Limitations of Available Tests for Diagnosis of Infectious Mononucleosis

GARY R. FLEISHER,[†]* MARJEANNE COLLINS, AND SAMUEL FAGER

Department of Pediatrics and Student Health Service, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Received 12 November 1982/Accepted 13 January 1983

Among 500 students seen at a university health service for illnesses resembling infectious mononucleosis (IM), the diagnosis of IM was established in 124 (25%) on the basis of the initial presence or subsequent emergence of the spectrum of Epstein-Barr virus (EBV) antibodies characteristic of a primary EBV infection. Of these 124 patients, 113 had an EBV-specific antibody pattern in the initial serum indicative of current primary infection; however, 11 (9%) had no detectable immunoglobulin G antibodies to EBV-specific antigens in their first serum. The sensitivity of this panel of EBV antibody assays was 91% and the specificity was 100%. Initial sera had detectable heterophil antibodies for 107 (86%) of the 124 students with IM and for 2 with other illnesses. Among our patients, the Monospot (Ortho Diagnostics Inc.) test had a sensitivity of 86% and a specificity of 99%. Reliance on hematological criteria (lymphocyte count $\geq 50\%$ and atypical lymphocyte count $\geq 10\%$) gave a sensitivity of only 39%, but a specificity of 99%. Students with IM who showed a delayed emergence of the spectrum of EBV-specific antibodies characteristic of an acute infection were compared with control patients who had such antibodies at the time of their initial visit to the health service. They were found to have a briefer duration of illness ($P > 0.05$), lower leukocyte ($P < 0.005$), lymphocyte ($P < 0.005$), and atypical lymphocyte ($P < 0.05$) counts, and a less frequent occurrence of heterophil antibodies ($P < 0.05$).

Sprunt and Evans (24) described the clinical features of infectious mononucleosis (IM) in 1920, and shortly thereafter Downey and McKinlay (3) added a detailed description of the hematological findings for the disease. In 1937, the heterophil antibody (HA) test with differential absorption was shown to detect IM-specific HA, providing a third criterion for the diagnosis of IM (2, 19). However, a specific etiological diagnosis did not become possible until three decades later, when Henle et al. (10) showed, in 1968, that the Epstein-Barr virus (EBV) caused this disease.

Initially, virus-specific serology was limited to immunoglobulin G (IgG) antibodies to viral capsid antigen (VCA) (9). Under these circumstances, the diagnosis of IM relied on the emergence of IgG anti-VCA in retrospective studies and on the finding of a high titer of this antibody in clinical practice; since 85% of patients with IM already had peak levels of IgG anti-VCA at the time of diagnosis, a fourfold rise in titer was seldom observed. Subsequently, antibodies to other EBV-related antigens were discovered and

proved to be useful to the practitioner, allowing in most cases for the diagnosis of IM by assay of a single acute-phase serum (9, 11, 12, 21, 23). Because of the overlap in IgG antibody titers to VCA that occurs between individuals with an acute primary EBV infection and those with infection in the distant past, it is now clear in retrospect that reliance on a high titer of IgG anti-VCA alone is not acceptable (12).

During a study on the treatment of IM, we encountered a number of patients, subsequently proven to have IM, who in the initial serum did not have antibodies to EBV, a positive test for HA (Monospot; Ortho Diagnostics Inc.), or characteristic hematological changes in the peripheral blood. This led us to reassess the use of these various diagnostic tools, focusing particular attention on patients with IM who lacked initially detectable antibodies to EBV.

MATERIALS AND METHODS

Subjects. Beginning in September 1980, all patients seeking treatment at the Student Health Service at the University of Pennsylvania with illnesses suggestive of IM had blood drawn for EBV-specific serological assays and the Monospot test. Total leukocyte (WBC) and differential counts were performed uniformly dur-

[†] Present address: Division of Infectious Diseases, The Children's Hospital of Philadelphia, Philadelphia, PA 19104.

TABLE 1. Results of diagnostic tests for IM

Diagnosis	No. of students	No. (%) meeting criteria for diagnosis of IM by:			
		EBV-specific serology		HA test	
		Initial serum	Later serum	Initial serum	Later serum
IM (EBV)	124	113 (91)	124 (100)	107 (86)	111 (90)
No-IM	376	0 (0)	0 (0)	2(0.4)	Not tested

ing weekdays and sporadically at other times. Sera from these students were logged consecutively. The majority of the patients diagnosed as having IM were enrolled in an ongoing therapeutic study, and the remainder were followed until they recovered clinically or did not return. Patients consenting to the therapeutic study were bled after 4, 12, and 26 weeks had elapsed. From those who were not diagnosed initially as having IM, additional blood samples were obtained only if the first EBV-specific assays were inconclusive or the physician caring for the patient elected to repeat the tests due to persistent illness.

