

## Value of Microimmunofluorescence for Diagnosis and Follow-up of *Bartonella* Endocarditis

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***Bartonella* endocarditis is a disease of emerging importance that causes serious complications and high rates of mortality. Due to the fastidious nature of *Bartonella* species and their high degrees of antibiotic susceptibility, cultures of clinical samples most often remain sterile and valvular biopsy specimens, the best specimens for PCR amplification, are seldom available. Therefore, serology appears to be the easiest diagnostic tool. In order to determine the best cutoff value for serology and its predictive values for the detection of *Bartonella* endocarditis, we studied 48 patients with culture- and/or PCR-confirmed *Bartonella* endocarditis. We also applied these serological criteria to 156 patients with blood culture-negative endocarditis. Furthermore, we compared the kinetics of the antibody responses to *Bartonella* spp. in order to estimate the value of serology for prediction of the occurrence of relapses. A titer of  $\geq 1:800$  for immunoglobulin G antibodies to either *Bartonella henselae* or *B. quintana* has a positive predictive value of 0.810 for the detection of chronic *Bartonella* infections in the general population and a value of 0.955 for the detection of *Bartonella* infections among patients with endocarditis. When this cutoff was applied to 156 patients with blood culture-negative endocarditis, we were able to diagnose *Bartonella* infections in an additional 45 patients with definite endocarditis for whom a positive *Bartonella* serology was the only evidence of infection. On follow-up, the kinetics of the decrease in antibody titers were significantly delayed in two patients with relapses. In conclusion, we recommend the determination of antibodies to both *B. quintana* and *B. henselae* and the use of a cutoff value of 1:800 for the diagnosis of *Bartonella* endocarditis. We propose that this criterion, which may also help with the detection of late relapses, be included as a major criterion in the Duke criteria for the diagnosis of infective endocarditis.**

Four *Bartonella* species (primarily *Bartonella quintana* and *B. henselae* and, in one case each, *B. elizabethae* [5] and *B. vinsonii* subsp. *berkhoffii* [27]) have been implicated as causes of endocarditis in humans. *Bartonella* endocarditis most often occurs in middle-aged patients. Clinical diagnosis relies on a combination of epidemiological and clinical features such as male sex, homelessness, chronic alcoholism, contact with body lice and the absence of a previously known valvulopathy for *B. quintana*, and contact with cats or cat fleas and the presence of a previously known valvulopathy for *B. henselae* (10). *B. henselae* also causes other chronic infections such as chronic cat scratch disease, peliosis hepatis, and bacillary angiomatosis; and *B. quintana* causes chronic bacteremia and bacillary angiomatosis (20).

The most widely used method for the laboratory diagnosis of *Bartonella* infections is serology, with indirect immunofluorescence being the reference technique (26). In our laboratory, two cutoff values are used for the diagnosis of *Bartonella* infections. A titer of immunoglobulin G (IgG) antibodies to *B. henselae* of  $\geq 1:50$  is diagnostic of acute infections such as cat scratch disease with an evolution of less than 3 months (26). For the diagnosis of endocarditis, we use a titer of  $\geq 1:1,600$  for IgG antibodies to either *B. quintana* or *B. henselae*. Although a definite diagnosis of *Bartonella* endocarditis relies on culture,

PCR, or immunohistochemical analysis of valvular biopsy specimens, we have previously demonstrated that such an antibody titer has a positive predictive value of 0.884 (25). However, this cutoff value had been determined with samples from a small number of patients with culture- and/or PCR-proven cases of *Bartonella* endocarditis (25). Moreover, the specificity of the serological method has been questioned due to cross-reactions among *Bartonella* species and between *Bartonella* species and *Coxiella burnetii* (16) and *Chlamydia* species (21). In a study described in a previous article (25), we tested sera from 11 patients with a previous diagnosis of *Chlamydia* endocarditis on the basis of serological data (9, 19). Adsorption procedures indicated that the reactive antibodies detected were most likely directed against *Bartonella* spp. and the existence of *Chlamydia* endocarditis should be questioned. To date, we have collected data for 38 patients with confirmed *B. quintana* endocarditis and 10 patients with *B. henselae* endocarditis.

The purpose of the study described in this report was (i) to determine the best serological cutoff value to be used for the diagnosis of *Bartonella* endocarditis, (ii) to detect additional cases of *Bartonella* endocarditis among patients with blood culture-negative endocarditis by use of this cutoff value, and (iii) to estimate the usefulness of serology for the prediction of relapses of *Bartonella* endocarditis by comparing patients who relapsed with those who recovered.

