Acta virol. **32** : 70—74, 1988

DIAGNOSIS OF ENDOCARDITIS IN ACUTE Q-FEVER BY IMMUNOFLUORESCENCE SEROLOGY

D. RAOULT[1], J. ÚRVÖLGYI[2], J. ETIENNE[3], M. ROTURIER[1], J. PUEL[4], H. CHAUDET[5]

[1]Centre National de reference des Rickettsies, CHU la Timone, 13385 Marseille Cedex 5 France, [2]Institute of Virology, Slovak Academy of Sciences, 81703 Bratislava, Czechoslovakia, [3]Service de Bacteriologie, hospital Louis Pradel, 69394 Lyon Cedex 3, [4]Laboratoire de Microbiologie, CHU Purpan, 31052 Toulouse Cedex, and [5]Faculte de Medecine-Boulevard Jean Moulin, Moulin, 13385 Marseille, France

Received February 4, 1987

Summary. — The authors compared two groups of 20 patients suffering from Q fever using microimmunofluorescence (micro IF) serology. One group had endocarditis and the other conventional symptoms of acute Q fever but no endocarditis. Determination of the levels of antibodies against the two phases of rickettsiae in each of the three immunoglobulin classes (IgG, IgM and IgA), allowed to determine the type of infection using a single serum sample. Patients having IgA class antiphase I antibodies at a level equal to/or higher than 1 : 25 as well as those whose antibody levels fulfilled the conditions for the equation (IgG anti-phase I $\geqq$ IgG anti-phase II) + (IgA anti-phase I $\geqq$ IgA anti-phase II) were suffering from endocarditis. The positive predictive value of these tests was 100% and 94.1%, respectively.

Key words: Coxiella burnetii; endocarditis; diagnosis; immunofluorescence

Introduction

Coxiella burnetii (*C.b.*), the etiologic agent of Q fever, is an obligate bacterial pathogen of eucaryotic cells. *C.b.* causes subclinical, acute or chronic endocarditis in man (Raoult *et al.*, 1987). Based on immunological tests, *C.b.* exhibits a phase variation phenomenon (Stoker and Fiset, 1956, Fiset, 1957). In nature or in laboratory animals, *C.b.* exists in the phase I; repeated passages through embryonated eggs results in conversion to phase II (Baca and Paretsky, 1983). The antiphase I antibody response in convalescent patients after acute disease is low, but it is very high (Kazár *et al.*, 1977) in patients with endocarditis. The laboratory diagnosis is usually based on serological tests of which the indirect IF test is frequently used (Hunt *et al.*, 1983, Murphy and Margo, 1980, WHO 1982). This test is economic and requires small amounts of antigen only (Brezina, 1985). The technique permits to distingish between the antibody classes as well as their phase I and

phase II specificity. Peacock *et al.* (1983) have demonstrated that IgG and IgA anti-phase I are diagnostic for endocarditis. The purpose of the present work is to confirm and extend these data.

Materials and Methods

Selection of sera. The patients were hospitalized in following university hospitals in France: Marseille (28 patients), Toulouse (7 patients), Lyon (4 patients) and Paris (1 patient). All patients had clinical features compatible with the diagnosis of Q fever, 15 of them had been reported to have endocarditis (Raoult *et al.* 1985). All patients were examined between years 1984—1986 and had serological evidence of recent Q fever infection as evidenced by seroconversion, or fourfold increase of antibody titre or due to the strong positivity of the micro IF test (equal or greater than 400). Depending on the clinical diagnosis, the patients were separated into following groups: endocarditis group and acute (or subacute) Q fever group; 20 patients were selected for each group. The first diagnostic serum of each patient was included into the study.

