Serological differentiation between acute (late control) and endocarditis Q fever

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Abstract

***Aims*—To differentiate the serological profiles of chronic (endocarditis) Q fever from the late follow up of acute cases.**

***Methods*—Twenty patients (10 diagnosed with acute and 10 with endocarditis Q fever) were studied. Those diagnosed with acute infection were followed up from 2·5 to 88 months (mean 35·8 months). Serological variables included indirect immunofluorescence against phase I and II of *Coxiella burnetii* (IgM, IgG, and IgA), complement fixation and rheumatoid factor (RF).**

***Results*—All patients with titres of IgA against phase I, after IgG removal, equal to or above 320 and a complement fixation value equal to or above 128 had endocarditis. No patient with acute Q fever had such a serological profile.**

***Conclusions*—The combination of IgA against phase I and complement fixation values may be sufficient to differentiate the serological profile of chronic (endocarditis) Q fever from the late follow up of acute cases.**

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The serological diagnosis of acute Q fever is based on the demonstration of a significant increase in the titre to the phase II antigen of *Coxiella burnetii* in sera collected during the febrile and convalescence phases of the illness.[1–11] On the other hand, chronic Q fever is characterised by the appearance of a significant titre of antibodies against the phase I antigen of *C burnetii*.[2 6 12–15] There are reports, however, which show that histologically confirmed cases of Q fever endocarditis occurred with lower titres[12] and also that the development of phase I antibodies may be part of the normal response to acute infection if the serological follow up of these patients were long enough.[1–3 7]

Peacock *et al* presented a clearer serological picture of acute and chronic Q fever by determining antibodies against phases I and II by complement fixation, microagglutination, and immunofluorescence (IgG, IgM, and IgA) tests.[6] Although our current knowledge of the serological profiles of acute and chronic (granulomatous hepatitis and endocarditis) Q fever is more complete, the "late" serological follow up of acute Q fever patients has not been extensively studied. The duration of the different antibodies produced after an acute infection by *C burnetii* is not well known. In many countries, including Spain,[16] Q fever is a rather common infection, so many people may have antibodies against *C burnetii*. The differentiation of these residual antibodies from those present in patients with chronic Q fever, particularly endocarditis, is essential because culture negative subacute bacterial endocarditis by micro-organisms other than *C burnetii* may occur in patients with residual antibodies after an acute episode of Q fever. In such cases the patient could be misdiagnosed with endocarditis: some subacute bacterial endocarditis infections are culture negative, when the patient has received antibiotics or the micro-organism is an enterococcus or a fastidious pathogen.[17]

In this study we evaluated the serological profiles of patients who had had an acute episode of Q fever and who were serologically followed up for up to 88 months. These results were compared with those obtained in culture histologically confirmed cases of Q fever endocarditis. The possible interference of an excess of IgG antibodies and rheumatoid factor were also evaluated by using anti-human IgG to remove this immunoglobulin from the samples.

Methods

Twenty patients diagnosed as having Q fever from 1983 to 1991 were selected. Ten of them had acute Q fever on the basis of clinical symptoms and complementary explorations including seroconversion for phase II antigen of *C burnetii* by complement fixation. The remaining 10 patients were diagnosed as having chronic Q fever based on clinical symptoms, complementary explorations, serological tests and, in four cases, by isolation of the micro-organism from tissues. All these patients had endocarditis; the clinical and microbiological data of three of these cases have been published before.[18] Sera obtained from these 20 patients were stored at −20°C until use.

Complement fixation was performed using a microdilution test[19 20] using *C burnetii* ATCC VR-616 (Virion, Switzerland) which contains phase II antigen.

Indirect immunofluorescence assay (IFA) against phases I and II from *C burnetii* was carried out by a microtechnique according to a previously described assay,[20 21] using antigens kindly supplied by MG Peacock (Rocky Mountain Laboratories, Hamilton, Montana, USA) at a final concentration of 5 μg/ml.

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Table 1 Reciprocal C burnetii titres and rheumatoid factor in patients with acute Q fever (late follow up)

Case No	Time*	Complement fixation	Phase I IgG	Phase I IgM	Phase I IgM†	Phase I IgA	Phase I IgA†	Phase II IgG	Phase II IgM	Phase II IgM†	Phase II IgA	Phase II IgA†	Rheumatoid factor (IU/ml)
1	33	32	640	<20	<20	<20	<20	320	<20	20	20	<20	<20
2	88	64	320	<20	<20	320	320	1280	20	40	<20	<20	<20
3	19	256	1280	40	20	80	80	⩾2560	320	640	320	320	<20
4	21	32	160	20	20	<20	<20	1280	320	320	40	40	<20
5	16	64	80	160	80	20	20	1280	640	320	80	80	<20
6	26	64	640	40	40	320	320	⩾2560	80	40	80	80	<20
7	8	256	⩾2560	160	320	40	40	⩾2560	40	320	20	20	<20
8	76·5	32	320	80	80	40	20	320	320	320	20	20	<20
9	2·5	<8	320	160	160	40	20	⩾2560	80	80	40	20	20
10	62	64	320	<20	<20	<20	<20	640	<20	<20	<20	<20	<20

*Months since the first (acute) serum sample. †Titres after IgG removal.