### MATERIALS AND METHODS

**Study design. (i) Case definition.** Patients were considered to have definite endocarditis on the basis of the criteria of Duke for the diagnosis of infective endocarditis (8). Patients were considered to be suffering from chronic cat scratch disease when the clinical evolution of a typical case of cat scratch disease

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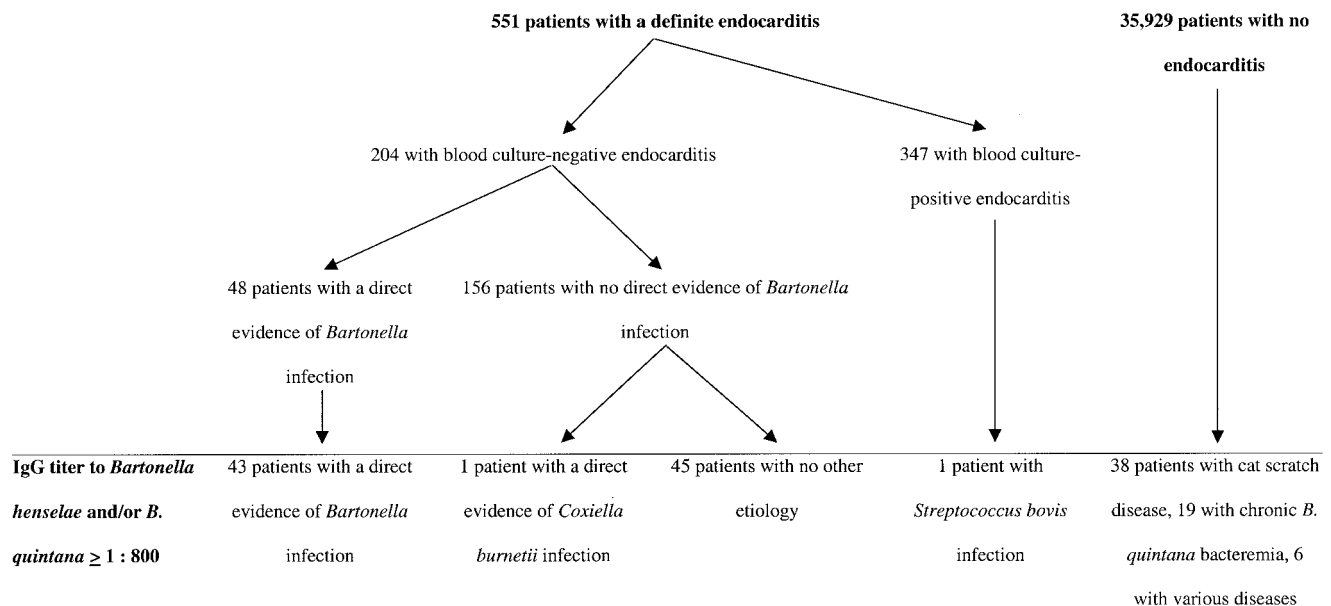


FIG. 1. Distribution of 36,480 patients whose serum samples were tested by the Unité des Rickettsies between January 1995 and December 2000 for antibodies to *B. henselae* or *B. quintana* by the MIF assay.

was longer than 3 months or when visceral involvement was diagnosed. A diagnosis of chronic bacteremia was considered in patients who suffered from persistent bacteremia, as proven by positive blood cultures over a period of several weeks, without any echocardiographic indication of endocarditis.

Patients were considered to be suffering from *Bartonella* infection following direct identification of *B. henselae* or *B. quintana* by culture, PCR, or immunohistochemical analysis of valvular tissue or lymph node biopsy specimens or by culture or PCR of blood specimens. In addition, patients with cat scratch disease who were positive for *Bartonella* by serology and granulomatous lymphadenitis by histological examination of a lymph node biopsy specimen were also considered to have *Bartonella* infection.

