr virus, anti-EBNA1 IgG, anti-VCA IgG avidity, Infectious mononucleosis, Hodgkin's lymphoma

INTRODUCTION

Testing of IgM and IgG against viral capsid antigen (anti-VCA IgM and anti-VCA IgG) and IgG against EBV nuclear antigen (anti-EBNA-1 IgG) is a routine serology triad for stage determination of EBV infection [1]. The EBV expresses EBNA proteins during the different latency programs and EBNA1 is detected in all EBV-related tumours, and EBV-infected proliferated cells in healthy carriers [2–5]. Antibodies against the antigen appear weeks or months after the beginning of the infection and indicate past infections [1]. They can appear significantly later (up to 8 months) in children [6], can appear very early in primary infections [6,7], cannot appear at all [8] or can be lost in immunosuppressed patients [1,9]. Profiles with positive VCA antibodies and negative anti-EBNA1 IgG are typical for acute infections. Normally, past infections show positive anti-EBNA1 IgG and anti-VCA IgG[1,10]. Anti-EBNA1 IgG antibodies can be a useful marker in long-term persistence of anti-VCA IgM and in patients with reactivation/reinfection with the reappearance of anti-VCA IgM[1].

Avidity determination of anti-VCA IgG is additionally required in cases with longer persistence of anti-VCA IgM, in reactivation/reinfection, in IgM negative primary infections, in past infection without anti-EBNA1 IgG. The IgG avidity (maturation) occurs several weeks after the infection and is a result of somatic hypermutation in IgG DNA-coding region, which generates antibodies with higher affinity and stronger binding properties [1,7,11].

In Bulgaria the EBV routine diagnostic is based on the determination of antibodies against VCA only. However, our clinical practice shows a significant number of challenging cases, which requires additional tests for proper interpretation. Therefore, we try with the current study to answer is there any benefit of using anti-EBNA1 IgG tests in the laboratory practice. We also compared the efficacy of anti-EBNA1 IgG tests with this of the anti-VCA IgG avidity tests.

MATERIALS AND METHODS

Eighty-two serum (plasma) samples collected in UMBAL "St. Marina", Varna and UMBAL "G. Stranski", Pleven were tested for anti-EBNA1 IgG. All of the patients were clinically diagnosed with infectious mononucleosis (IM) or other EBV-related diseases. The samples were also tested for anti-VCA IgM and anti-VCA IgG. From the 82 samples tested, 46 were chosen for avidity determination – 33 with negative anti-VCA IgM and IM diagnosis and 13 with positive anti-VCA IgM and other diagnoses.

Indirect ELISA (Euroimmun, Luebeck, Germany) was used according to the manufacturer's instructions for all serological tests – anti-VCA IgÌ/anti-VCA IgG, anti-EBNA1 IgG and anti-VCA IgG avidity. IgG values were quantitatively determined via calibration curves, while IgM was determined with the semi-quantitative method. The avidity results were calculated as Relative avidity index (RAI%) – a proportion of OD of a urea-treated sample and OD of untreated sample. SPSS v.23 statistical software was used for data analyses. Quantitative variables were reported as mean, and standard deviation (mean±SD) and the qualitative variables were reported as a number and a relative proportion (%). P<0.05 was considered statistically significant.

RESULTS

The mean age of all patients tested was 26.5(SD±20.3) (range 8 months to 69 years) and 61% of them were males. The serological profiles determined with the classical routine serology were distributed as follows: 57 (69.5%,95%CI:58.4%-79.2%)were anti-VCA IgM(-)/anti-VCA IgG (+)and25(30.5%,95%CI: 20.8%-41.6%)were anti-VCA IgM(+)/anti-VCA IgG (+).

We found positive anti-EBNA1 IgG in 74.4% (95% CI:63.6% - 83.4%, n=61) of all tested samples. The mean age of patients with positive antibodies was significantly higher (30.5;SD±20.5)when compared to this of patients without anti-EBNA1 IgG (14.5; SD±14.1) ($p < 0.05$).Cases with detectable anti-EBNA1 IgG dominated in all serological profiles (Figure 1).

