ORIGINAL ARTICLE

Correlation between the immunological condition and the results of immunoenzymatic tests in diagnosing infectious mononucleosis

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Abstract. *Background and aim of the work:* A symptom-based diagnosis of infectious mononucleosis is not sufficiently accurate, since some clinical symptoms of infectious mononucleosis are also detected in other virally induced diseases. Moreover, not all patients suffering from infectious mononucleosis show circulating atypical lymphocytes, which are considered characteristic of this disease. Therefore, when this disorder is suspected, serum analyses are carried out to detect the presence of certain immunoglobulins associated with infectious mononucleosis in the patient's blood. The aim of this study was to evaluate the sensitivity and the specificity of a rapid test detecting heterophil antibodies in diagnosing infectious mononucleosis in a paediatric population. *Methods:* We considered 163 paediatric patients with suspected infectious mononucleosis and we tested their serums to detect heterophil antibodies (using an inexpensive and rapid test) and specific immunoglobulins directed against Epstein-Barr virus (EBV) (these assays are known to be characterized by high sensitivity and specificity, but are more expensive and time-consuming). *Results:* By comparing the results of the rapid test with those of the other assays, we found that the sensitivity of the first test was 61.8%, whereas its specificity was sufficiently high (about 90%). *Conclusions:* We show that, in paediatric patients, the detection of heterophil antibodies is not a very sensitive test, therefore the determination of immunoglobulins against specific antigens of EBV is recommended. (www.actabiomedica.it)

Key words: Infectious mononucleosis, diagnosis, immunoenzymatic assay

Introduction

Infectious mononucleosis is an acute self-limited disease, caused by the Epstein-Barr virus (EBV) of the Herpesviridae family (1 2).

In humans the primary infection by this virus is generally asymptomatic in infants, but tends to be associated with the following clinical symptoms in adolescence and/or adulthood: fever, lymphoadenopathy and, in some patients, the presence of atypical lymphocytes (3, 4).

Standard laboratory tests to confirm the clinical symptoms include measuring the level of immunoglobulins directed against specific antigens of the Epstein-Barr virus, or the determination in the patient's serum of the so-called heterophil antibodies capable of agglutinating ram or horse erythrocytes (5, 6). The relationship between antibody production to the EBV antigens (IgM and IgG to the viral capsid antigen (VCA), IgG to the early antigen (EA) and IgG to Epstein-Barr Nuclear Antigen (EBNA) and EBV-related diseases is frequently evaluated (4, 7, 8).

By combining the results obtained from these determinations, it is possible to divide the patients into four main categories: 1) patients without a history of infection. Determination of all the mentioned im-

munoglobulins is negative; 2) patients with acute EBV-infection. IgM anti-VCA and often IgG anti-EA are observed in the patient's serum. The levels of IgG anti-VCA gradually increase during the phase of acute infection and subsequently persist throughout the patient's life. Since the concentration of IgM anti-VCA rapidly decreases during recovery, the positive value of IgG anti-VCA indicates that an infection is in course only if a positive value of IgM anti-VCA is simultaneously detected; 3) patients with a history of infection. They show high IgG anti-VCA and IgG anti-EBNA antibody levels; 4) patients with reactivation of the latent virus. This group is positive for IgM anti-VCA, IgG anti-VCA and IgG anti-EBNA. These patients simultaneously show signs of the current infection but also of the previous one. A similar pattern of the serum levels can also be observed in patients who have been recently affected by the infection, and consequently it is not easy to interpret results of this kind (9).

An infection with EBV can be diagnosed not only by determining serum levels of the various immunoglobulins, but also by assessing the presence of agglutinins (the so-called heterophil antibodies) in the patient's blood. These agglutination tests (e.g. combining the patients' serum with horse erythrocytes) are particularly quick and easy to perform. A major draw back of these tests, however, is their lack of sensitivity and specificity because heterophil antibodies are also produced under other pathological conditions, such as serum sickness, some viral disorders not related to infectious mononucleosis, and some lymphoproliferative conditions. Moreover, these agglutinins are absent in the serum of 10-20% of adults affected by infectious mononucleosis, and this percentage increases to 50% in children younger than twelve years of age. Finally, heterophil antibodies remain in the serum up to six-twelve months after the infection (10, 11).

False positive samples in the agglutination test due to causes other than EBV-instigated infectious mononucleosis can be avoided by absorbing the patient's serum with a homogenate of guinea pig kidney (Forssman antigen) before the reaction with erythrocytes. Pre-treatment with Forssman antigen allows inactivation of the agglutinins produced in the course of diseases that are not caused by the Epstein-Barr virus (12).

The aim of this study was to evaluate the sensitivity and specificity of the infectious mononucleosis (MNI) test (Bouty, Sesto San Giovanni, Italy), a cheap and rapid agglutination test detecting heterophil antibodies by comparing the results obtained in this analysis with results of determinations performed with more expensive and time-consuming immunoenzymatic assays specific for measuring IgM anti-VCA, IgG anti-VCA and IgG anti-EBNA.

Materials and methods

We evaluated 163 different human sera, which were received from the Hematology Laboratory of the Children's Hospital IRCCS Burlo Garofolo (Trieste, Italy) and were derived from both male and female patients of paediatric age (< 18 years old) with suspected infectious mononucleosis or suspected production of heterophil antibodies.

