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Investigation of Anti-EA (D) IgG in Patients with Haematological Diseases

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Abstract

Epstein-Barr virus is associated with a wide range of clinically relevant diseases - from infectious mononucleosis to malignant diseases of epithelial and lymphoid origin. The use of serological markers, such as predictors of malignant disease or the detection of viral reactivation, are debatable and unsupported by many studies. The purpose of this study is to establish positivity in the anti-EA (D) IgG test in patients with haematological diseases as a possible marker for viral reactivation.

We examined 91 patients with haematological diseases (acute leukemias and non-Hodgkin's lymphomas, including chronic lymphocytic leukemia), of which 54.9% (95% CI: 44.2-65.2) were male. The age range was from 1 to 83 years, with predominance of older patients. Patients were tested for anti-EA (D) IgG with tests of Euroimmun, Germany. The patients positive for anti-EA (D) IgG were also tested for EBV-DNA.

We found 13.2% (95% CI: 6.2 -20.1, n = 12) positive for anti-EA (D) IgG. Patients with non-Hodgkin lymphomas predominated (23.1% 95%CI: 11.1-39.3, n=9). Two of the patients were in a gray zone. All positive patients were over 60 years of age.

The positive anti-EA (D) IgG samples represent a small proportion of the investigated patients with predominance in those with non-Hodgkin's lymphomas.

Based on our previous experience and the results of this study, we consider that the use of anti-EA (D) IgG are not sufficiently reliable in defining EBV reactivation in these patients.

Keywords: *Epstein-Barr virus*, anti EA (D) IgG, leukemias, non-Hodgkin's lymphomas.

Резюме

Epstein-Barr вирусът се асоциира със широк спектър от клинично значими заболявания - от добокачествено протичаща инфекциозна мононуклеоза до малигнени заболявания от епителен и лимфоиден произход. Използване на серологични маркери, като предиктори на малигнено заболяване или за установяване на вирусна реактивация са дискутабилни и неподкрепени с много проучвания. Целта на това проучване е да установим позитивност в anti-EA (D) IgG теста при пациенти с хематологични заболявания, като евентуален маркер за вирусна реактивация.

Изследвахме 91 пациенти с хематологични заболявания (остри левкемии и неходжкинови лимфоми, вкл. хронична лимфоцитна левкемия), от които 54.9% (95%CI:44.2-65.4) мъже. Възрастовият диапазон е 1 г. - 83 г., с преобладаване на по-възрастните пациенти. Пациентите са изследвани за anti-EA (D) IgG с тестове на Euroimmun, Germany. Позитивните anti-EA (D) IgG пациенти бяха тествани сравнително за определяне на EBV-DNA.

Нашите данни показват 13.2% (95%CI:6.2-20.1, n=12) положителни за anti-EA (D) IgG, с преобладаване на пациентите с Неходжкинови лимфоми (23.1%; 95%CI:11.1-39.3, n=9). При двама от пациентите резултатите са в сива зона. Всички положителни пациенти са във възраст над 60 г.

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Положителните анти-EA (D) IgG проби представляват малка част от изследваните пациенти. Преобладават случаите с неходжкинови лимфоми. Въз основа на предишния ни опит и резултатите от това проучване считаме, че използването на анти-EA (D) IgG, не е достатъчно надеждно за установяване на EBV реактивация при тази група пациенти.

Introduction

The *Epstein-Barr virus* (EBV) is a DNA virus which, after primary infection, persists in B lymphocytes. This determines the possibility of reactivation, especially in immunocompromised and immunosuppressed patients. As a result of multiple studies, EBV has been associated with a number of oncogenic diseases in humans (Cohen, 2000).

Usually, the diagnosis of primary infection is based on the detection of a combination of serological markers (anti-VCA IgM/IgG and anti-EBNA 1 IgG) (De Paschale, Clerici, 2012). Viral reactivation, however, remains a major challenge for laboratory diagnostics and there are still no uniform criteria for EBV positivity.