Furthermore, we divided our patients into three groups based on their clinical diagnosis and on the preliminary serology (Table 1).The first group of patients showed the lowest mean age (9.9; SD±7.0)and had the highest number of anti-EBNA1 IgG negative results (Table 1). The mean age in the second group was 41.2(SD±14), and the predominant serological profile was anti-VCA IgM (-)/anti-VCA IgG (+). Negative for anti-EBNA 1 IgG was 12% of all patients at different ages (19, 34 and 51 years). In this group, one patient showed positive results for both anti-VCA IgM/anti-VCA IgG and ànti-EBNA 1 IgG. In the third group anti-EBNA 1 IgG positivity was found in 70.8% of the studied samples (Table 1). The negative patients were of significantly lower mean age (16.5; SD±17.2), compared to the positive patients (40.1; SD±22.1).

All patients in group I was also tested for anti-VCA IgG avidity (Table 2). Excluding the indeterminate results, one patient with high avidity is negative for ànti-EBNA 1 IgG.

We tested the avidity of 13 patients from the third group (positive for anti-VCA IgM and IgG). The results of both tests were in total accordance (Table 3).

Fig. 1. Distribution of samples according to anti-EBNA1 IgG and anti-VCA IgM serology (all patients were anti-VCA IgG positive).

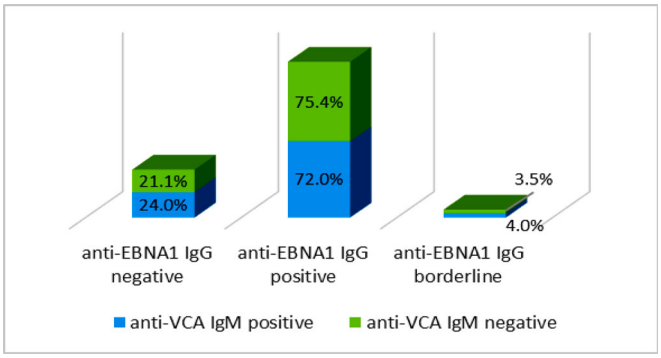

Table 1. Number and proportion of the tested patients with different anti-EBNA1 IgG serological profiles.

Group	N (%)	anti-EBNA1 IgG (+) N (%; 95% CI)	anti-EBNA1 IgG (-) N (%; 95% CI)	anti-EBNA1 IgG -(+/-) N (%; 95% CI)
I. Primary infection (IM) anti-VCA IgM(-)/anti-VCA IgG (+)	33 (40.2%)	23 (69.7%; 95% CI: 51.3%-84.4%)	9 (27.3%; 95% CI: 13.3%-45.5%)	1 (3.0%; 95% CI: 0.1%-15.8%)
II. Hodgkin's lymphoma (HL) anti-VCA IgM(±)/anti-VCA IgG (+)	25 (30.5%)	21 (84.0%; 95% CI: 63.9%-95.5%)	3 (12.0%; 95% CI: (2.5%-31.2%)	1 (4.0%; 95% CI: 0.1%-20.4%)
III. Other diagnoses anti-VCA IgM(+)/anti-VCA IgG (+)	24 (29.3%)	17 (70.8%; 95% CI: 48.9%-87.4%)	6 (25.0%; 95% CI: 9.8%-46.7%)	1 (4.2%; 95% CI: 0.1%-21.1%)
Total	82	61	18	3

Table 2. Comparison of anti-EBNA 1 IgG and anti-VCA IgG avidity tests in patients with infectious mononucleosis and anti-VCA IgM (-)/anti-VCA IgG (+).

Anti-VCA IgG avidity	Anti-EBNA 1 IgG (+)	Anti-EBNA 1 IgG (-)	Anti-EBNA 1 IgG (+/-)
Low	0	7	1
High	23	1	0
Indeterminate	0	1	0

Table 3. Comparison of anti-EBNA 1 IgG and anti-VCA IgG avidity tests in patients with other diagnoses and anti-VCA IgM(+)/anti-VCA IgG (+)

Anti-VCA IgG avidity	Anti-EBNA 1 IgG (+)	Anti-EBNA 1 IgG (-)
Low	0	4
High	9	0

DISCUSSION

We found anti-EBNA1 IgG in 74.4% of tested patients, regardless of the antibodies against VCA. The anti-EBNA1 antibodies dominated in the older age groups. In a previous study anti-EBNA1, IgG was detected in 69.4% among 555 healthy and ill individuals [12].