(ii) **Patients.** The Unité des Rickettsies is a reference center for the diagnosis and study of rickettsioses and collected data for patients with blood culture-negative endocarditis between January 1995 and December 2000. During that period, we tested 45,000 serum specimens for anti-*Bartonella* antibodies. The serum specimens were obtained from 36,480 patients suspected of having various diseases including arthropod-transmitted infections, pneumonia, endocarditis, pericarditis, myocarditis, meningoencephalitis, or prolonged fever of unknown etiology. For patients for whom several specimens were available, we used the sample with the highest antibody titers. Of these 36,480 patients, 551 had endocarditis, including 204 patients with blood culture-negative endocarditis (Fig. 1). When available, blood or heart valve tissue from these patients was also sent to our laboratory. Systematic testing included *Bartonella* serology and PCR amplification and culture of valve and blood specimens. Among the 204 patients with blood culture-negative endocarditis, 48 patients had a diagnosis of *Bartonella* endocarditis on the basis of microbiological and/or PCR findings. Of these, 10 were infected with *B. henselae* and 38 were infected with *B. quintana*. On follow-up, the physicians in charge of these 48 patients were asked for serum specimens every 3 months for 1 year following the diagnosis. The treatment and outcome for each patient in the year following the diagnosis of culture-negative endocarditis were noted. Of the 48 patients, 46 were considered clinically and microbiologically cured following therapy, but 2 were considered to have relapses. Among the 156 patients with blood culture-negative endocarditis for whom no direct evidence of infection with a *Bartonella* sp. was obtained, data for 45 patients that we considered to have *Bartonella* endocarditis on the basis of elevated titers of antibodies to *B. quintana* and/or *B. henselae* were excluded from the determination of cutoff values.

(iii) **Serology.** *B. quintana* Oklahoma, *B. quintana* Marseille (isolated from a French patient from the present series), *B. henselae* Houston-1 (ATCC 49882<sup>T</sup>; American Type Culture Collection), and *B. henselae* Marseille (6) were used as antigens. The bacteria, grown in human endothelial cells (ECV 304 cells), were harvested, pelleted, and used as crude antigen in a microimmunofluorescence (MIF) assay (4, 26). The pellets were then resuspended in sterile distilled water

so that each suspension had the same density of organisms, as determined optically following Gimenez staining. These antigens were applied with a drawing pen at opposing poles of each well on 30-well microscope slides (Dynatech Laboratories Ltd., Billingshurst, United Kingdom), air dried, and fixed in acetone for 10 min. Twofold serial dilutions of the sera (from 1:50 to 1:3,200) were made in phosphate-buffered saline with 3% nonfat powdered milk and applied to the antigens, and the mixtures were incubated in a moist chamber for 30 min at 37°C. After three washes in phosphate-buffered saline for 10 min for each wash, the slides were air dried and the reactive antibodies were detected with 1:300 dilutions of fluorescein isothiocyanate-conjugated goat anti-human IgG ( $\gamma$  chain; Fluoline G; BioMerieux, Marcy l'Etoile, France) by using the incubation times and the washing procedure described above. The slides were mounted in buffered glycerol (Fluoprep; BioMerieux) and read with a Zeiss fluorescence microscope at  $\times 400$  magnification.

In order to determine the best cutoff value for the diagnosis of *Bartonella* endocarditis, the sensitivity, specificity, and positive and negative predictive values of titers of 1:100, 1:200, 1:400, 1:800, 1:1,600, and  $\geq 1:3,200$  for antibodies to *B. henselae*, *B. quintana*, and either species were compared for the whole population of 36,480 patients. The predictive values of the various cutoffs studied were evaluated in relationship to the expected prevalence of the disease among patients with endocarditis by use of Bayes's theorem, as follows: positive predictive value =  $(SE \times PR) / [(SE \times PR) + (1 - SPE) \times (1 - PR)]$  and negative predictive value =  $[SPE \times (1 - PR)] / [SPE \times (1 - PR) + (1 - SE) \times PR]$ , where PR is the prevalence of the disease in the population concerned, SE is the sensitivity, and SPE is the specificity (Fig. 2). We excluded from this calculation the data for the 45 patients with a positive serology as sole evidence of *Bartonella* infection, as in this preliminary study correct interpretation of the results was not possible.

These serological criteria were then applied to 156 patients with blood culture-negative endocarditis with no etiological diagnosis.

**Graphs and statistical analysis.** Bayesian curves and slopes of linear regression curves of the antibody titers were obtained by use of Microsoft Excel (version 7) software. The slopes of the linear regression curves of antibody titers were compared as variances by Student's *t* test with Epi info (version 6.0) software, which was also used to compare means. The observed differences were considered significant when the *P* value was  $< 0.05$  by two-tailed tests.

## RESULTS

The data used for the diagnosis of *Bartonella* endocarditis in the 48 patients with *Bartonella*-proven endocarditis are presented in Table 1.