On the basis of the initial and follow-up EBV serological assays, patients were categorized as having: (i) IM with the expected pattern of EBV-specific antibodies (group 1), (ii) IM with delayed antibody response to EBV (group 2), or (iii) illnesses other than IM.

Controls. Two patients from group 1 were selected as controls for each patient in group 2 by choosing the cases of IM immediately preceding and following the entries for the group 2 patients in the log book.

Hematology. Total WBC counts were performed with an automatic cell counter (Coulter Electronics Inc.), and differential cell counts were done in a routine fashion in the hematology laboratory of the hospital of the University of Pennsylvania. The reported counts were interpreted as diagnostic of IM when the relative lymphocyte count was $\geq 50\%$ and the atypical lymphocyte count (expressed as a percentage of the total WBC count) was $\geq 10\%$ (14).

HA tests. A Monospot test was performed in the laboratory at the hospital of the University of Pennsylvania and repeated by one of us (G.R.F.). Additionally, HA was measured by the immune adherence hemagglutination assay (IAHA), with fetal bovine serum used as a source of heterophil antigen (5, 16). Titers of greater than 40 were considered significant.

EBV-specific serology. Initially, the sera were titrated for IgG antibodies to VCA and to the diffuse and restricted components of the early antigen complex (EA) by indirect immunofluorescence and for antibodies to the EBV-associated nuclear antigen (EBNA) by anticomplement immunofluorescence (9, 11, 21, 23). Specimens that showed an antibody pattern compatible with an acute, primary EBV infection or were not clearly interpretable were assayed for IgM antibodies to VCA (12). The diagnosis of a current primary EBV infection was based on the following criteria: (i) presence of IgM antibodies to VCA, (ii) a high titer of VCA-specific IgG antibodies, (iii) detection of antibodies to the EA diffuse component, and (iv) absence or low titers (≤ 5) of antibodies to EBNA (5, 12).

RESULTS

EBV-specific serology. Serum specimens from 500 students were tested between September 1980 and August 1981 (Table 1). IM was diagnosed in 124 students (25%) on the basis of the initial presence or subsequent emergence of the spectrum of EBV antibodies characteristic of a primary EBV infection, as defined above. Of these 124 patients, 113 (group 1) had an EBV-specific antibody pattern in the initial serum that was indicative of a current, primary infection (Table 2). The IgG anti-VCA titer varied from 1:10 to 1:2,560 in these 113 patients, 105 (93%) of them already having a high titer (>160); patients with low levels (1:10 to 1:40) of IgG anti-VCA uniformly showed a fourfold or greater rise in titer on subsequent testing. Altogether, 76% of the patients had no detectable levels of anti-EBNA, and in those with a low level of anti-EBNA, IM was diagnosed on the basis of IgM anti-VCA or anti-diffuse component responses.

Eleven patients (group 2) with IM had no detectable IgG antibodies in their first serum to VCA, EA, or EBNA even when the tests were repeated and carefully scrutinized with sera retrieved from the freezer after the diagnosis of IM had been established. EBV-specific antibodies subsequently emerged in each of these 11 students (Table 3). Those who were retested within several weeks had the expected pattern of a current primary infection, and one patient tested 6 months later had modest levels of IgG anti-

TABLE 2. EBV-specific antibodies in 113 patients with initial diagnosis of IM

Antibody	No. (%) of positive samples ($n = 113$)	Reciprocal titer (range)	Geometric mean titer
IgM anti-VCA	113 (100)	10-320	109
IgG anti-VCA	113 (100)	10-2,560	366
Anti-EA (diffuse component)	74 (65)	10-160	43
Anti-EA (restricted component)	9 (8)	10-40	15
Anti-EBNA	27 (24)	2-5	3

TABLE 3. Emergence of EBV-specific antibodies in patients with IM whose initial sera were interpreted as falsely negative before being tested for IgM anti-VCA

Specimen interval	Specimen no.	Reciprocal titer of:			
		IgM anti-VCA	IgG anti-VCA	Anti-EA	Anti-EBNA
4 wk	1	<10	<10	<10	<2
	2	10	160	<10	<2
2 wk	1	<10	<10	<10	<2
	2	160	1,280	80(D) ^a	5
5 days	1	20	<10	<10	<2
	2	80	160	40(D)	<2
8 days	1	<10	<10	<10	<2
	2	40	40	<10	<2
1 wk	1	<10	<10	<10	<2
	2	40	640	20(D)	<2
2 wk	1	<10	<10	<10	<2
	2	20	640	40(D)	2
26 wk	1	<10	<10	<10	<2
	2	<10	640	40(D)	5
9 wk	1	<10	<10	<10	<2
	2	160	2,560	20(D)	<2
8 days	1	40	<10	<10	<2
	2	320	640	80(D)	<2
2 days	1	<10	<10	<10	<2
	2	40	20	<10	<2
3 wk	1	80	<10	<10	<2
	2	160	640	80(D)	<2

^a D, Diffuse component of EA.

