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Latex Agglutination Test for Rapid and Retrospective Diagnosis of Meningococcal Infection

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Patients with clinical manifestations of meningococcal infection were examined for verification of diagnosis. The authors conclude that repeated combined analysis of the cerebrospinal fluid and serum is needed for verification of meningococcal infection in patients with typical clinical symptoms.

Key Words: *meningococci; infection; latex; agglutination; diagnosis*

Early diagnosis of meningococcal infection (MI) and serotyping are important for adequate etiotropic therapy as well as for predicting the epidemiological situation and the use of highly effective serogroup A, C, Y, and W_{135} meningococcal polysaccharide vaccines.

Early prehospital antibacterial therapy sharply inhibits the growth of meningococcal culture and makes the detection of microorganisms with clear-cut morphological signs more difficult. That is why laboratory immunochemical and serological methods aimed at detection of meningococcal antigen in biological fluids, are of special importance for verification of the diagnosis of MI. Among such methods are countercurrent immunoelectrophoresis, enzyme immunoassay (EIA), and latex agglutination test (LAT). LAT has many advantages, as it requires no sophisticated and expensive equipment and reagents, is reproducible at any laboratory, can be performed under field conditions, and detects meningococcal group-specific polysaccharides in the cerebrospinal fluid (CSF) within several minutes [4,8].

LAT is successfully used for the diagnosis of MI; it is far superior to bacteriological methods and is comparable to EIA by sensitivity and specificity [4]. Accumulation of specific meningococcal agents in the CSF precedes an increase in the titers of specific antibodies in the serum of patients with MI. Increase in antibody titer depends on the disease course and is more intensive in meningococcal meningitis than in meningococcal sepsis; it is less expressed in younger children and can be easily detected by various immunochemical methods. EIA showed that during the first week the level of specific antibodies to meningococcus in adult patients increases 6-fold for IgG and 14 and 5-fold for IgA and IgM, respectively [8]. The time course of antibody production and their levels are well demonstrated by LAT, which allows differentiation of antibody isotypes. IgM predominate in the agglutination tests and it is sufficient for diagnostic purposes, because the increment in the level of these antibodies is observed in almost all patients with MI [1,2,6]. It was interesting to compare the diagnostic potentialities and predictive value of latex antigen and antibody meningococcal test systems in serological diagnosis of MI.

MATERIALS AND METHODS

Antigen latex test systems were prepared on the basis of colored polystyrene microspheres (2 μ) with reac-

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tive epoxide groups [2,3,6]. Purified capsular polysaccharides of meningococcus serogroups A, B, and C were isolated as described previously [7] with modifications. LAT was performed in micromodification [2,3]. Meningococcal antigens of serogroups A, B, and C in the liquor were detected using antibody latex diagnostic kits (Merieux). LAT was performed on glass against a dark background. A drop of liquor was added to a drop of the diagnostic agent and shaken on a shaker for 2 min. Agglutination with the appropriate latex within several minutes indicated the presence of group-specific antigen. CSF and serum from patients with suspected MI was obtained in Clinical Hospital for Infectious Diseases No. 2, Moscow. A total of 243 patients were observed, hospitalized during an outbreak of MI in Moscow in 1996 and sporadic cases in 1997 and 1998. SCF samples from 120 patients were obtained on admission and others in the course of MI.

TABLE 1. Laboratory Verification of MI by Analysis of CSF from Patients Hospitalized with Typical Symptoms

Method of detection	Number of positive CSF samples, %
Meningococcal culture growth	14 (11.7)
Meningococcal culture growth+ positive LAT	34 (28.3)
Positive LAT (no meningococcal culture growth)	32 (26.6)
Positive counter-current immunoelectrophoresis	2 (1.7)
Total number of positive CSF samples	82 (68.3)
Total number of CSF samples examined	120 (100)
Number of CSF samples without laboratory detection of the agent	38 (31.7)

RESULTS

Analyses of 120 CSF samples from patients hospitalized with symptoms of MI (Table 1) showed growth of infection agent only in 14, meningococcal group-specific polysaccharides were detected by LAT in 32, and growth of meningococci and the presence of meningococcal antigen detected by LAT were revealed in 34 sera. In two cases meningococcal antigen was detected by counter-current immunoelectrophoresis. In 38 patients the etiology of disease was not confirmed by laboratory tests.