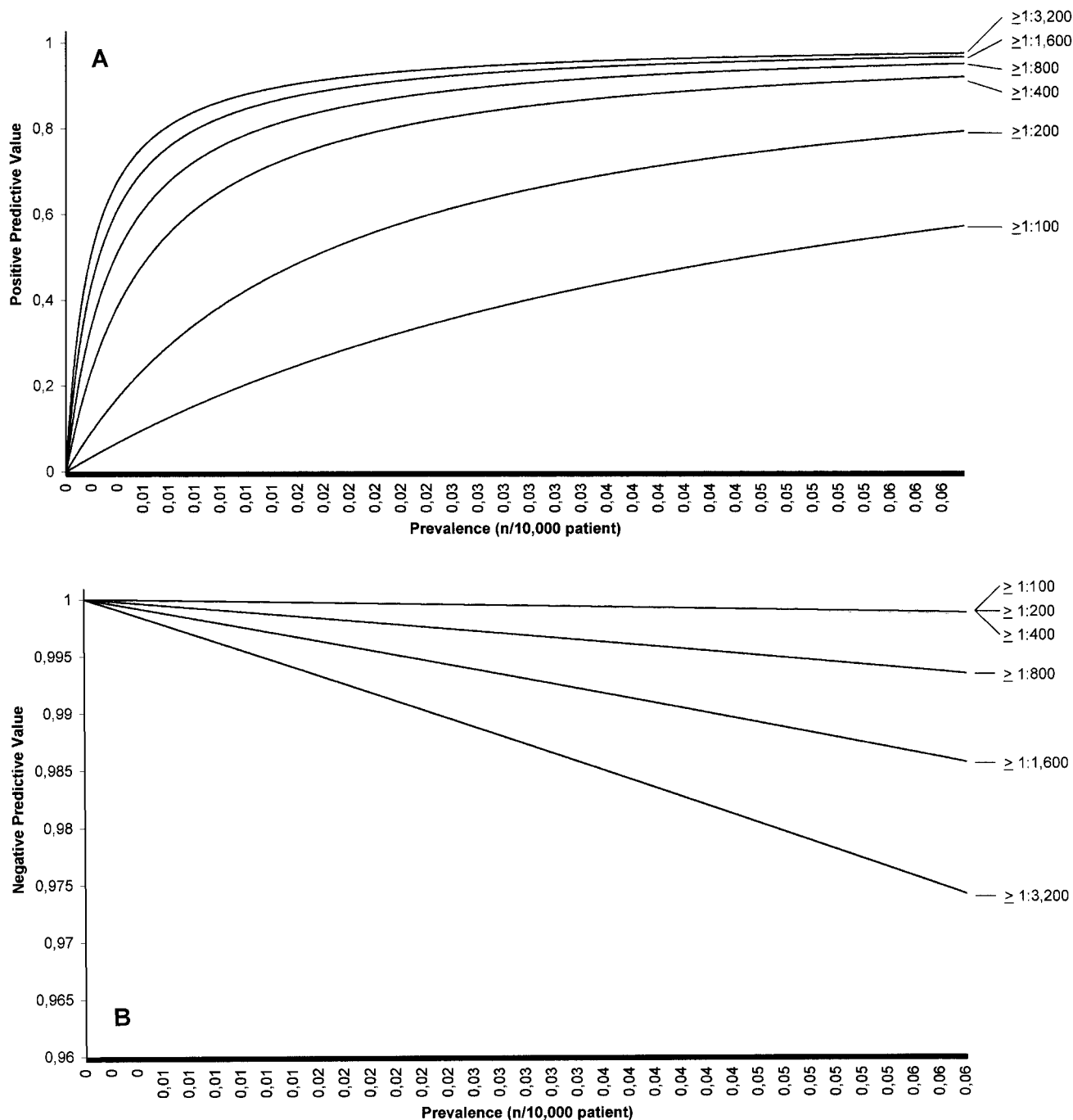


FIG. 2. Evaluation of the positive (A) and negative (B) predictive values of the various cutoffs in titers of antibodies to *Bartonella* spp. for the detection of *Bartonella* endocarditis, tested by comparison of the predictive value of each titer in relationship to the expected prevalence of the disease by use of Bayes's theorem.

