

ASSESSMENT OF THE EFFICACY OF AN IgM-ELISA AND MICROSCOPIC AGGLUTINATION TEST (MAT) IN THE DIAGNOSIS OF ACUTE LEPTOSPIROSIS

P. CUMBERLAND, C. O. R. EVERARD, AND P. N. LEVETT

Infectious Disease Epidemiology Unit, London School of Hygiene and Tropical Medicine, London, United Kingdom; Leptospira Laboratory, St. Michael, Barbados; School of Clinical Medicine and Research, and Leptospira Laboratory, University of the West Indies, Bridgetown, Barbados

Abstract. In a prospective study in Barbados between 1979 and 1989, 321 cases were diagnosed in 638 patients presenting at a hospital with symptoms of leptospirosis. Initial diagnosis was based on patient history and characteristic signs and symptoms. In 92 cases (29%), diagnosis was confirmed by isolation of organisms from the blood, urine, or dialysate fluid; in the remaining 229 cases (71%) diagnosis was confirmed by serology alone. Results of an IgM-ELISA and microscopic agglutination test (MAT) in cases with isolates and in non-leptospirosis cases were used to assess the sensitivity and specificity of the tests. The sensitivity of IgM detection by ELISA was 52% in the first acute-phase specimen, increasing to 89% and 93% in the second acute-phase and convalescent specimens, respectively. The specificity of the IgM-ELISA was high ($\geq 94\%$) in all specimens. The sensitivity of the MAT was low (30%) in the first acute-phase specimen, increasing to 63% in the second acute-phase specimen and 76% in the convalescent specimen. The specificity of the MAT was $\geq 97\%$ in all specimens.

Leptospirosis is a common cause of acute febrile illness throughout the wet tropical regions of the world. Early diagnosis is essential, since untreated the illness can progress rapidly and mortality rates are high in severe cases. It is therefore important to differentiate leptospirosis from other acute febrile illnesses. A definitive diagnosis is made by isolation of organisms from blood or urine, but it takes time for the organism to develop in culture and growth is unreliable, so diagnosis usually depends on clinical assessment and serologic tests. The definitive serologic test is the microscopic agglutination test (MAT), in which live antigen suspensions are titrated with patients' sera and then inspected microscopically for agglutination.^{1,2} However, this assay requires significant expertise to perform and interpret³ as well as the laboratory maintenance of a battery of live culture antigens; thus, other serologic approaches have been developed, including the use of an ELISA for both IgM and IgG antibodies.^{4,5}

One of the limitations of serodiagnosis by MAT is the prolonged period after recovery for which agglutinating antibodies can be detected. In an endemic population, a single elevated titer cannot be relied upon for diagnosis and it is usual to examine paired (acute-phase and convalescent) sera. A 4-fold increase in titer between these paired sera is usually accepted in confirming a current case leptospirosis. However, a presumptive diagnosis of leptospirosis can be made in the presence of clinical symptoms suggestive of the disease and a single elevated titer. The threshold titer for such presumptive diagnosis depends upon the prevalence of leptospirosis in the population, and may be as low as 1:200⁶ or as high as 1:800.¹ It is noted that high MAT titers may be retained for several months after acute infection.⁷ Moreover, individuals who have had leptospirosis can retain high levels of IgM and IgG antibodies for months and even years.⁸⁻¹⁰

This study compares the efficacy of an IgM-ELISA and the MAT for diagnosis of acute leptospirosis over time. Three blood samples were usually taken, 2 in the acute phase of illness and 1 in the convalescent phase. During the study period the overall seroprevalence at a titer of 1:50 was 18.5% in rural and urban communities in Barbados,^{11,12} while the prevalence in children was 12.5%.¹³

PATIENTS AND METHODS

Patients were admitted to a diagnostic protocol if they presented with an acute febrile disease and there was clinical suspicion of leptospirosis. The diagnostic protocol has been described elsewhere.¹⁴ Cultures of blood, urine, and dialysate fluid (when available) were made in EMJH medium.² The period of this study was 11 years, from January 1979 to December 1989 inclusive. The study was approved by the Ethics Committees of the Ministry of Health and the Environment, Barbados, the Queen Elizabeth Hospital, University of West Indies, Barbados, and the Medical Research Council in the United Kingdom. Informed consent was obtained from each study subject before entry into the protocol.

Blood samples were taken from patients at the time of hospitalization (median = 5 days after onset of symptoms) and 4-7 days later (median 8 days after the onset of symptoms), in the acute phase of illness and then later in the convalescent phase of illness (median = 28 days after onset).

The two serologic tests used were a genus-specific ELISA and the MAT. The IgM-ELISA used either a *Leptospira biflexa* patoc 1 or an *L. interrogans* serovar *copenhageni* wijnberg antigen.⁵ A current case was confirmed by a 4-fold increase in titers between any 2 samples or an IgM titer ≥ 160 in a single sample. The MAT was performed using standard microtiter methodology.¹⁵ A current case was confirmed by a 4-fold increase in titers between any 2 samples or an MAT titer ≥ 800 in any sample.