Antigens. Phase I (strain Nine Mile *C.b.*, egg passage (EP) 6) and phase II (prepared from strain Nine Mile *C.b.* EP 6 by potassium periodate treatment according to Schramek *et al.* 1972) antigens were provided by the World Health Organisation reference centre, Institute of Virology, Slovak Academy of Sciences (Bratislava, Czechoslovakia). The antigens were mixed with bovine albumin and disposed on 30 spot slides (Dynatech) and fixed for 20 min in cold acetone.

Serological procedures. The sera were diluted in phosphate buffered saline (PBS) (from 1 : 25 to 1 : 6400). After serum distribution, the slides were incubated for 30 min at 37 °C in a moist chamber and washed 3 times in PBS. Conjugated anti-serum was then added (bio-Merieux goat IgG fraction either to anti-human immunoglobulin G or to IgM or goat IgG fraction to anti-human IgA prepared in the Institut Pasteur). It was allowed to react 30 min in a moist chamber at 37 °C and then washed 3 times in PBS. Then the slides were air dried and mounted in buffered glycerol. The slides were read with a Nikon fluorescent microscope with a ×40 objective. The first experiments showed big discrepancies between the results of consecutive trials. These facts prompted us systematically to remove the serum IgG before determining the serum IgM and IgA levels. The Behring Rheumatoid factor absorbant was used for this purpose. It was diluted in water (1 : 5) and then mixed with the serum diluted in PBS (1 : 5) and centrifuged.

Statistical analysis. The purpose of this work was to compare two series of patients with endocarditis or without endocarditis in terms of serological parameters measured to phase I and phase II. Three variables were analysed:

— the geometric mean of each immunoglobulin (IgG, IgM and IgA) against the two phases of rickettsiae in both groups which were compared using the student's test.

— an automatic classification was performed on two variables IgG phase I $\geq$ IgG phase II and IgA phase I $\geq$ IgA phase II. These data were analysed by the hierarchic ascendent classification of Russel and Rao (Benzecri, 1973).

— the predictive value of a single serum of IgA phase I greater than or equal to 25.

Results

The data are presented in Tables 1 and 2. With respect to IgA and IgG there was a significant difference between patients with endocarditis and simple acute cases (with $p < 0.001$). We also found a significant difference when comparing the results of the two phases inside the acute case group (S above the 0.05 limit). Using the Student's test we did not notice any difference between IgG and IgA phase I and phase II within the endocarditis group. In the acute cases group IgG, IgM and IgA phase II were significantly higher than IgG, IgM and IgA phase I. In differentiating endocarditis from other acute cases, the IgA phase I levels higher than or equal to 25 revealed a specificity of 100%. Using the equation (IgG phase I $\geq$ IgG

Table 1. Titres of anti-phase I and II antibodies to Coxiella burnetii as determined by the mieroimmunofluorescence in 20 cases of acute Q fever

Case	Sex	Age	Phase I			Phase II		
			IgG	IgM	IgA	IgG	IgM	IgA
1	M	47	6400	0	0	6400	400	400
2	M	26	0	0	0	800	400	0
3	M	41	200	0	0	3200	400	0
4	M	35	0	0	0	200	200	0
5	M	55	200	0	0	800	200	0
6	M	51	0	0	0	800	400	100
7	M	17	800	0	0	1600	100	0
8	M	60	0	0	0	200	200	50
9	F	82	100	0	0	1600	100	25
10	F	3	0	0	0	1600	200	25
11	F	60	400	50	0	800	800	0
12	M	33	0	0	0	1600	1600	1600
13	M	36	0	0	0	3200	400	100
14	M	35	400	400	0	6400	800	100
15	M	40	0	200	0	800	1600	100
16	M	60	25	25	0	800	100	0
17	M	40	200	25	0	800	100	0
18	M	37	0	0	0	800	800	800
19	M	67	800	0	0	1600	100	400
20	M	70	800	0	0	1600	100	400

phase II) + (IgA phase I $\geqq$ IgA phase II) we found that 16 out of 20 patients from the endocarditis group were positive as compared to 1 positive out of 20 conventional cases. The difference is significant using the χ^2 test ($p < 0.01$). The sensitivity of the test was 80% (true positive/true positive + false negative), the specificity was 79% (true negative/true negative + false positive), the positive predictive value was 94.1% (true positive + false positive) and the negative predictive value was 92.6% (true negative/true negative + false negative). Adjusting the positive predictive value of the test according to the fact that frequency of endocardits represents about 10% of the sera in a diagnostic laboratory (Tellez *et al.*, 1985), the result is 64%.