**Serology results.** None of the 48 patients had IgM antibodies reactive against *Bartonella* by MIF tests. IgG titers ranged from 0 to 1:51,200. Cross-reactions between the *Bartonella* species tested were observed for all patients with a positive result by serology. Of the 10 patients with *B. henselae* endocarditis, 8 (80%) had titers of IgG antibodies to *B. henselae* greater than those to *B. quintana*, 1 had equal titers of antibodies to both antigens, and 1 had negative results by serological tests with

the three antigens tested. In all nine patients with positive serological test results, the titers of antibodies to both *B. henselae* strains tested were the same. Among the 38 patients with *B. quintana* endocarditis, 12 (31.6%) had titers of antibodies to *B. quintana* greater than those to *B. henselae*, 11 (28.9%) had equal titers of antibodies to *B. quintana* and *B. henselae*, and 15 (39.5%) had lower titers of IgG antibodies to *B. quintana* than to *B. henselae*. When the *B. henselae* and *B.*

TABLE 1. Serology, culture, and DNA amplification results for 48 patients with *Bartonella*-induced endocarditis

Patient (reference)	IgG titer by MIF assay		Results of <sup>a</sup>				Proven diagnosis
	<i>B. henselae</i>	<i>B. quintana</i>	Specific blood culture	PCR with fresh blood	Valvular culture	PCR with valve tissue	
1 (25)	3,200	400	ND	—	—	<i>B. henselae</i>	<i>B. henselae</i>
2 (25)	1,600	800	—	ND	—	<i>B. henselae</i>	<i>B. henselae</i>
3 (25)	12,800	400	—	—	<i>B. henselae</i>	<i>B. henselae</i>	<i>B. henselae</i>
4 (25)	0	0	—	—	—	<i>B. henselae</i>	<i>B. henselae</i>
5 (6)	6,400	1,600	<i>B. henselae</i>	<i>B. henselae</i>	ND	ND	<i>B. henselae</i>
6	3,200	3,200	—	—	<i>B. henselae</i>	<i>B. henselae</i>	<i>B. henselae</i>
7	1,600	200	ND	ND	—	<i>B. henselae</i>	<i>B. henselae</i>
8	3,200	1,600	—	ND	—	<i>B. henselae</i>	<i>B. henselae</i>
9	1,600	800	ND	ND	ND	<i>B. henselae</i>	<i>B. henselae</i>
10	3,200	1,600	<i>B. henselae</i>	<i>B. henselae</i>	ND	ND	<i>B. henselae</i>
11 (25)	12,800	51,200	—	—	—	<i>B. quintana</i>	<i>B. quintana</i>
12 (25)	3,200	3,200	—	—	—	<i>B. quintana</i>	<i>B. quintana</i>
13 (25)	51,200	51,200	<i>B. quintana</i>	<i>B. quintana</i>	ND	ND	<i>B. quintana</i>
14 (25)	51,200	6,400	—	—	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>
15 (7)	3,200	3,200	<i>B. quintana</i>	<i>B. quintana</i>	—	<i>B. quintana</i>	<i>B. quintana</i>
16 (7)	3,200	1,600	<i>B. quintana</i>	<i>B. quintana</i>	—	<i>B. quintana</i>	<i>B. quintana</i>
17 (7)	3,200	3,200	<i>B. quintana</i>	<i>B. quintana</i>	—	<i>B. quintana</i>	<i>B. quintana</i>
18 (18)	12,800	800	—	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>
19	3,200	12,800	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>
20	400	50	ND	ND	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>
21	800	1,600	ND	ND	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>
22	200	400	<i>B. quintana</i>	ND	—	<i>B. quintana</i>	<i>B. quintana</i>
23	200	12,800	—	—	—	<i>B. quintana</i>	<i>B. quintana</i>
24	1,600	800	—	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>
25	6,400	3,200	ND	ND	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>
26	3,200	1,600	ND	ND	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>
27	6,400	800	—	ND	—	<i>B. quintana</i>	<i>B. quintana</i>
28	3,200	1,600	—	ND	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>
29	1,600	1,600	—	ND	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>
30	3,200	3,200	—	ND	—	<i>B. quintana</i>	<i>B. quintana</i>
31	3,200	1,600	ND	ND	ND	<i>B. quintana</i>	<i>B. quintana</i>
32	800	400	—	—	—	<i>B. quintana</i>	<i>B. quintana</i>
33	800	1,600	—	—	<i>B. quintana</i>	—	<i>B. quintana</i>
34	400	400	—	—	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>
35	400	800	ND	ND	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>
36	400	400	—	ND	—	<i>B. quintana</i>	<i>B. quintana</i>
37	1,600	3,200	—	ND	—	<i>B. quintana</i>	<i>B. quintana</i>
38	1,600	800	—	ND	—	<i>B. quintana</i>	<i>B. quintana</i>
39	1,600	1,600	—	ND	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>
40	3,200	1,600	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>
41	3,200	1,600	—	ND	—	<i>B. quintana</i>	<i>B. quintana</i>
42	1,600	3,200	ND	ND	ND	<i>B. quintana</i>	<i>B. quintana</i>
43	3,200	1,600	ND	ND	ND	<i>B. quintana</i>	<i>B. quintana</i>
44	3,200	3,200	ND	ND	ND	<i>B. quintana</i>	<i>B. quintana</i>
45	200	800	—	ND	ND	<i>B. quintana</i>	<i>B. quintana</i>
46	200	800	—	ND	ND	<i>B. quintana</i>	<i>B. quintana</i>
47	200	800	—	ND	<i>B. quintana</i>	<i>B. quintana</i>	<i>B. quintana</i>
48	200	800	—	ND	ND	<i>B. quintana</i>	<i>B. quintana</i>

