Acta virol. **35**: 587–592, 1991

COMPARISON OF SEROLOGIC METHODS FOR THE DIAGNOSIS OF MEDITERRANEAN SPOTTED FEVER

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Received March 25, 1991

Summary. – During summer Mediterranean spotted fever (MSF) is prevalent in the subcostal part of Croatia (North Dalmatia) as well as in other areas of the Mediterranean coast. We compared the specificity and sensitivity of complement fixation (CF), latex agglutination (Latex-*R. conorii*), microimmunofluorescence (micro-IF) and enzyme-linked immuno assay (ELISA) for detection and measuring antibodies against *Rickettsia conorii* in 78 sera from 46 patients with/or suspected Mediterranean spotted fever. The seroreactivity with SFG antigens containing the *Rickettsia conorii*-antigen(s) as a common determinant, was positive in all four serological tests suggesting that *Rickettsia conorii* was the probable causative agent of infection in our patients.

Key words: Mediterranean spotted fever; Rickettsia conorii; serological methods

Introduction

Rickettsial disease, especially murine typhus, Mediterranean spotted fever and Rocky Mountain spotted fever continue to constitute an important public health problem in certain areas of the world (Moraga *et al.*, 1982; Harris, 1986; McDonald *et al.*, 1988). Mediterranean spotted fever (MSF) is prevalent during the summer in the North Dalmatian area of Yugoslavia (Borčić *et al.*, 1983), as well as in other parts along the Mediterranean coast (Tartaglia *et al.*, 1939; Punda *et al.*, 1984). In spite of this, until 1987 there was no convenient serological method for diagnosis of MSF in Yugoslavia (Dželalija *et al.*, 1990). Laboratory diagnosis of MSF is usually accomplished by identifying specific antibody titres against *Rickettsia conorii* (Gross *et al.*, 1982; Borčić *et al.*, 1985). In this study we compared complement fixation (CF), latex agglutination (Latex-*R. conorii*), microimmunofluorescence (micro-IF) and immunoperoxidase reaction (IP) for their specificity and sensitivity in detecting and measuring antibodies against *Rickettsia conorii* in 78 sera from patients with/or suspected MSF in the North Dalmatian area.

Materials and Methods

Sera. Seventy-eight sera specimens from 46 patients were submitted from the North Dalmatian area for rickettsial serology during the 1989 season. The patients were classified according to their clinical diagnosis into two groups: patients with MSF (N=27) (tick bite, rash, fever) and patients with suspected MSF (N=19) (febrile patients hospitalized during the same epidemiological period with or without history of tick attachment). Selected sera from 78 blood donors from the same region were controls. All sera were examined for the presence of antibodies to SFG Rickettsia (*R. conorii)* with four serological methods: CF, Latex-*R. conorii,* micro-IF, and IP test.

Complement fixation test. Antigen of SFG rickettsia for CF was kindly provided by dr. Úrvölgyi, Slovak Academy of Sciences, Institute of Virology, Bratislava; it was prepared by ether extraction of rickettsia infected yolk sac, extensive washing and stored at 4 °C. The complement fixation test was performed as published previously (Lennette and Schmidt, 1969).

Latex-Rickettsia conorii test. The group antigen used to coat the latex particles was erythrocyte-sensitizing substance, which was extracted from purified *Rickettsia conorii* (Morrocan strain) kindly provided by dr. Karim Hechemy, Wodsworth, Center for Laboratories and Research, New York State, Albany. The Latex-*R. conorii* test was performed as previously described (Hechemy *et al.,* 1986).

Micro-immunofluorescence. The micro-IF test was performed as described by Philip *et al.* (1976). *Rickettsia conorii*-Spote IF by BioMerieux were used. Interference of rheumatoid factor in the determination of anti-*R. conorii* IgM was avoided by the prior removal of IgG. The reagent was obtained from Behringwerke AG, Germany.

Indirect immuno-peroxidase reaction. Antigen of SFG rickettsia for IP test was kindly provided by prof. dr. Tsunehisa Suto, Department of Microbiology, Akita University School of Medicine, Akita, Japan; the IP reaction was performed according to his recommendations (Suto, 1985).