Discussion

The results of this study indicate that the method, proposed by Peacock *et al.* (1983) is very efficient. An important step is to remove IgG prior to testing the IgA and the IgM. This is necessary not only because the rheumatoid factor, ofter present in the Q fever endocarditis, may cause false positive IgM, but also because the very high levels of IgG can saturate antigen sites and cause false negative results for both IgM and IgA. Indeed som sera has IgG level as high as 1,000.000. In this work we evaluated the sensi-

Table 2. Titres of phase I and phase II antibodies to Coxiella burnetii as determined by the microimmunofluorescence in 20 cases of Q fever endocarditis

Case	Sex	Age	Phase I			Phase II		
			IgG	IgM	IgA	IgG	IgM	IgA
1	M	44	3200	100	200	3200	200	200
2	5	64	1600	0	100	1600	0	0
3	M	19	3200	0	100	3200	0	800
4	M	48	6400	400	400	5400	200	200
5	F	51	1600	0	100	1600	0	100
6	M	59	6400	100	25	1600	100	25
7	M	52	6400	6200	6400	64000	6400	6400
8	M	47	1600	100	200	1600	100	200
9	F	78	3200	100	100	1600	100	25
10	F	67	6400	100	1600	6400	100	1600
11	M	75	6400	800	100	3200	800	25
12	M	60	6400	1600	200	6400	1600	200
13	F	53	6400	1600	100	6400	1600	100
14	F	56	6400	1600	1600	6400	1600	1600
15	F	56	6400	200	1600	6400	200	800
16	M	45	1600	25	400	1600	0	400
17	M	49	6400	200	800	6400	200	1600
18	M	64	6400	800	800	6400	400	800
19	M	53	3200	50	1600	6400	50	3200
20	F	56	6400	800	1600	6400	800	1600

tivity, the specificity and the predictive value of the micro IF in determining the type of infevtion: the acute and the chronic endocarditis. To our knowledge, this is the first work including enough patients to perform a statistical analysis. It is confirming that IgG phase I and IgA phase I antibodies are diagnostic for endocarditis as reported by Peacock *et al.* (1983), Edlinger (1985) and Warwick and Marmion (1985). Our study is different from Peacock's work because we did not segregate granulomatous hepatitis from acute cases due to the fact that hepatic granuloma is usually present in acute cases and the liver biopsy is not often available to confirm granulomatous hepatitis. This explains why the levels of IgG phase I could be elevated as reported in hepatitis cases. Our work differs also from the report from Edlinger that some patients with endocarditis have no IgA phase I antibody which in his work seemed correlate with the prognosis. We introduced two different approaches to the serological diagnosis of endocarditis: the significant level of positivity was found to be 1 : 25 for IgA phase I (100% of patients) and a level of 100 was found to detect 95% of the patients. Otherwise, we compared the levels of IgG and IgA anti-phase I and anti-phase II in the two groups. Keeping as the upper dilution 1 : 6 400, we found that 80% of patients with endocarditis had IgG and IgA phase I antibody equal to/or higher than phase II. If a patient has two parameters positive, the probability he has an endocarditis is high. In our work the IgM

levels, either anti-phase I or anti-phase II, of endocarditis patients were impredictable. This result is in agreement with the work of Peacock *et al.* (1983).