<sup>a</sup> +, positive; —, negative; ND, not determined.

*quintana* antigens were studied separately, titers of  $\geq 1:800$  for antibodies to *B. henselae* were observed in 9 of 10 patients (90%) with *B. henselae* endocarditis but only 29 of 38 patients (76.3%) infected with *B. quintana*. Titers of  $\geq 1:800$  for antibodies to *B. quintana* were observed in 33 of 38 patients (86.8%) with *B. quintana* endocarditis and 6 of 10 patients (60%) infected with *B. henselae*. The specificity ranged from 0.955 to 0.999 for sera with titers of  $\geq 1:100$  and  $\geq 1:3,200$ , respectively. The negative predictive value for the diagnosis of endocarditis was 0.999 for all titers tested. Titers less than 1:800 had a positive predictive value of  $< 0.398$  for the diagnosis of endocarditis, whereas titers  $\geq 1:3,200$  had a sensitivity of only 0.583. The positive and negative predictive values of all

titers tested are presented in Fig. 2. The 1:800 dilution cutoff had a lower positive predictive value than the 1:1,600 dilution cutoff (0.398 versus 0.672) but a higher sensitivity (0.895 versus 0.771). Therefore, the 1:800 dilution was considered the best cutoff and was adopted in our study. The determination of titers of antibodies to *B. henselae* and *B. quintana* had similar sensitivities, specificities, and negative predictive values, but the positive predictive value was lower for antibodies to *B. henselae* (range, 0.005 and 0.206 for titers of  $\geq 1:100$  and  $\geq 1:3,200$ , respectively) and higher for antibodies to *B. quintana* (range, 0.042 to 0.765).

The distributions of the 36,480 patients tested with regard to their *Bartonella* serologies are presented in Fig. 1. Among