The biggest proportion of positive samples was among patients with HL diagnosis (84.0%), and we clearly classified them as past infections, as anti-VCA IgM were negative and anti-VCA IgG were positive. Nearly all of the researchers consider serological diagnostic not enough informative in immunocompromised patients or in patients with EBV-related malignant diseases. In such patients, low titers of anti-EBNA-1 IgG are registered [1,9]. In 12% of the samples in this group (II) we did not detect anti-EBNA-1 IgG (all of the samples were positive only for anti-VCA IgG). The reason can be related to their loss during the treatment or with no formation of anti-EBNA-1 IgG in, some infected individuals. In addition, in 5% of immunocompetent people,anti-EBNA-1 IgG do not appear [8]. *De Paschaleet al*. reported 9% of undetectable anti-EBNA 1 IgG samples among adults – such isolated positive anti-VCA IgG without anti-EBNA-1 IgG suggest past infections [11]. IgG avidity tests are more helpful in these difficult cases. The higher proportion of individuals without anti-EBNA1 IgG in our study could be a result of the small sample size or of the cytostatic and corticosteroid therapy of these patients.

On the other hand, anti-EBNA-1 IgG is discussed in correlation with anti-EBNA2. Anti-EBNA-2 IgG appears early and can be detected in 30% of the primary infections. Then, their number decreases to undetectable levels. As ànti-EBNA1appear later, several months after the primary infection the proportion anti-EBNA1/anti-EBNA 2 is normally < 1.0 for 6-12 months [1,13]. Then, in healthy carriers, the proportion becomes higher than 1 but decreases in immunocompromised or immunosuppressed individuals because of the poor immune control [14]. Patients with HL have anti-EBNA1/anti-EBNA2 < 1 [13,15]. As anti-EBNA1/anti-EBNA2 <1.0 is significantly more frequent in HL patients this can be used as a predictive risk factor for HL in EBV positive samples [14,15]. In the group of HL patients, one serum showed anti-VCA IgM (+)/anti-VCA IgG (+)/anti-EBNA-1 IgG+ profile. In this case, additional testing is required for the correct interpretation of the result.

We also found a high anti-EBNA1 IgG positivity (70.8%) in the group of anti-VCA IgM (+)/anti-VCA IgG (+) patients. These cases are past infections, and the anti-VCA IgM (+) could be due to reactivation, reinfection or false positivity. It is also possible that anti-VCA IgM persists for a long time if the primary infection is in older age. Anti-EBNA1 IgG can appear early, as shown in cases of low avidity and detectable EBV DNA [6]. In another study, 42% of all investigated samples were of patients with primary infection and positive anti-EBNA1IgG [7]. In the same study, an EBNA1 seroconversion was registered 18 days after the appearance of the first symptoms. On the other hand, the increased sensitivity of the tests with the use of recombinant, synthetic peptides leads to earlier antibody detection during the primary infection [16]. EBNA1 presence can also be a result of a non-specific reactivity, as proven in the immunoblot of 12 out of 18 patients with primary infection [7]. In our study, primary infection is suggested (because of the avidity results) for 4 of 6 anti-EBNA1 IgG (-) patients.

The smallest proportion of anti-EBNA1 IgG positive individuals (69.7%) was found among patients with IM and negative anti-VCA IgM. The same patients were of high avidity, which suggested a past EBV infection.

The lack of EBNA1 IgG and the presence of anti-VCA IgG can lead to misdiagnosis in children or immunocompromised patients. In such cases, avidity tests are more helpful. In our study, patients with anti-VCA IgM (-)/anti-VCA IgG (+)/anti-EBNA1 IgG (-) profile were of low avidity. Only one patient was with high avidity and without anti-EBNA1 IgG. This is in contrast to previous studies, where a significantly higher proportion of individuals without anti-EBNA1 IgG and high avidity was found (44.4%) [6]. Therefore, patients positive only for anti-VCA IgG and negative for anti-EBNA1 IgG should be also tested for IgG avidity.

As different serological profiles exist during the EBV infection, to include additional testing of anti-EBNA1 IgG can assist the correct stage determination. Corrales I. et al.

(2014), based on a chemiluminescent immune assay (CMIAs), offer a panel of antibodies (anti-VCA IgM / IgG and anti-EBNA1 IgG) to facilitate laboratory work [17].

The presented results are in agreement with the existing data where anti-VCA IgG are widely considered as a better marker for seroprevalence definition, because of the possible loss or no formation of anti-EBNA1. But anti-EBNA1 IgG can be a useful supplementary marker for stage determination of the EBV infection.