Thus, of 82 cases of laboratory verified MI, LAT was 1.4-fold more accurate and confirmed the diagnosis in 66, while culture growth only in 48 patients. Moreover, in 32 patients the diagnosis was serologically confirmed only by LAT; this notably surpassed potentialities of bacteriological tests which verified the diagnosis in only 14 samples. On the whole, MI was confirmed by laboratory findings in 68.3% examined patients, which is a sufficiently high value. However, almost one-third of MI cases were not confirmed by laboratory findings, which agrees with reports of other scientists and necessitates the search for additional methods of serological diagnosis of this infection. We suppose that LAT with latex antigen diagnosticum developed by us [1-3,6] can be used for this purpose.

Previously we determined diagnostic titers of antimeningococcal serogroup antibodies: 1:128 for serogroup A occuring in 2.5-3% normal human sera, 1:64 for serogroup B normally occuring in 4.5-5.0% cases, and 1:32 for serogroup C occuring in 5% normal sera [1,2,6].

However LAT titration of sera from patients with MI confirmed by laboratory data on the agent serogroup showed that diagnostic titers of serogroup A are rarely detected on days 1-2 after hospitalization (in 7% cases), while after 7-10 days their number increases to 93% (Table 2). Moreover, the number of patients with notable increment in the titers of serogroup A-specific antibodies (4-fold and more) increased to 95% of the total number of examined patients. The number of patients in this group with diagnostic titers of A-specific antibodies in LAT and seroconversion was as high as 98%.

Similar changes were observed in patients with groups B and C meningoccal infection verified by laboratory tests (Table 2).

TABLE 2. LAT Titration of Sera from Patients with MI Confirmed by Laboratory Tests and Identified Serogroups

Identified meningococcus serogroup	LAT titer	Number of examinees, %			Total number of patients with diagnostic titers and positive seroconversion, %
		with diagnostic titer in LAT		with positive seroconversion	
		days 1-2	days 7-10		
A (n=43)	≥1:128	3 (7)	40 (93)	41(95)	42 (98)
B (n=36)	≥1:64	5 (14)	30 (83.3)	31 (86)	33 (92)
C (n=9)	≥1:32	2 (22)	7 (78)	7 (78)	8 (89)

TABLE 3. Serological Verification of MI by LAT with Sera from Patients with Clinical Picture of MI not Verified by Laboratory Tests

Serogroup of detected antibodies	Number of patients with verified MI diagnosis	Serologic diagnosis based on LAT detection of	
		diagnostic antibody titer	seroconversion
A	10	8	2
B	12	9	3
CC	2	2	2
AB	3	2	1
AC	1	1	1
BC	2	2	2
None	5	—	—
Total	35	—	—
With identified serogroup	30	—	—

On days 1-2, the diagnostic titers were detected by LAT in 14% patients, while after 7-10 days this parameter increased to 83.3%. Tests for seroconversion of B-antibodies in 86% patients in this group increased the percentage of serologically confirmed B-meningitis to 92%. The same regularities were observed in LAT with sera from patients with relatively rare C serogroup: 22% diagnostic titers on days 1-2, 78% on days 7-10 and the same number of seroconversions, with the total number of verified diagnoses 89%. Thus, starting from the second week, the diagnostic titers or seroconversion of antimeningococcal serogroup-specific antibodies were detected in the sera of the majority of MI patients.