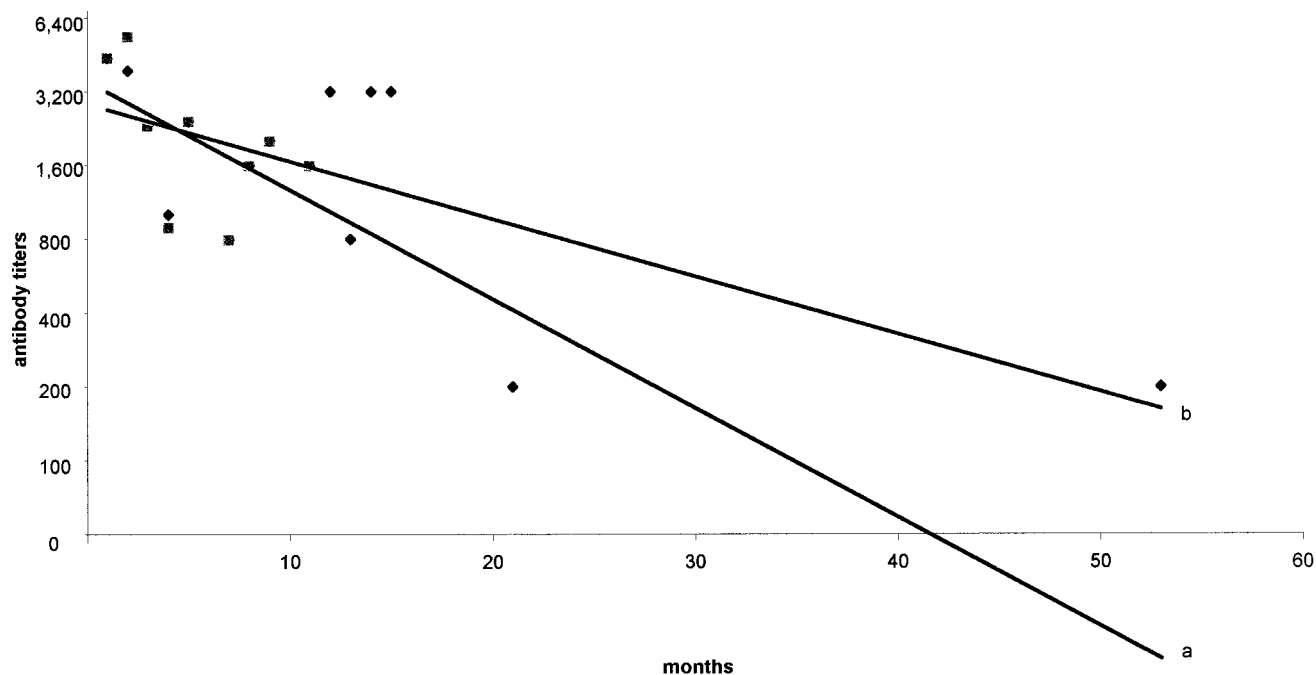


FIG. 3. Comparison of kinetics of titers of IgG antibodies to either *B. quintana* or *B. henselae* in 20 patients with *Bartonella* endocarditis who recovered (curve a) and 2 patients who relapsed (curve b), shown as linear trend curves. Grey squares, titers in serum of patients who recovered; black diamonds, titers in serum of patients who relapsed.

these 36,480 patients, 153 had titers of antibodies to a *Bartonella* species of 1:800 or more. Of these, 90 had endocarditis on the basis of the Duke endocarditis service criteria (8). Among these 90 patients, 43 had endocarditis proved to be caused by *Bartonella*, 45 had positive *Bartonella* serologies as the main evidence of infection and were classified as definitely having endocarditis, 1 had a documented *C. burnetii* infection and the positive serology was considered a cross-reaction (16), and 1 was infected with *Streptococcus bovis*. As none of the other patients from our series positive for endocarditis by blood culture exhibited a positive result for *Bartonella* by serological testing, the last patient described above may have had a dual infection. Sixty-three additional patients with titers of IgG antibodies to a *Bartonella* sp.  $\geq 1:800$  did not have endocarditis, 38 had cat scratch disease (including 17 with chronic infections), 19 were homeless and suffered from chronic *B. quintana* bacteremia, 1 presented with unexplained prolonged fever, 1 had unexplained pericarditis, 1 was diagnosed with a primary cytomegalovirus infection, 1 had legionellosis, 1 had a lymphoma, and 1 had leptospirosis. Altogether, a cutoff of  $\geq 1:800$  had a positive predictive value of 0.810 for the diagnosis of chronic *B. henselae* or *B. quintana* infections.

When the data for the 45 patients who we considered to be infected with a *Bartonella* sp. on the basis of a positive serological test result are discounted, among the 551 patients from our series with endocarditis, the cutoff value of 1:800 had a positive predictive value of 0.955 for the detection of *Bartonella* endocarditis, a negative predictive value of 0.990, a sensitivity of 0.895, and a specificity of 0.996.

When these diagnostic criteria were applied to 156 patients who had endocarditis but who were blood culture negative, 46

had a positive serological test result, including 1 patient with chronic Q fever and 45 patients with no other evidence of infection. Among these 45 patients, 19 (45.5%) had exposure factors consistent with *B. quintana* infection (13 were homeless, 6 were exposed to body lice, and 17 were alcoholics), 10 (22.2%) had exposure factors consistent with *B. henselae* infection (9 reported either cat scratches or bites and 5 reported cat flea bites), and 16 (35.5%) had no specific exposure factor. Follow-up serum specimens were not available from these 45 patients.

Follow-up serum specimens were obtained from 22 of the 48 patients with proven *Bartonella* infections (including the 2 patients who relapsed). The mean number of serum samples from these patients was  $3.5 \pm 1.5$ . The delay of sampling from the onset of therapy ranged from 1 to 53 months. Follow-up results are reported in Fig. 3. Compared to patients who recovered, the kinetics of the decrease in antibody titers in patients who relapsed was significantly delayed ( $P < 0.01$ ) (Fig. 3). However, a single dilution difference was observed 1 year after the diagnosis, as patients who recovered had a median titer of 1:800 and those who relapsed had a titer of 1:1,600 (Fig. 3).