Statistical methods. To determine differences between four serological methods we used coefficient correlation by Pearson (Petz, 1970).

Results and Discussion

Sera were collected from patients with Mediterranean spotted fever (rash, fever, tick bite) (N=27), patients with suspected Mediterranean spotted fever

Table 1. Assay of sera for antibodies to SFG Rickettsia (*R. conorii)* in North Dalmatian area (Zadar, Yugoslavia)

	Patients with MSF			suspected MSF			Control		
	total number	positive N.	%	total number	positive N.	%	total number	positive N.	%
CF	48	40	83.3	30	21	70.0	78	3	3.8
IFA	48	43	89.5	30	24	80.0	78	4	5.1
Latex-*R.conorii*	48	41	85.4	30	22	73.3	78	2	2.5
IP	48	42	87.5	30	23	76.6	78	3	3.8

(N=19) and blood donors (N=46) from the North Dalmatian area (Yugoslavia). They were examined for the presence of antibodies to SFG Rickettsiae (*Rickettsia conorii*) with four different serological methods: complement fixation (CF test), micro-immunofluorescence (micro-IF), latex agglutination (latex-*R. conorii* test) and immunoperoxidase reaction (IP test).

The sera from all groups contained antibodies to SFG rickettsiae (*Rickettsia conorii)* that were detected more efficiently by micro-IF than CF technique ($r = 0.53$; $p<0.01$) (Table 1). The titres of sera reactive by CF test ranged from 1:16 - 1:32 for patients with MSF, from 1:4 - 1:32 for patients with suspected MSF and from 1:2 - 1:4 for blood donors in the North Dalmatian area (Fig. 1).

The significantly higher seropositivity in all samples by micro-IF than by CF test emphasizes the better sensitivity using micro-IF (Edlinger, 1979). The titres of sera reactive by micro-IF ranged from 1:160 - 1:2560 for patients with MSF, from 1:40 - 1:320 for patients with suspected MSF and lower than 1:40 for blood donors (Fig. 2).

Approximately equal results like by micro-IF we obtained by IP test in all groups (IP test: patients with MSF - 87.5 % positivity ws. 89.5 % by micro-IF, patients with suspected MSF - 76.6 % positivity ws. 80.0 % by micro-IF, and blood donors - 3.8 % positivity ws. 5.1 % by micro-IF) (Table 1). The titres of sera reactive by IP test ranged from 1:320 - 1:5120 for patients with MSF, from 1:80 - 1:1280 for patients with suspected MSF and were lower than 1:40 in

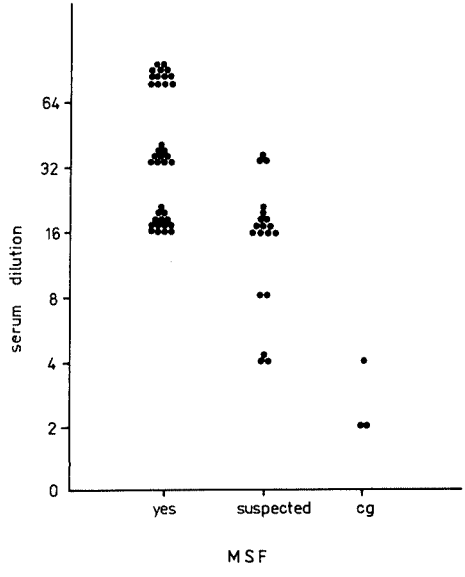

Fig. 1
Antibody titres against *R. conorii* as determined by complement fixation test