We conclude that examination of a single serum sample by micro IF can differentiate recent acute cases and endocarditis in Q fever. If the equation: (IgG anti-phase I $\geqq$ IgG anti-phase II) + (IgA anti-phase I $\geqq$ IgA anti-phase II) is positive and if the level of IgA is higher than 1 : 25 the patient may have endocarditis. It is very important to determine whether the patient has an endocarditis, because in such case the duration of therapy is long and the prognosis is poor (Raoult *et al.*, 1987).

References

Baca, O. G., and Paretsky, D. (1983): Q fever and *Coxiella burnetii:* a model for host parasite interactions. *Microbiol. Rev.* **47**, 127—149.

Benzecri, J. P. (1973): L'analyse de donnees. Tome 2: l'analyse des correspondance. Dunod ed. Paris.

Brezina, R. (1985): Diagnosis and control of rickettsial diseases, pp. 409—430. In J. Kazár (Ed.): *Rickettsiae and Rickettsial diseases.* Publishing House of the Slovak Academy of Sciences, Bratislava 1985.

Edlinger, E. (1985): Immunofluorescence serology. A tool for prognosis of Q fever. *Diag. Microbiol. Infect. Dis.* **3**, 343—351.

Fiset, P. (1957): Phase variation of *Rickettsia (Coxiella) burnetii:* response in guinea pigs and rabbits. *Can J. Microbiol.* **3**, 435—445.

Hunt, J. G., Field, P. R., and Murphy, A. M. (1983): Immunoglobulin responses to *Coxiella burnetii* (Q fever): single serum diagnosis of acute infection using an immunofluorescence technique. *Infect. Immun.* **39**, 977—981.

Kazár, J., Schramek, Š., and Brezina, R. (1977): Analysis of serum immunoglobulin in a patient with Q fever and endocarditis. *Bratisl. Lek. Listy* **67**, 109—113.

Murphy, A. M., and Magro, L. (1980): IgM globulin response in Q fever *(Coxiella burnetii)* infections. *Pathology* **12**, 391—396.

Peacock, M. G., Philip, R. N., Williams, J. C., and Faulkner, R. S. (1983): Serological evaluation of Q fever in humans: enhanced phase I titers of immunoglobulin G and A are diagnostic for Q-fever endocarditis. *Infect. Immun.* **41**, 1089—1098.

Raoult, D., Drancourt, M., De Micco, C., Durand, J. M., Neari, M., Charrel, C., Bernard, J. P., Callais, H., and Casanova, P. (1985): Les hepatites de la fievre Q, a propos de 14 cas. *Sem. Hop. Paris*, **62**, 997—999.

Raoult, D., Etienne, J., Massip, P., Iacono, S., Prince, M. A., Beaurain, P., Benichou, S., Auvergnat, J. C., Mathieu, P., Bachet, P., and Serradimigni, A. (1987): Q fever endocarditis in the south of France. *J. Infect. Dis.* **155**, 570—573.

Schramek, Š., Brezina, R., and Úrvölgyi, J. (1972): A new method of preparing diagnostic Q fever antigen. *Acta virol.* **16**, 487—492.

Stoker, M. G. P., and Fiset, P. (1956): Phase variation of the Nine Mile and other strains of *Rickettsia burnetii. Can. J. Microbiol.* **2**, 310—321.

Tellez, A., Sainz, C., Echevarria, C., De Carlos, S., and Fernandez, M. V. (1985): Q fever in Spain. Evaluation of acute and chronic cases seen during 1982—1983, pp. 398—405. In J. Kazár (Ed.): *Rickettsiae and Rickettsial Diseases.* Publishing House of the Slovak Academy of Sciences, Bratislava.

World Health Organisation (1982): Rickettsiosis: a continuous disease problem. *Bull. Wrld. Hlth Org.* **60**, 157—164.

Worswick, D., and Marmion, B. P. (1985): Antibody responses in acute and chronic Q fever and in subjects vaccinated against Q fever. *J. Med. Microbiol.* **19**, 281—296.