## DISCUSSION

Eighty-one cases of *Bartonella* endocarditis have been reported in the international literature, mostly by our group (1–3, 5–7, 10–15, 18, 23, 24, 28, 29). In a previous study, we estimated that bartonellae are responsible for 3% of all cases of endocarditis (30). To date, we have identified 48 patients with *Bar-*

*tonella* endocarditis on the basis of positive culture and/or PCR results.

The serological tests available at present may not reliably distinguish between antibody responses to *Bartonella* species and *C. burnetii* (16) or *Chlamydia* species (21). Moreover, the demonstrated antigenic cross-reactivity between *Bartonella* and *Chlamydia* species has raised questions about the reliability of the diagnoses of cases of *Chlamydia* endocarditis described in the literature.

Using the MIF assay, we have previously demonstrated that a titer of 1:1,600 had a positive predictive value of 0.884 for the detection of *Bartonella* endocarditis in the general population (25). In the present study, such a cutoff value had a positive predictive value of 0.672 for the detection of *Bartonella* endocarditis in the general population but a sensitivity of only 0.771, whereas a titer of 1:800 had a positive predictive value of 0.398 and a sensitivity of 0.895. The lower positive predictive value observed in the present study compared to that observed in our previous study (25) may be explained by the recent inclusion in our series of homeless patients who suffer from chronic *B. quintana* bacteremia and who thus exhibit elevated antibody titers.

Given the potential severity of *Bartonella* endocarditis and the good outcomes achieved when patients with *Bartonella* endocarditis are treated with aminoglycosides (D. Raoult, unpublished data), we considered it more useful to adopt the titer which had the greatest sensitivity, i.e., a titer of 1:800 for antibodies against *B. quintana* and/or *B. henselae*. We also determined that this cutoff had a positive predictive value of 0.955 for the detection of *Bartonella* infections among patients with endocarditis (Fig. 2).

The specificities of serological tests for the identification of the species causing *Bartonella*-induced endocarditis have been questioned due to the cross-reactivities among *Bartonella* species. In our study, we observed cross-reactions among *Bartonella* species for almost all patients. Moreover, 68% of patients with proven *B. quintana* infections even exhibited higher titers of antibodies to *B. henselae* than to *B. quintana*. The combination of results of tests for both antigens allowed the diagnosis of *Bartonella* endocarditis in 43 of 48 patients with proven *Bartonella* endocarditis, whereas testing for antibodies only to *B. henselae* or *B. quintana* led to the diagnosis of *Bartonella* infection in only 38 and 39 patients, respectively. Therefore, we recommend the determination of titers of antibodies to both *B. quintana* and *B. henselae* for the diagnosis of *Bartonella* endocarditis and propose that a cutoff of 1:800 for the titer of the antibody to either *B. quintana* and/or *B. henselae* be included as a major criterion in the diagnostic score of the Duke endocarditis service (17).

The 1:800 cutoff titer had a positive predictive value of 0.810 for the detection of chronic *Bartonella* infections, including endocarditis, in our population of 36,480 patients tested. This high predictive value of elevated antibody titers for the detection of chronic *Bartonella* infections appears to be specific to our laboratory, as many other series reported in the literature, in which other antigens were used, have reported elevated antibody titers even for patients with acute infections (31).

Using MRL-BA slides (MRL Diagnostics, Cypress, Calif.), we commonly obtained titers of >1:400 for patients with acute cat scratch disease, whereas using our own antigens, we most

commonly determined titers of 1:50 to 1:200 (unpublished data). The difference in the antibody responses between patients with acute and chronic *Bartonella* infections observed in our laboratory is not unique. Such a phenomenon is also observed in patients with Q fever, with a cutoff for the detection of acute infections of  $\geq 1:200$  for phase II IgG antibody and a cutoff for chronic infections of  $\geq 1:800$  for phase I IgG antibody (22). In patients with chronic *Bartonella* or *C. burnetii* infections, the recurrent presence of bacteria in the bloodstream may result in a prolonged antigenic stimulation and thus antibody titers higher than those during acute infections, during which the antigenic stimulation is of short duration.

When studying the evolution of antibodies to *Bartonella* following the diagnosis of endocarditis, we observed significantly delayed responses in two patients who relapsed in comparison with the response times in those who recovered (Fig. 3). The follow-up of patients with positive results for *Bartonella* by serological tests may be suitable for the prediction of late relapses, but further studies are needed to confirm these data.

In conclusion, in our study, the MIF assay had a high predictive value for the detection of chronic *Bartonella* infections, especially endocarditis, when it was applied to the sera of patients with blood culture-negative endocarditis and was also helpful in detecting late relapses in patients with delayed decreases in antibody titers.

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