A battery of 12 pathogenic serovars, representing 12 serogroups, were used as antigens throughout the study. An additional 10 serovars were added in November 1983 after they had been isolated and found to be new serovars. These included *Autumnalis bim* and *Australis bajan* and *barbadensis*. Seven serogroups of *L. interrogans* were recorded as most commonly reacting with the sera from our patients. These serogroups (serovars) were as follows: *Ballum (arborea, ballum)*, *Canicola (canicola)*, *Pyrogenes (pyrogenes)*, *Icterohaemorrhagiae (copenhageni)*, *Autumnalis (bim, fortbragg)*, *Panama (panama)*, and *Australis (bajan, barbadensis, bratislava)*. The non-pathogenic patoc 1 strain of *L. biflexa* (serogroup Samaranga, serovar *patoc*) was used as a

TABLE 1
Summary of ELISA IgM test and microscopic agglutination test (MAT) results: data and tests of efficacy*

	First acute-phase specimen			Second acute-phase specimen			Convalescent specimen		
	Isolate case	Non-lepto case	Total	Isolate case	Non-lepto case	Total	Isolate case	Non-lepto case	Total
ELISA IgM									
Positive	48	15	63	67	5	72	62	12	74
Negative	44	298	342	8	216	224	2	185	190
MAT									
Positive	28	4	32	47	4	51	51	5	56
Negative	64	309	373	28	217	245	16	192	208
ELISA IgM or MAT									
Positive	52	19	71	70	9	79	64	17	81
Negative	40	294	334	5	212	217	3	180	183
N	92	313	405	75	221	296	67	197	264
Summary of tests of efficacy									
	%	95% CI		%	95% CI		%	95% CI	
ELISA IgM									
Sensitivity	52	41–63		89	80–95		93	83–98	
Specificity	95	92–97		98	95–99		94	90–97	
PPV	76	64–86		93	85–98		84	73–91	
MAT									
Sensitivity	30	21–41		63	51–74		76	64–86	
Specificity	99	97–100		98	95–100		97	94–99	
PPV	88	71–96		92	81–98		91	80–97	
ELISA IgM or MAT									
Sensitivity	57	46–67		93	85–98		96	87–99	
Specificity	94	91–96		96	92–98		91	87–95	
PPV	73	61–83		89	79–95		79	69–87	

* lepto = leptospirosis; CI = confidence interval; PPV = positive predictive value.

genus-specific antigen for detection of antibodies against any serogroup.

Sensitivity and specificity were assessed using 2 acute-phase specimens (A1 and A2) and the convalescent phase specimen (C) to assess test efficacy in specimens taken at different times. The positive diagnosis of 71% of the patients in this study was dependent on serologic tests. Since diagnosis in these cases was not independent of the screening tests, they were not used when assessing the sensitivity and specificity of the tests. Only the cases positively diagnosed by isolation of leptospires, independent of any serologic test, were used in the calculation of sensitivity and specificity.

RESULTS

During the 11-year study period, 638 patients presented with symptoms suggestive of leptospirosis and were admitted into the diagnostic protocol. There were 321 confirmed cases of leptospirosis and 317 non-leptospirosis cases. The sex ratio was similar in both leptospirosis and non-leptospirosis cases. The age distribution in men was similar in leptospirosis and non-leptospirosis cases ($P = 0.125$, by Wilcoxon rank sum test), with a median of 37 years (range = 14–85 years). The age distribution in non-leptospirosis cases in women was similar to that in men; however, the age distribution of women with leptospirosis had a median of 55 years (range = 23–79 years) ($P = 0.003$, by Wilcoxon rank sum test). The higher proportion of leptospirosis cases in young men compared with women probably reflects the lev-

els of exposure in younger men entering the working environment and the type of work they undertake. More men than women are outdoor workers, particularly agricultural workers, who may be exposed to contaminated soil and water.¹⁶

Of the 321 leptospirosis cases, there were 92 cases confirmed by isolation of leptospires (28.7%). Of these culture-positive patients, all 92 had a blood specimen for serology taken at the time of hospitalization (A1), 75 had a second specimen taken during the acute phase (A2), and 67 had a specimen taken in the convalescent phase (C). Of the 317 non-leptospirosis cases, 313 had a blood specimen for serology taken at hospitalization (A1), 221 had a second specimen taken during the acute phase (A2), and 197 also had a specimen taken in the convalescent phase (C). Sixteen of the 92 culture-proven cases died in hospital, after having a single blood sample taken. In these 16 cases, leptospirosis was confirmed as the cause of death subsequently by growth of an isolate in culture.

