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Relevance of Avidity Testing of VCA IgG in EBV Diagnostics

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Abstract

Diagnostics of *Epstein-Barr virus (EBV)* primary infection is usually based on the results of ELISA determination of IgM and IgG against viral capsid antigen (anti-VCA IgM and anti-VCA IgG) and of IgG against *EBV nuclear antigen* (anti-EBNA-1 IgG). In cases of difficult interpretation, the use of additional tests, such as measurement of IgG avidity, may help to determine the infection stage. IgG maturation occurs several weeks after primary infection, and the presence of high-grade antibodies is a marker for a previous infection. In this study, we determined the significance of the avidity of anti-VCA IgG avidity test for patients with infectious mononucleosis or suspected *EBV* reactivation. Serological ELISA was used to determine the avidity of IgG against viral capsid antigen (anti-VCA IgG) in 46 single serum samples. Low anti-VCA IgG avidity was found in 26% (95% CI: 14% - 41%) of all tested samples, which could be interpreted as acute infection. Our results confirmed the presence of isolated IgG models in 24% (95% CI: 11%-42%) of primary *EBV* infections, while possible reactivation or non-specific reactivity of anti-VCA IgM was suggested for 69% (95% CI: 39%-91%) of the positive anti-VCA IgM/IgG patients.

Our results show that the laboratory confirmation of patients with clinical evidence of infectious mononucleosis and absence of IgM should include the use of avidity tests. We believe that avidity tests may be useful for the discrimination between reactivation and primary infections.

Keywords: *Epstein-Barr virus*, anti-VCA IgG avidity, Infectious mononucleosis, *EBV* reactivation.

Резюме

Диагностиката на първична инфекция с *Epstein-Barr* вируса (*EBV)* обикновено се основава на определянето на IgM и IgG антитела срещу вирусния капсиден антиген (anti-VCA IgM и anti-VCA IgG) и IgG антитела срещу ядрения антиген (anti -EBNA- 1 IgG). Съществуват серологични профили, в които използвайки само тези маркери не е достатъчно да се определи стадия на инфекцията. Използването на допълнителен тест за определяне на IgG авидността, може да помогне в интерпретацията на резултатите. Узряването на IgG става няколко седмици след първичната инфекция и наличие на антитела с висока зрялост е маркер за минала инфекция. В това изследване определихме значимостта на теста за авидността на anti-VCA IgG при пациенти с инфекциозна мононуклеоза и предполагаема реактивация на *EBV*. Включихме 46 серумни проби първично тествани за anti-VCA IgM/anti-VCA IgG, разделени в две групи в зависимост от диагнозата и серологичните профили. Използвахме ELISA за определяне на авидността на anti-VCA IgG. Установихме 26% (95% CI: 14% - 41%) проби с ниска anti-VCA IgG авидност, определящи ги като остра инфекция. Наличието на изолирани IgG модели има в 24% (95% CI: 11% -42%) от случаите с първична *EBV* инфекция, докато реактивация или неспецифична реактивност на anti -VCA IgM има в 69% (95% CI: 39% -91%).

Нашите резултати показват, че лабораторното потвърждение на пациентите с клинични данни за инфекциозна мононуклеоза и липсата на IgM трябва да включва използването на тестове за авидност. Тестовете могат да бъдат полезни за диференциране на първични инфекции и реактивация.

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Introduction

The *Epstein-Barr virus (EBV)* is usually diagnosed on the basis of the results of ELISA determination of IgM and IgG against viral capsid antigen (anti-VCA IgM and anti-VCA IgG) and of IgG against *EBV nuclear antigen* (anti-EBNA-1 IgG). The correct interpretation of the serological pattern is difficult as the immune response to viral antigens is highly specific in each patient. Normally, in primary infection anti-EBNA-1 IgG are not present, and the typical anti-VCA IgM may not appear either. In addition, anti-VCA IgM may persist for longer periods or may appear during reactivation of *EBV*. In these cases, positivity of anti-EBNA-1 IgG is frequent, but in a small proportion of patients, IgG antibodies are not formed or may be lost if immunosuppression is present (Nystad and Myrmel, 2007; De Paschale and Clerici, 2012). IgG- and IgM-antibodies to viral capsid antigen (VCAIn cases of difficult interpretation, the use of additional tests, such as measurement of IgG avidity may help to determine the infection stage (Gray, 1995). IgG maturation occurs several weeks after primary infection. It is a result of somatic hypermutation in the IgG DNA-coding region, which generates higher affinity to the antigen and stronger binding. Low avidity is common in acute infections, while high avidity is typical for past infections (Hess, 2004). Avidity tests are not a routine practice and we chose to investigate the avidity of anti-VCA IgG to help the interpretation in cases where standard ELISA serology could not give a clear diagnostic decision.