Polymerase chain reaction (PCR), particularly in immunocompromised patients, is considered to be the best option (Brengel-Pesce *et al*., 2002; Holmes *et al*., 2002), but it is not routinely used in the diagnostics of EBV reactivation in our country due to the high cost. Based on a literature review, we tried to establish a serological marker that could be used as initial screening for viral reactivation. Some authors have detected reappearance of anti-VCA IgM , but in a small (5.8%) percentage of cases (Obel *et al.,* 1996). Others have found an increase in the amount of anti-VCA IgG (Rahman e*t al*., 1991), seroconversion of anti-EA IgM, concomitant anti-EA IgM and anti-EBNA1 IgG seropositivity (Obel *et al*., 1996), quantitative determination of anti-EA in paired serum samples (Wohlrabe *et al*., 1989).

The aim of this study was to establish positivity in the anti-EA (D) IgG test in patients with haematological diseases as a possible marker for viral reactivation.

Materials and Methods

Study population

Ninety-one patients with hematological diseases (acute leukemia and non-Hodgkin's lymphomas including chronic lymphocytic leukemia) were investigated for anti - EA (D) IgG, of them 54.9% (95%CI: 44.2-65.4) were male. The average age was defined as 50.7 years (SD ± 19.96; range of 1 - 83 years).

Anti-VCA IgM/IgG data were available for anti-EA (D) IgG positive patients only. Serological samples were examined once without monitoring of dynamic changes.

Methods

1. Serological methods

Indirect ELISA was used for anti-EA (D) IgG and anti VCA IgM/IgG. When calculating the IgM results, the semi-quantitative method was applied: Ratio = Extinction of the sample/Extinction of calibrator. Positive samples had a ratio > 1.1; negative samples had a ratio < 0.8; and ratios between 0.8 and 1.1 were considered borderline. For IgG, the quantitative method for defining positive and negative samples was used by constructing a calibration curve (Cal 1 = 200 RU/ml, Cal 2 = 20 RU/ml, Cal 3 = 2 RU/ml, where RU/ml is relative units/ml). Positive results were ≥ 22 RU/ml; negative samples < 16 RU/ml; and borderline results were between 16 and 22 RU/ml.

2. PCR methods

DNA was extracted from 150 µl plasma using Kit Ribo Virus (Sacace Biotechnologies S.r.l., Como, Italy). Amplification was performed with EBV Real-TM Quant (Sacace Biotechnologies S.r.l., Como, Italy) following the standard manufacturer's instructions in reaction volumes of 25µl and using the QuantStudio Dx Real-Time PCR Instrument (Thermo Fisher Scientific, Waltham, MA USA). The target amplification region is the latent membrane protein (LMP) gene of EBV and the sensitivity of the kit is reported to be > than 200 copies/ml with a linear range of 500-10^6 EBV DNA copies/ml.

3. *Statistical methods*

Proportion, confidence intervals, average age, and standard deviation were calculated. We used chi square test to compare the data. Values of $p < 0.05$ were considered statistically significant.

Results

Of the patients investigated for anti-EA (D) IgG, more than half (57.1%) were with acute leukemia and most notably with acute myeloid leukemia (Fig. 1).

Positive results were found in 13.2% of the patients (95% CI: 6.2-20.1, n=12), with the highest proportion of patients with follicular lympho-