The results of serologic tests performed on all specimens are summarized in Table 1. At the time of hospitalization, in the acute phase (A1), the test sensitivities were low: 52% for the IgM-ELISA and 30% for the MAT. The sensitivity of serology increased to 57% when a positive result was considered as either a positive ELISA or MAT result. The positive predictive value was 76% for the ELISA and 88% for the MAT. Of the 317 non-leptospirosis cases, 3.5% had IgM titers of 160 and 2.5% had IgM titers >160 (range = 320–20,480) in the acute (A1) sample. Two non-leptspi-

rosis cases had a 4-fold increase in IgM titer from 40 to 160 in the paired acute-phase sera.

When the second acute-phase specimens (A2) were tested, the sensitivities increased to 89% and 63% for the ELISA and MAT, respectively. When ELISA and MAT results were combined, the sensitivity of serology increased to 93%. The positive predictive values for A2 sample results were higher than those for A1 samples: 93% for the ELISA and 92% for the MAT.

In the convalescent specimens (C), the sensitivity of the ELISA was 93% and the sensitivity of the MAT increased to 76%. The sensitivity of serology increased to 96% if ELISA and MAT results were combined. The positive predictive value was 84% for the ELISA and 91% for the MAT.

Leptospira biflexa strain Patoc 1 antigen was not an effective genus-specific antigen for use as a screening test under our microtiter conditions. It is most sensitive using the second acute-phase specimen, but still only 34% of the cases had a titer \geq 400.

DISCUSSION

Detection of IgM antibodies by ELISA is now widely used in the diagnosis of leptospirosis in specialized laboratories.^{4,5} It has both high sensitivity and specificity if the blood sample is taken several days after the onset of symptoms are first noted, when the IgM antibodies have had time to develop (Table 1). In the acute phase of illness, there may be little correlation between IgM antibody titers and MAT titers. Most patients will first show an IgM response, but those individuals who have had a previous clinical or sub-clinical infection may develop an anamnestic IgG response with high levels of residual antibodies from the previous infection.

The MAT detects both IgG and IgM antibodies,^{7,17} but titers in the MAT increase later than those in other assays.³ The upper limit of sensitivity of the MAT may be as high as 74% by the time of the second acute-phase specimen, collected a median of 8 days after onset of symptoms, as shown in our study. When combined with the detection of IgM by the ELISA, the sensitivity increases to 93% (85–98%) in the second acute-phase specimen.

The specificity of both the IgM-ELISA and MAT was relatively high in the first acute-phase specimen, but the predictive values were low. However, results from the second acute-phase specimen gave predictive values greater than 93% for the IgM-ELISA and greater than 89% for the MAT.

A comparison of the sensitivities between the first (A1) and second (A2) acute-phase specimens demonstrates that using a single specimen for diagnosis is unreliable. It should therefore be mandatory to take paired blood specimens, at least 3 or more days apart, to demonstrate a 4-fold increase in titer to confirm a current case. The interval between paired samples may be short, provided they are taken very early in the acute phase and then a few days later, as in our patients. However, this requires patients to have sought medical assistance within a few days of the onset of symptoms and a high degree of clinical suspicion on the part of the physician, which will be dependent in part upon the range and severity of symptoms. Since the early symptoms of leptospirosis are often regarded as non-specific, in many poorer rural popu-

lations, where medical attention may be both difficult to obtain and costly, it is less likely that early acute-phase samples will be taken. If patients are not ill enough to require hospitalization, then a longer interval between samples may be appropriate, as recommended by the Centers for Disease Control and Prevention (Atlanta, GA).⁶ Frequently, cases are not diagnosed because patients are not tested adequately. This occurs particularly when a single sample is taken at an inappropriate time after the onset of symptoms, and the reason for the negative result is not explained to the requesting physician, often because the date of onset of symptoms is not provided to the laboratory.

Detection of IgM antibodies by ELISA was more sensitive than the MAT in all 3 specimens, and of equivalent specificity. The highest sensitivity was attained when both the IgM-ELISA and MAT were performed on each specimen. In addition to its diagnostic value, the MAT can provide useful epidemiologic data in the form of presumptive serogroup. Several new assays for detection of anti-leptospiral IgM have been recently described.¹⁸ These enable rapid diagnosis early in the course of clinical disease when treatment is most likely to be effective. A negative test result in an initial specimen should require the testing of a convalescent sample, since a small proportion of patients may not produce antibodies until the second or third week after the onset of symptoms.

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Authors' addresses: P. Cumberland, Infectious Disease Epidemiology Unit, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, United Kingdom. C. O. R. Everard, 90 Anchor Road, Calne, Wiltshire, SN11 8EA, United Kingdom. P. N. Levett, Leptospira Laboratory, Enmore 2, Lower Collymore Rock, St. Michael, Barbados.

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