Material and methods

Tested population

Forty-six single serum samples were tested in the current study. Depending on the profile of the initial serology for anti-VCA IgM/IgG and the clinical diagnosis, they were divided into two groups: the first group included 33 (71.7%) patients with clinical diagnosis of infectious mononucleosis (IM), negative anti-VCA IgM and positive anti-VCA IgG. The second group included 13 (28.3%) patients with positive anti-VCA IgM, positive anti-VCA IgG, a diagnosis other than IM and suspected EBV reactivation.

Methods

To determine the avidity of anti-VCA IgG, an ELISA test was performed (Euroimmun, Luebeck, Germany). The serums were tested simultaneously in two replicates – one untreated and one treated with 8M urea for 10 minutes at room temperature. We calculated the relative avidity index (RAI%) by dividing the OD of the sample treated with urea by the OD of the sample without urea, according to the manufacturer‘s instructions. The values < 40% were interpreted as low avidity, samples with RAI > 60% – as high avidity, and RAI% between 40% and 60% – as undetectable, possibly due to advanced acute infection, according to the standard instructions of the manufacturer.

Statistical analysis

To determine the means, confidence intervals, Spearman’s rank correlation coefficient and Chi-square test of independence, the results were processed with SPSS, vs 23.

Results

The actual age in the sample ranged from 8 months to 69 years (mean age = 16.3 years, median age = 13 years) and males were 61% of all tested patients. The mean age in the first group (patients with infectious mononucleosis, anti-VCA IgM-negative and anti-VCA IgG-positive) was 9.9 years, significantly lower than the mean age in the second group (patients with a clinical diagnosis other than IM, anti-VCA IgM-positive and anti-VCA IgG-positive) – 32.5 years (p = 0.002).

Out of the 46 samples tested, 12 (26%; 95% CI:14% - 41%) were with low anti-VCA IgG avidity, which could be interpreted as acute infection, one sample was indeterminate and could be interpreted as advanced acute infection, and 33 (72%; 95% CI: 57% - 83%) were with high avidity (Fig. 1).

In the first group, 8 out of 33 samples (24%; 95% CI:11% - 42%) were with low avidity, while in the second group 4 samples (31%; 95% CI: 9% - 61%) were of low avidity. There was a nonsignificant difference between the low avidity frequencies in both groups (Table 1).

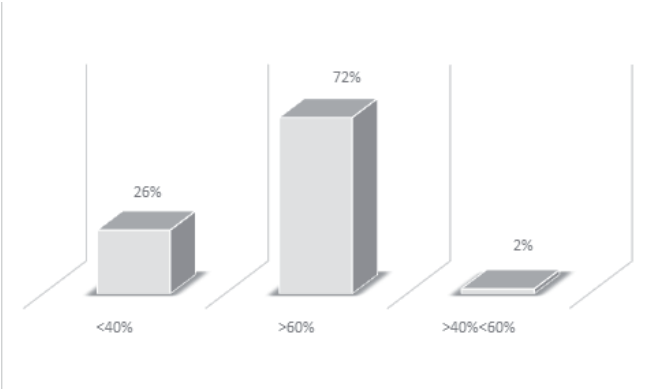

Fig. 1. Proportion of patients studied in the two target groups

Table 1. Proportion of patients with low ELISA IgG avidity in the two target groups

Group	N	<40%	40% 60%	> 60%	Proportion (95%CI)	Chi-square	P value
I	33	8	1	24	24% (11%-42%)	0.21	0.65
II	13	4	0	9	31% (9%:61%)		

In the second group, the mean age of low avidity patients was 24 years and the mean age of high avidity patients was 36 years. However, the Spearman's rank correlation between the avidity value and the age of the tested patients was 0.2 (p-value = 0.2). Two of the four patients with low avidity in the second group were diagnosed with *Non-Hodgkin lymphoma* and two – with hepatitis, negative for the habitual causative agents. For all of them, primary *EBV* infection was proven with the avidity test used.