Briefly, 10 μl of antigen suspension was placed on 10 spot slides, fixed with cold acetone for 10 minutes, air dried, and stored at −70°C until required. Sera (20 μl) diluted in phosphate buffered saline (PBS) (pH 7·2) ranging from 1 in 20 to 1 in 2560, was added and allowed to react at 37°C for 30 minutes. After a 10 minute wash in PBS and air drying, 20 μl of specific fluorescein conjugate rabbit antihuman IgG (γ chain), IgM (μ chain), or IgA (α chain) (Behring, Marburg, Germany) at appropriate dilution was added. The slides were then incubated at 37°C for 30 minutes, washed for 10 minutes in PBS and rinsed in distilled water. After being air dried, the slides were mounted in FA mounting fluid, pH 9·0 (Difco, Detroit, Michigan, USA) and examined under epifluorescence microscopy. Positive and negative controls were included with each run.

Removal of IgG was done by using IgG inactivation with GullSORB (Gull Laboratories, Salt Lake City, Utah, USA) following the manufacturer's recommendations.

Rheumatoid factor (RF) titres of sera were determined by Rapi Tex-RF (Behring, Marburg, Germany).

Results

Table 1 shows the serological profiles of the late follow up of acute Q fever cases. These 10 patients were followed up from 2·5 to 88 months (mean 35·8) since the first (acute) serum sample was taken. Most had high titres of IgG against phases I and II of *C burnetii* but the titres of IgM and IgA, with and without IgG absorption, against both phases were rather low as was the complement fixation test. The removal of IgG antibodies did not affect significantly the results of IgM and IgA determinations as a whole, but in one case antibodies of the IgM class against phase II increased after IgG was removed. Rheumatoid factor was negative or very low.

Table 2 shows the serological profiles of endocarditis Q fever cases. High titres against *C burnetii* determined by complement fixation as well as by indirect immunofluorescence assay against phases I and II were found. Great variation in the titres, however, was observed among most immunoglobulins, the most constant being the high titres of IgA and IgG against phase I (equal to or above 640 in all cases). The complement fixation value was always positive at titres equal to or greater than 128. The removal of IgG antibodies affected some serological results, especially the IgM titres against *C burnetii* phase II, and to a lesser extent, against phase I, such titres being, in all except one case, lower after immunoglobulin removal.

As far as the titres of IgA is concerned, there were no significant changes in the results obtained with or without IgG removal in six out of the 10 endocarditis cases. In cases 3 and 5 a pronounced reduction of the IgA titres against phase II was obtained after IgG removal. On the contrary, in case 2 a higher titre of IgA against phase II was obtained when IgG was removed. Rheumatoid factor appeared, usually at high titres, in all endocarditis cases.

Patients with endocarditis had high titres of IgA against phase I of *C burnetii* as well as high titres by complement fixation these being higher than in patients with acute Q fever (late follow up). Combining these two sets of data it is possible to discriminate between the two processes: all patients with

Table 2 Reciprocal C burnetii titres and rheumatoid factor in patients with Q fever endocarditis

Case No	Complement fixation	Phase I IgG	Phase I IgM	Phase I IgM†	Phase I IgA	Phase I IgA†	Phase II IgG	Phase II IgM	Phase II IgM†	Phase II IgA	Phase II IgA†	Rheumatoid factor (IU/ml)
1	⩾1024	640	640	1280	⩾2560	⩾2560	1280	1280	320	640	1280	⩾320
2	⩾1024	⩾2560	1280	160	640	640	1280	320	40	40	⩾2560	160
3	⩾1024	640	⩾2560	⩾2560	⩾2560	⩾2560	1280	1280	160	⩾2560	20	160
4	512	640	80	40	1280	320	160	320	640	1280	1280	40
5	⩾1024	⩾2560	⩾2560	1280	⩾2560	⩾2560	160	⩾2560	320	⩾2560	160	⩾320
6	⩾1024	⩾2560	1280	1280	⩾2560	⩾2560	⩾2560	⩾2560	⩾2560	⩾2560	⩾2560	40
7	⩾1024	⩾2560	1280	1280	⩾2560	⩾2560	⩾2560	640	20	⩾2560	⩾2560	320
8	128	⩾2560	640	160	1280	1280	⩾2560	320	40	160	160	40
9	⩾1024	⩾2560	160	640	640	640	⩾2560	160	40	640	1280	20
10	⩾1024	⩾2560	640	640	1280	1280	⩾2560	1280	320	⩾2560	⩾2560	160

†Titres after IgG removal.

titres of IgA against phase I, after IgG removal, equal to or greater than 320 and a complement fixation value equal to or greater than 128 had endocarditis. On the contrary, no patient with acute Q fever had such a serological profile.