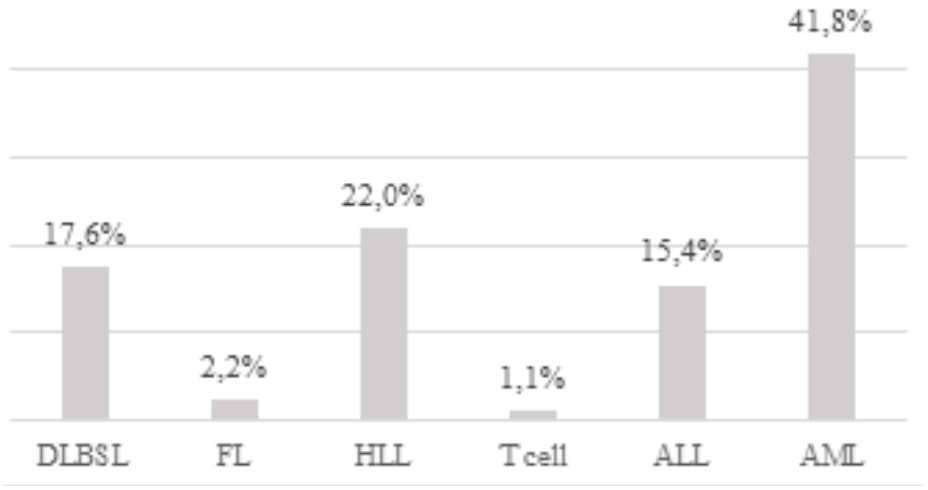

Fig. 1. Distribution of the patients depending on the histological variant

ma - 50.0%, who represented, however, a very small proportion of the patients studied (Table 1). Overall, positive results prevailed in patients with non-Hodgkin's lymphomas, unlike patients with acute leukemia ($p = 0.03$). For two of the patients the results were in the gray area. All positive results were in patients over 60 years of age. Those who were anti-EA (D) IgG positive were also anti-VCA IgG (+) / anti-VCA IgM (-).

The patients positive for anti-EA (D) IgG were also tested for EBV-DNA. In three of the samples with NHL (2-CLL, 1-FL) EBV-DNA was detected. The viral load was below 500 copies/ml (Table 2).

Discussion

While in immunocompetent individuals the primary goal is to identify the type of infection – whether it is primary or past, or to rule it out (Gärtner *et al.*, 2003), in immunocompromised individuals the diagnosis is mainly related to early detection of viral replication, which is of high prognostic value (Gärtner *et al*., 2000).

Table 1. Characteristics of the patients positive for anti EA (D) IgG

Subtype	N, (%)	+ anti- EA (D), n (%)
DLBCL	16 (17.6)	
FL	2 (2.2)	1 (50.0)
HLL	20 (22.0)	8 (40.0)
Peripheral T cell	1 (1.1)	
ALL	14 (15.4)	
AML	38 (41.8)	3 (7.9)

The early antigen (EA) complex is expressed during the lythic phase of the EBV life cycle and the antibodies against EA (D) of the IgG class appear in the course of primary infection in some patients. Typically, titres increase in the first 3-4 weeks and remain steady for about 3-4 months. Their detection is also associated with the reactivation of the infection due to impaired immune system control on viral replication (De Paschale and Clerici, 2012).

The patients tested for anti-EA (D) IgG in this study exhibited no evidence of primary infection. Although anti-EA (D) IgG are positive up to 3 months after the onset of symptoms, in some cases they can be detected years later (De Paschale and Clerici, 2012).

Anti-EA (D) IgG have been subject of another study of ours, in which patients with infectious mononucleosis, non-Hodgkin's and Hodgkin's lymphomas, and other haematological diseases were tested. The highest percentage (55.6%) of positive results was detected in patients with primary infection. In the remaining patients, the positive results were close to those found in this study (8.5%).

Immunocompromised patients have dysfunction in the production of antibodies, which may lead to atypical serological profiles (Wagner et al., 1992*)*. Molecular biological methods and especially Quantitative Real-time PCR are the methods of choice since viral load determination can be useful to identify those at high risk of developing complications caused by EBV (Brengel-Pesce *et al*., 2002; Holmes *et al*., 2002).

EBV-DNA was found in 25.0% of the positive patients in the serological test. A limitation in our study is the lack of PCR data for the anti-EA (D) IgG negative patients.

Table 2. Patients with detectable EBV-DNA

No	Diagnosis	anti- EA (D)	EBV-DNA
141	CLL	162.5 RU/ml	50 c/ml
1012	FL	42.1 RU/ml	228 c/ml
1239	CLL	65.6 RU/ml	32 c/ml

Conclusion

Based on the results obtained in this study, we believe that a single anti-EA (D) IgG test is not a convincing marker for detection of viral reactivation in patients with haematological diseases.

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