We also carried out a retrospective LAT analysis of sera from 35 patients with clinical picture of MI without laboratory verification (for various reasons). In the majority of patients blood was collected twice, during the first and second weeks of the disease (Table 3).

Diagnostic titers of antibodies or their positive seroconversion were detected by LAT in 30 of 35 patients (85.7%). Serogroup B sera predominated. This agrees with epidemiological situation in Moscow, where serogroup B predominates in the MI structure. Serogroup A ranked second: high titers of A-antibodies were detected by LAT in 10 patients. Antibodies to relatively rare serogroup C agent were detected in only 2 cases.

Increment of antibody titers to two serogroups described by other authors deserves special attention [5]. In cases with A and B antibodies it is most probably that MI was caused by serogroup A meningococcus, and the increment in B-antibodies is due to polyclonal stimulation of lymphocyte clones producing these antibodies, because they are always present in the serum of healthy subjects and are produced in response to *E. coli* capsular polysaccharides structurally similar to serogroup B *Neisseria meningitidis* polysaccharides.

The increment in B+C and A+C antibodies suggests that the infection is most probably caused by serogroup C meningococci, almost never cross-reacting with normal human flora, while A-polysaccharides occur in nonpathogenic *N. lactamica* in the nasopharynx of healthy carriers.

Hence, the use of antigen latex diagnosticum helps to identify the etiology of MI in cases when the disease could not be diagnosed by laboratory tests of CSF or due to other causes. In our study the serogroup of meningococci was not identified only in 5 patients.

Previously we reported successful trials of LAT with diagnosticum for rare serogroups, such as X, Y, and Z [3]. Presumably, LAT with these diagnosticums and polysaccharide-sensitized *Haemophilus influenzae* can further reduce the percentage of nonconfirmed cases of MI.

Hence, LAT possesses very high diagnostic and prognostic potentialities in urgent diagnosis of MI (analysis of CSF) and in retrospective analysis of sera from patients with MI at different terms of the disease.

Moreover, we previously reported [3] that LAT screening of sera from high-risk subjects in MI helped to detect (by the level of antibodies) subjects who had contacts with the infection agent (asymptomatic carriership, nasopharyngitis, *etc.*) and need no meningococcal vaccination, which notably decreased the number of subjects for whom vaccination with A, A+C, or A+C+Y+W_{135} meningococcal vaccines was obligatory.

REFERENCES

1. A. P. Alliluev, Yu. Ya. Vengerov, I. S. Koroleva, *et al.*, *Biomedical Technologies*, No. 8, Moscow (1998), pp. 85-93.

2. A. P. Alliluev, Yu. Ya. Vengerov, O. V. Kotel'nikova, *et al., Ibid.* Moscow (1996), No. 4, pp. 82-86,.
3. A. P. Alliluev, O. V. Kotel'nikova, N. V. Yashina, *et al., Ibid.*, No. 2, pp. 79-83, Moscow (1995).
4. A. A. Demina, I. M. Samsonova, A. M. Blinkovskii, *et al., Zh. Mikrobiol.*, No. 9, 94-98 (1987).
5. Yu. V. Martynov, *Immunoepidemiological Regularities of Meningococcal Infection*, Abstract of Doct. Med. Sci. Dissertation, Moscow (1991).
6. A. V. Kharitonova, *Test-Systems for Indications of Antibodies to Capsular Meningococcal Polysaccharides Based on Polymeric (Latex) Microspheres*, Abstract of Cand. Med. Sci. Dissertation, Moscow (1997).
7. E. C. Gotshlich, T. X. Liu, and M. S. Artenstein, *J. Exp. Med.*, **129,** 1349-1365 (1969).
8. S. Hartug, E. Rosenqvist, E. A. Hoiby, *et al., J. Clin. Microbiol.*, **24,** No. 6, 947-953 (1986).
9. M. Leinonen and E. Herva, *Scand. J. Infect. Dis.*, **9,** 187-191 (1977).