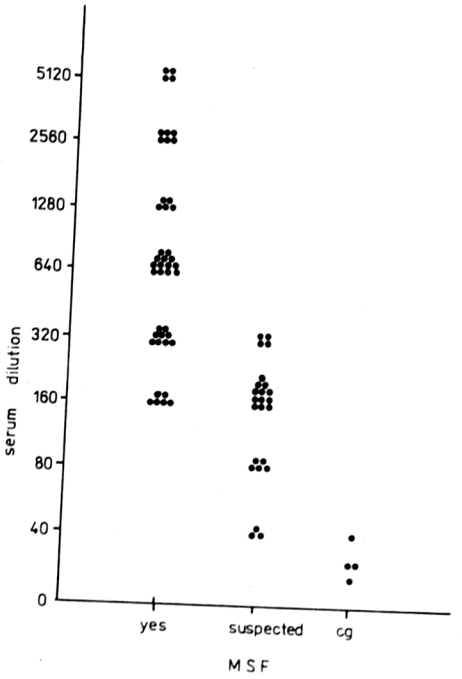

Fig. 2
Antibody titres against *R. conorii* detected by micro immunofluorescence test

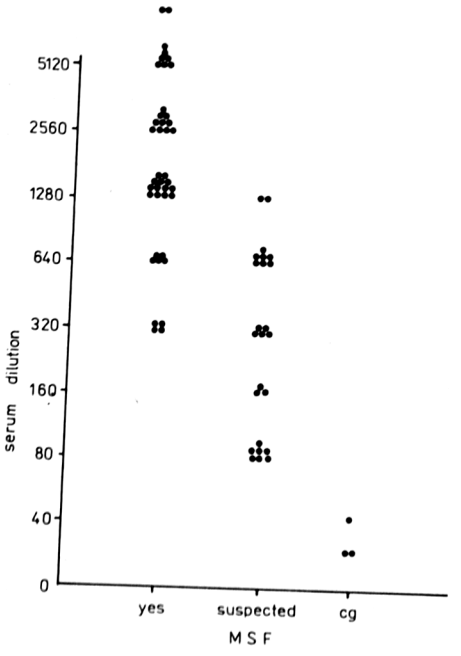

Fig. 3
Antibody titres against *R. conorii* detected by immunoperoxidase test

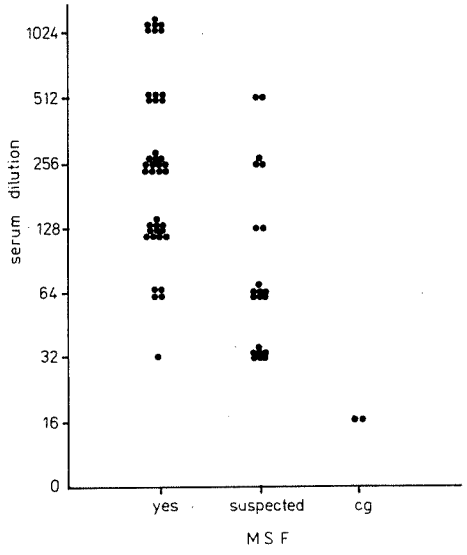

Fig. 4
Antibody titres against *R. conorii* determined by latex agglutination test

blood donors (Fig. 3). It is obvious that in our condition the IP test was as sensitive and specific for SFG Rickettsiae antibody detection as the micro-IF test.

Somewhat lower value of seropositivity we obtained by Latex-*R. conorii* test in comparison with micro-IF and IP tests in each group (Latex-*R. conorii* 85.4 % seropositivity for patients with MSF; 73.3 % seropositivity for patients with suspected MSF and 2.5 % seropositivity in blood donors, donors, Table 1) (IF : $r = 0.66$; $p>0.01$; IP: $r=0.63$; $p>0.01$). This can be explained by using sera from patients in late phase of acute disease or in the convalescent phase. The titres of sera reactive by Latex-*R. conorii* test ranged from 1:32 - 1:1024 for patients with MSF, from 1:32 - 1:512 for patients with suspected MSF and lower than 1:32 in blood donors (Fig. 4).

Summing up, the seroreactivity with SFG antigens containing as a common determinant the *Rickettsia conorii*-antigen(s), was positive in all four serological tests. This suggests that *R. conorii* was probably the causative agent of infection in all our patients. Our results indicate that microimmunofluorescence is the method of choice under our conditions.

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