VCA and anti-EBNA, as are seen with an infection in the distant past. As is our practice, IgM anti-VCA was not initially assayed in the sera that lacked EBV-specific IgG antibodies; however, subsequent testing showed the presence of IgM anti-VCA in 3 (27%) of these 11 sera.

Excluding the IgM test, the sensitivity of this panel of EBV antibody assays was 91%, the false-negative rate was 9%, and the negative predictive value was 97%. Had the IgM test been performed initially on all sera, the sensitivity of the expanded panel would have been 94%, the false-negative rate 6%, and the negative predictive value 98%. By our definition of IM, no student had falsely positive EBV-specific antibody titers.

A total of 376 students did not have acute, primary EBV infections. Of these, 86 were seronegative and 290 had serological evidence of infection in the distant past.

HA test. The Monospot test was reported as

positive in the first sera from 94 of 124 (76%) students with IM. Thirteen additional sera reported as negative by the hospital laboratory produced easily recognizable agglutination when retested by one of us. The presence of HA in these 13 sera with discrepant results was confirmed by the IAHA test; thus, 107 of 124 (86%) of the initial sera were HA positive (Table 1). Two (0.4%) patients had a positive Monospot test, repeated twice, but had an EBV-specific antibody pattern indicative of a past rather than a current EBV infection. Neither of these sera showed evidence of HA by IAHA; IAHA was not performed on the other 374 sera from patients who did not have IM. The Monospot test had a sensitivity of 86% and a specificity of 99% based on the results obtained in these 500 patients. The positive predictive value was 98%, and the negative predictive value was 96%.

From 5 of the 17 IM patients who were Monospot negative initially, follow-up sera were

FIG. 1. Mean WBC, lymphocyte, and atypical lymphocyte counts in 82 of 124 students with IM from groups 1 and 2 and in 208 of 376 students with non-IM illnesses. Horizontal bar indicates 1 standard deviation.

drawn after an interval of 1 to 4 weeks. Four of these five students developed an HA response; the single student who remained negative had the test repeated after only 1 week had elapsed.

WBC counts. WBC counts were performed at the time of the initial presentation on 82 of 124 (66%) students with IM and 208 of 376 (55%) students who subsequently proved not to have this disease (Fig. 1). The mean total WBC, total lymphocyte, and atypical lymphocyte counts were significantly higher in the students with IM than in those with illnesses not due to EBV ($P < 0.01$, $P < 0.005$, and $P < 0.005$, respectively, by Student's t test); however, the total WBC and lymphocyte counts showed a considerable overlap. Using a lymphocyte count of $\geq 50\%$ as diagnostic of IM gave a sensitivity of 62% and a specificity of 96%. Reliance on an atypical lymphocyte count $\geq 10\%$ was slightly less sensitive (54%) but minimally more specific (98%). The requirement that both of the aforementioned hematological criteria be met for the diagnosis of IM led to the highest specificity (99%) but the lowest sensitivity (39%).

Comparison of patients in groups 1 and 2. The 11 IM patients with initially negative EBV serologies (group 2) were compared with 22 control patients, selected as described above from group 1, for duration of illness before the first visit, type of IM syndrome, presence of adenopathy and hepatosplenomegaly, and laboratory findings (Tables 4 and 5).

The WBC ($P < 0.0005$), lymphocyte ($P < 0.005$), and atypical lymphocyte ($P < 0.05$) counts were significantly higher in group 1 (Student t test). Although the patients in group 2 were sick for an average of 4.5 days before their first visit, compared with 8 days for those in group 1, and were more likely to have an angiose syndrome, these differences were not significant (chi-square test). However, group 2 patients were less likely to have a positive Monospot test ($P < 0.05$), a WBC count $> 10,000/\text{mm}^3$ ($P < 0.005$), a lymphocyte count $\geq 50\%$ ($P < 0.005$), or an atypical lymphocyte count $\geq 10\%$ ($P <$

0.05). They met the hematological criteria for the diagnosis of IM significantly less often ($P < 0.01$) than did the patients in group 1.

DISCUSSION

IM has long been known as a frequent infection in young adults, particularly on college campuses (6–8, 22, 25). Incidence rates for college students have been reported to vary from 1,000 to 4,000 per 100,000 annually. Studies at universities in the United States and Great Britain have shown, by periodically screening students for antibodies to EBV, that both overt and silent infections occur, the ratio being between 1:2 and 2:1 (6, 22, 25).