Discussion

We found low avidity of anti-VCA IgG in 24% of all patients suspected for infectious mononucleosis without anti-VCA IgM, thus confirming primary infection. This result is more significant than the proportions found in previous studies – 4.5% in individuals younger than 10 years (De Paschale *et al.,*2009) and 1.8% of low avidity in IgM negative patients and 6.7% – in patients with indeterminate IgM serology (Vilibic-Cavlek *et al.,* 2011)especially in viral capsid antigen (VCA. A higher number of low avidity samples (55.6%) was found in a study of 27 anti-VCA IgM-negative and anti-EBNA 1 IgG-negative patients, 73.3% of which were also positive for *EBV DNA* (Chan *et al.*, 2001).

The majority of the patients in our study was in the age range typical for primary *EBV* infection in Bulgaria (Kostadinova *et al.,* 2016). The youngest patient with IM and low IgG avidity was 10 months old, while the oldest one was 19 years old. Children up to 5 years were predominant among the cases with low avidity samples and negative anti-VCA IgM. This age group is likely to have more significant variations in the anti-VCA IgM response. In patients with high IgG avidity, other viruses (such as human cytomegalovirus) can be implicated in the disease etiology. The presence of isolated anti-VCA IgG serological profiles in primary infection is obviously a rare event, but because of the variability in anti-VCA IgM formation, should be considered. On the other hand, as anti-EBNA 1 IgG is not present in primary infection, avidity tests are more informative in these cases.

In the second group of patients (28.3% of all studied samples), nearly 1/3 was with low IgG avidity and therefore determined as acute cases. *EBV* reactivation or reinfection is possible in the patients with anti-VCA IgM/IgG positivity and high anti-VCA IgG avidity. Given the life-long persistence of the virus, reactivation of *EBV* is a normal phenomenon in both immunocompetent and immunosuppressed individuals (Adler *et al.,* 2002). High IgG avidity and therefore possible reactivation has been previously shown in 49% of the patients with positive anti-VCA IgM (Nystad and Myrmel*,* 2007), while in our study the proportion of these samples was 69%. It is important to discriminate the primary infections from the cases of reactivation, as reactivation has a low clinical importance in immunocompetent patients (Nystad and Myrmel, 2007). Most of our patients with possible reactivation were older than 35 years. These results correlate well with a previous seroepidemiological study, where a lower risk of primary infection was associated with older ages (Kostadinova *et al.,* 2016). However, one of the patients was an 8-month-old baby. Usually maternal antibodies can be detected in 2-8 month-old infants (Chan *et al.,* 2003). It can be speculated that *EBV* infection has occurred in the past and IgM antibodies persist longer. It is also possible that IgG were of maternal origin and the IgM positivity was a false positive result. In Bulgaria, early infections during the first year of life are not excluded – in this age group the IgM positive samples are 11.9% (Kostadinova *et al.,* 2016). In this particular case, the IgM Immunoblot did not show presence of antibodies against VCA-p19 and VCA-gp125 (data not shown).

Determination of viral reactivation is a serious problem in the serological diagnosis of *EBV,* as unified criteria are missing. Testing the avidity of anti-VCA IgG can assist proper diagnosis, as it can discriminate primary from reactivation infections. With this method, false positive IgM in high avidity patients cannot be completely eliminated and an additional Immunoblot must be performed.

Conclusion

Determination of IgG avidity is a standardized and automated method, easy to apply in routine practice. Despite the small number of tested samples, our results show that in patients with difficult to interpret serology, avidity tests can be very helpful for the final diagnostic decision.

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