Discussion

Acute Q fever was diagnosed by clinical and serological procedures. Most patients had pneumonitis or fever and only those with clear seroconversion by complement fixation test were selected for this study. Q fever endocarditis was diagnosed by clinical, serological, and in four cases by histopathology and isolation of the micro-organism using a previously published method.[18] The serological profile of acute and chronic Q fever is relatively well known.[1 7-10 14 15] There is little information, however, on the late follow up of acute Q fever,[1-4 7] because most studies have not been followed up for more than one year. The main objective of this work was to find the serological differences between acute Q fever cases with long follow up and Q fever endocarditis. All patients with acute Q fever, studied for long periods of time, were asked about their clinical symptoms and only those who were considered healthy at late follow up were included in the study. The serological follow up of these uncomplicated acute Q fever cases showed a decrease of antibodies of IgM class against phases I and II as well as those detected by complement fixation test (unpublished observations). On the other hand, titres of IgG against phases I and II increased in some patients with time (unpublished observations) in spite of the patients being considered completely cured. So patients cured after an acute Q fever episode do continue forming antibodies of IgG class, which confirms the findings of previously published studies with shorter follow up,[1-3 7] although other authors have stated that antibodies against phase I do not persist for more than one year after the acute infection.[4] In the long follow up of our patients, however, antibodies of the IgG class against phase II but also against phase I increased with time, being detectable 88 months after the acute infection. Moderate titres of IgA against phases I and II were not infrequently found in the late follow up of our acute Q fever cases but most titres were lower than 160. In one case the titre of IgM against phase II was falsely low as was shown after IgG removal.

Endocarditis Q fever was characterised by high IgG, IgM, and IgA titres against phases I and II of *C burnetii*, high titres being determined by complement fixation and the presence of rheumatoid factor. The most constant and characteristic serological pattern of endocarditis Q fever was a high IgA titre against phase I of *C burnetti* which confirms previously published data.[6 11 14 15 22] Among the 20 patients diagnosed with Q fever, all those with titres of IgA against phase I antigen equal to or above 640 belonged to the endocarditis group.

Antibodies of IgA class against phase II also appeared in endocarditis Q fever but the titres varied widely, ranging from 40 to equal to or higher than 2560.

The possible interference of an excess of IgG and rheumatoid factor in the IgM titrations was confirmed, showing that in endocarditis Q fever titres of IgM may be falsely raised by IgG interference.[23] Such interference occurs with phases I and II and seems to be due to the immune complex formed with the IgM rheumatoid factor which could either give false positive reactions or greatly exaggerate the IgM titre.[6] This interference was also observed, but to a lesser extent, for IgA against phase II and I, most titres being lower after IgG removal.[6]

The serological profile of endocarditis Q fever was different from that of the late follow up of acute Q fever. In most cases, therefore, antibodies appearing in Q fever endocarditis may be distinguished from residual antibodies of acute Q fever if IgA against phase I, with or without IgG removal, of *C burnetii* and complement fixation titres are detected at high titres. This confirms the findings of previous reports,[6 11-15 24] although there is a report[22] in which IgA against phase I was detected only in three of the six endocarditis cases, and against phase II in just one of the six aforementioned endocarditis cases. Such differences obtained by different authors must be due to the differences in the antigen preparations. Rheumatoid factor appeared in all cases of Q fever endocarditis when diagnosed and titres decreased only after treatment. Although rheumatoid factor only appeared in one case, and at a very low titre, at late follow up of acute Q fever, it must be taken into account that rheumatoid factor is found in 40–50% of other subacute bacterial endocarditis cases, especially when the illness lasts more than six weeks.[25 26]

Acute Q fever is easily diagnosed by complement fixation test, especially if acute and convalescence sera show a seroconversion. Although many of these patients will continue to have high titres of antibodies (mainly IgG against phases I and II) for a long time, the high titres of IgA against phase I are typical of endocarditis Q fever.

In summary, titres of IgA against phase I antigen equal to or above 640 correlate with endocarditis, and none of our patients with acute Q fever in late follow up had this. As the IgA titre against phase I of *C burnetii* may decrease after IgG removal, the combination of such a titre (equal to or greater than 320) with the complement fixation value (equal to or above 128) correlates equally well with endocarditis, and can differentiate these patients from those healthy subjects who had an acute episode of Q fever several months or years earlier.

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