CONCLUSION

Determination of anti-EBNA1 IgG together with anti-VCA should be considered in the initial serological testing in EBV diagnostics. As different immune responses against the EBNA1 antigen exist, clinicians should interpret the results carefully with regard to the clinical symptoms, the age, the immune status and the laboratory markers. We found anti-EBNA1 IgG ELISA tests exceptionally useful to distinguish primary and past infections in anti-VCA IgM(+)/anti-VCA IgG (+) patients.

REFERENCES:

1. 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]

2. Babcock GJ, Decker LL, Freeman RB, Thorley-Lawson DA. Epstein-barr virus-infected resting memory B cells, not proliferating lymphoblasts, accumulate in the peripheral blood of immunosuppressed patients. J Exp Med. 1999 Aug 16;190(4):567-76. [PubMed]

3. Hochberg D, Middeldorp JM, Catalina M, Sullivan JL, Luzuriaga K, Thorley-Lawson DA. Demonstration of the Burkitt's lymphoma Epstein-Barr virus phenotype in dividing latently infected memory cells in vivo. Proc Natl Acad Sci. 2004 Jan 6;101(1):239-44. [PubMed] [Crossref]

4. Kennedy G, Komano J, Sugden B. Epstein-Barr virus provides a survival factor to Burkitt's lymphomas. Proc Natl Acad Sci. 2003 Nov 25; 100(24):14269-74. [PubMed] [Crossref]

5. Kieff E, Rickinson AB. Epstein –Barr virus and its replicationNo Title. In: Fields Virology. 2007. p. Fields Virology.

6. Chan KH, Ng MH, Seto WH, Peiris JS. Epstein-Barr virus (EBV) DNA in sera of patients with primary EBV infection. J Clin Microbiol. 2001 Nov 1;39(11):4152-4. [PubMed] [Crossref]

7. 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]

8. 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]

9. Gartner BC, Kortmann K, Schafer M, Mueller-Lantzsch N, Sester U, Kaul H, et al. No correlation in Epstein-Barr virus reactivation between serological parameters and viral load. J Clin Microbiol. 2000 Jun;38(6):2458. [PubMed]

10. 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]

11. De Paschale M, Agrappi C, Manco MT, Mirri P, Vigano EF, Clerici P. Seroepidemiology of EBV and interpretation of the "isolated VCA IgG" pattern. J Med Virol. 2009 Feb; 81(2): 325-31. [PubMed] [Crossref]

12. Teoharov PB. [Study on etiology of viral hepatitis, epidemiology and specific prophylaxis of major hepatotropic viruses and the prevalence of other potentially hepatotropic viruses in Bulgaria.] [dissertation] National Center of Infectious and Parasitic Diseases, Sofia; 2013. 281 p. [in Bulgarian]

13. Henle W, Henle G, Andersson J, Ernberg I, Klein G, Horwitz CA, et al. Antibody responses to Epstein-Barr virus-determined nuclear antigen (EBNA)-1 and EBNA-2 in acute and chronic Epstein-Barr virus infection. Proc Natl Acad Sci U S A. 1987 Jan; 84(2):570-4. [PubMed]

14. Mueller NE, Lennette ET, Dupnik K, Birmann BM. Antibody titers against EBNA1 and EBNA2 in relation to Hodgkin lymphoma and history of infectious mononucleosis. Int J Cancer. 2012 Jun 15;130(12): 2886-91. [PubMed] [Crossref]

15. Levin LI, Chang ET, Ambinder RF, Lennette ET, Rubertone MV, Mann RB, et al. Atypical prediagnosis Epstein-Barr virus serology restricted to EBV-positive Hodgkin lymphoma. Blood. 2012 Nov 1;120(18):3750-5. [PubMed] [Crossref]

16. Gartner 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]

17. Corrales I, Gimenez E, Navarro D. Evaluation of the Architect Epstein-Barr Virus (EBV) viral capsid antigen (VCA) IgG, VCA IgM, and EBV nuclear antigen 1 IgG chemiluminescent immunoassays for detection of EBV antibodies and categorization of EBV infection status using immunofluorescence assays as the reference method. Clin Vaccine Immunol. 2014 May; 21(5):684-8. [PubMed] [Crossref]

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