On each serum sample a set of three different tests were performed through immunoenzymatic assay in order to determine :

1) the presence of IgM anti-VCA with the Beia EBV VCA IgM Quant kit (Bouty, Sesto San Giovanni, Italy)
2) the presence of IgG anti-VCA with the Beia EBV VCA IgG Quant Kit (Bouty, Sesto San Giovanni, Italy)
3) the presence of IgG anti-EBNA with the Beia EBV EBNA-1 IgG Quant kit (Bouty, Sesto San Giovanni, Italy)

In the same samples we have determined the presence of heterophil antibodies with the MNI test (Bouty, Sesto San Giovanni, Italy). This is a rapid agglutination test which does not require the Forssman antigen, although the horse erythrocytes have undergone a particular treatment to ensure test specificity.

Results

Patients were divided into eight different groups, on the basis of the results obtained in the three immunoenzymatic tests (Table 1).

We repeated the experiment with the MNI test to assess its specificity and sensibility. The samples be-

Table 1. Patient groups divided on the basis of the results obtained in the three immunoenzymatic tests

Group	Total number of samples ?	IgM anti-VCA	IgG anti-VCA	IgG anti- EBNA
1		negative	negative	negative
2		negative	positive	positive
3		positive	positive	negative
4		negative	positive	negative
5		positive	negative	positive
6		negative	negative	positive
7		positive	negative	negative
8		positive	positive	positive

longing to groups 1 and 2 were analysed to assess the specificity of the test, whereas the samples belonging to group 3 were studied to assess the sensitivity of the MNI test (Table 2).

Discussion

The diagnosis of infectious mononucleosis is often based on laboratory tests determining the presence of antibody molecules associated with this disorder and measuring them. Immunoenzymatic methods allow measurement of concentrations of different types of immunoglobulins directed against viral antigens. Through correct interpretation of the test results, it is possible to establish the patient's immunological condition with regard to the Epstein-Barr virus. These analyses are costly and time consuming, although they provide reliable results. On the other hand, erythrocyte agglutination tests are available: they assess the presence of heterophil antibodies in the patient's serum. In contrast with the immunoenzymatic assays these tests are faster and less expensive, but the results are not as precise or detailed (5, 6).

In the present study we analyzed the specificity and sensitivity of the MNI test – a rapid agglutination test for the in vitro diagnosis of infectious mononucleosis. The analysis is performed with horse erythrocytes that have undergone a specific treatment to improve test specificity. Therefore, the serum does not have to be absorbed with guinea pig kidney extract before performing the agglutination test.

As shown in Table 2, the specificity of the MNI test, based on the samples of group 1 (including patients without history of infection) and group 2 (including patients with previous infection) was 92.6% and 84.8% respectively.

The test sensitivity, calculated by analyzing the sera of group 3, was 61.8%. Some of these samples could be false negatives because they were derived from paediatric patients, since. young patients do not often produce heterophil antibodies, even during the acute phase of infectious mononucleosis (11).

The results observed in group 4 could be related to an active phase of the infection, but also to a condition of immunodepression whereby the patient does not synthesize the IgG anti-EBNA. Since the data obtained with the immunoenzymatic tests were not clear, the results of the MNI test could not be used to evaluate its sensitivity or specificity.

Results obtained from samples belonging to groups 5 and 6 were unusual, and no data to help in the understanding of these observations were found in the literature. It would thus be very interesting to analyse further serum samples from groups 5 and 6 by, for example, determining the IgG anti-EA levels.

Table 2. Results of the MNI test in the eight groups and specificity and sensitivity of the MNI test

Group	Total number of samples	Samples negative at IMN test	Samples positive at IMN test	Samples uncertain at IMN test	Specificity of IMN test	Sensitivity of IMN test
1	54	50	3	1	92.6%	_
2	92	1	8	6	84.8%	_
3	21	8	13	0	–	61.8%
4	13	13	0	0	–	–
5	1	0	0	1	–	–
6	1	1	0	0	–	–
7	2	0	0	2	–	–
8	3	2	1	0	–	–

The serum-samples of group 7 probably belonged to patients at a very early phase of the infection, since only IgM levels were found to be positive. IgM is the first immunoglobulin produced after the antigen enters into the circulation. Both samples were probably scored as negative in the MNI test, because the synthesis of agglutinins in serum occurs at a later stage of the infection. However, the negative MNI test result may also be related to the young age of the patients. In the light of these results, it would be advisable for the patients to repeat the immunoenzymatic and agglutination test after a period of time.

No sound interpretation could be provided for the immunoenzymatic test results obtained for patients in group 8. Similar to the patients in groups 4, 5 and 6, their immunological profile may be connected to a period of convalescence, but also to reactivation of the latent virus. Consequently, these samples could not be used to determine the sensitivity and specificity of the MNI test.

Although the MNI test has shown to have a good degree of specificity (group 1, 2) and acceptable values of sensitivity (group 3), it is recommended to confirm the test with the determination of immunoglobulins against specific antigens of the Epstein-Barr virus when diagnosing infectious mononucleosis. Only by performing these more accurate analyses is it possible to establish whether or not the patient is affected by acute EBV-related infectious mononucleosis. This approach is particularly important when the patients are children, since in most cases they do not produce agglutinins in the course of the disorder.

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