Reliance on the presence of HA and the hematological findings is the general practice for college students (4, 12, 14). Studies of patients with IM have shown that the majority have a positive test for HA and an absolute lymphocytosis, with 10% or more atypical cells (1, 4, 13, 15, 17, 18, 20). Evans and colleagues (4) found

TABLE 4. Comparison of students with IM in group 2 (false-negative EBV titers) with control subjects with IM in group 1 (positive EBV titers)

Clinical feature or abnormality	No. with feature/total (%) in:	
	Group 2	Group 1
Angiose syndrome	9/11 (82)	12/22 (55)
Lymphadenopathy	8/11 (73)	16/22 (73)
Hepatosplenomegaly	2/11 (18)	5/22 (23)
Ili more than 7 days	1/7 (14)	10/20 (50)
WBC count at first visit $\geq 10,000/\text{mm}^3$	0/11 (0)	12/22 (55)
Lymphocytes $\geq 50\%$	1/11 (9)	17/22 (77)
Atypical lymphocytes $\geq 10\%$	2/11 (18)	13/22 (59)
Positive Monospot test	2/11 (18)	22/22 (100)
Serum glutamic oxalacetic transaminase (aspartate aminotransferase) ≥ 40 IU	0/2 (0)	4/9 (44)

TABLE 5. Clinical and laboratory findings

Group	Feature and range (mean $\pm$ SD)			
	Days ill before first visit	WBC count (no./mm ³)	Lymphocytes (%)	Atypical lymphocytes (%)
2	2-12 (4.6 $\pm$ 3.5)	3,600-7,800 (5,500 $\pm$ 1,200)	9-50 (36.7 $\pm$ 13.0)	0-20 (5.3 $\pm$ 7.3)
1	1-30 (8.5 $\pm$ 8.1)	5,000-16,900 (10,500 $\pm$ 3,600)	14-77 (53.8 $\pm$ 16.9)	0-59 (14.7 $\pm$ 14.5)

the HA test accurate for the diagnosis of IM in a series of 53 cadets at the U.S. Military Academy at West Point, N.Y.

We studied 500 students, diagnosing IM in 124. These 124 patients had, either initially or on subsequent testing, the expected EBV-specific antibody pattern for IM: IgM anti-VCA, high or rising titers of IgG anti-VCA and anti-diffuse component (65%), and no or low anti-EBNA. Our data confirm the frequent occurrence of IM in this age group and the high positivity rate of the Monospot test, when properly performed, for this disease. In contrast, they point to a previously unreported lack of sensitivity in the total WBC and differential counts and emphasize the limitations of all assays, alone or in combination, for the initial diagnosis of IM. The initial Monospot test was negative in 17 of 124 students with IM, and the WBC and differential counts did not meet the established criteria for diagnosis in 61% of those tested.

There are two potential explanations for the low sensitivity of the hematological criteria as compared with earlier reports. First, repeat WBC counts were not performed in our study; often the most abnormal of a series of counts was the one included in other studies. Second, the use of EBV-specific serology for diagnosis led to the inclusion of cases of IM that were HA negative, particularly early in their course. Such cases may be less likely to manifest the characteristic hematological changes of IM.

EBV-specific serology enabled us to make a definitive diagnosis of IM in all cases. However, 11 of 124 students initially had no detectable antibodies to EBV detected by the usual panel in our laboratory, which does not routinely include a test for IgM anti-VCA. Even if the assay for IgM anti-VCA had been performed, the diagnosis would have been missed in 8 of 124 patients. The sensitivity of the routine panel of antibody tests was 91%, and the false-negative rate was 9%; adding the IgM anti-VCA test increased the sensitivity to 93% and decreased the false-negative rate to 7%.

The EBV-specific serology was most likely to be negative initially in patients who had the angiose syndrome and had been ill for only a few days at the time of their first visit. Such patients were unlikely to have diagnostic HA or hematological tests. However, within 1 to 2 weeks

antibodies to EBV-specific antigens appeared in the expected spectrum and titers. Usually, but not always, an HA response developed as well in patients from whom follow-up specimens were available.

We offer the following recommendations, based on our findings, for the diagnosis of IM among adolescents and young adults with persistent mononucleosis-like illnesses. (i) Initially, perform a Monospot test and a WBC count with differential. (ii) If these assays are not definitive and the situation does not mandate an urgent diagnosis, repeat the Monospot test and WBC count with differential weekly for 2 weeks, or, if an urgent diagnosis is important, obtain EBV-specific serology. (iii) If the initial EBV-specific serology shows no IgG antibodies, perform an IgM test on that specimen. (iv) If neither IgG nor IgM antibodies are detected, repeat the EBV-specific serology weekly for 2 weeks, particularly for patients with angiose disease of brief duration.

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