Immunoglobulin G Anticardiolipin Antibodies and Progression to Q Fever Endocarditis

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Background. Immunoglobulin G (IgG) anticardiolipin (aCL) antibodies are associated with valvulopathy and endocarditis in patients with lupus and other diseases. During acute Q fever, high IgG aCL prevalence has been reported, but the clinical significance remains unknown.

Methods. To test if increased IgG aCL at acute Q fever diagnosis is associated with an increased risk of progression to endocarditis, all patients diagnosed in the French National Referral Center for Q fever from January 2007 to December 2011 were included and followed regularly until January 2013 in a 5-year prospective cohort study. Q fever endocarditis was defined according to recently updated criteria.

Results. Seventy-two patients were followed for a median time of 31 months (interquartile range, 18–47 months). Of these, 13 patients with valvulopathy without antibiotic prophylaxis progressed to endocarditis. IgG aCL levels were highly prevalent (57%) and significantly higher in the presence of a valvulopathy (P = .005). Using Cox regression analysis, highly increased levels of IgG aCL (adjusted hazard ratio [AHR], 12.95; 95% confidence interval, 2.85–58.95; P = .001) and high levels of phase II immunoglobulin M (IgM; AHR, 6.59; 95% CI, 1.37–31.62; P = .018) were the only independent predictors of progression to endocarditis.

Conclusions. Rapid progression from acute Q fever to endocarditis is associated with high levels of IgG aCL and high levels of phase II IgM, findings that should be critical in the prevention of endocarditis.

Keywords. Q fever; Coxiella burnetii; antiphospholipid; anticardiolipin; endocarditis.

Q fever is a worldwide zoonosis caused by an obligate intracellular bacterium, Coxiella burnetii. Acute clinical manifestations correspond to primary infection and vary in range from asymptomatic seroconversion to severe disease [1]. Infective endocarditis, with an occurrence ranging from 0.6% during an outbreak [2] to 7% in a tertiary center [3], is one of the most frequent chronic infections and is usually fatal without treatment [4]. Acute clinical presentation is strain-specific, but all identified genotypes have been associated with endocarditis that was previously conditioned to the presence of a preexisting valvulopathy [3]. Based on the broadest studies [5–8], endocarditis is associated with surgery in 15%–73% of cases with a death rate between 5% and 65% and a large number of relapses when inadequately treated. Because at least 16% of endocarditis cases are preceded by acute Q fever [7], the identification of poor prognostic markers is critical as antibiotic prophylaxis has been shown to be effective in high-risk patients [3].

Coxiella burnetii infection is associated with many autoimmune manifestations [9, 10]. Antiphospholipid antibodies (APLAs), including anticardiolipin antibodies, are found in 42%–84% of patients, disappear when specific immunity is established, and have been linked to the presence of fever and thrombocytopenia [11]. In
contrast, Libman-Sacks endocarditis associated with APLAs was first described in 1985 in a patient who had no antinuclear antibodies [12]. Since then, the specific association between immunoglobulin G (IgG) anticardiolipin antibodies (aCLs) and increased risk for valvulopathy, including Libman-Sacks endocarditis, has been confirmed in patients with systemic lupus erythematosus [13] or primitive antiphospholipid syndrome [14] and patients referred for valve replacement [15].

In this context, an evaluation of IgG aCL can be helpful in identifying acute Q fever patients with a high risk of progression to endocarditis who should be treated with antibiotic prophylaxis [3]. For this purpose, we compared IgG aCL levels according to the presence of a valvulopathy at acute Q fever diagnosis and performed a cohort study to test the prognostic value of IgG aCL for progression to endocarditis in patients with acute Q fever.

METHODS

Patients, Samples, and Ethics
All patients with acute Q fever were assessed and followed in our center (French National Referral Centre for Q fever) by one of the study authors (D.R.) from January 2007 to December 2011. Patients underwent systematic transthoracic echocardiography and IgG aCL determination upon acute Q fever diagnosis. Clinical, laboratory, and echocardiographic data were collected prospectively in a standardized questionnaire. Follow-up was performed until January 2013 according to our current recommendations [16]. Pregnant women and patients without IgG aCL determination at diagnosis, without echocardiography, diagnosed with only 1 serological assay or deceased without elimination of Q fever endocarditis were excluded. Patients lost during follow-up were considered to be without endocarditis until the date of last visit or last serologic tests according to the recently published criteria for Q fever endocarditis [17]. For the first analysis, IgG aCL levels were compared among patients with acute Q fever, patients with C. burnetii endocarditis (the last patients with serum analyzed in our center), controls (blood donors), patients with valvulopathy but excluded endocarditis [18], and patients with endocarditis from other causes treated in our center. Each patient provided written consent, and the study was approved by the local ethics committee.

Diagnosis of C. burnetii Infection
Immunoglobin G, immunoglobin M (IgM), and immunoglobulin A (IgA) titers against phase I and phase II C. burnetii antigens were quantified using an indirect immunofluorescence assay [19]. Sera were incubated with an RF Absorbent (Siemens, Marburg, Germany) before titrations of IgM and IgA to prevent the presence of rheumatoid factor. For molecular detection, DNA was extracted using the QIAamp Tissue Kit (Qiagen GmbH, Hilden, Germany), and these extracts were used as templates for polymerase chain reaction (PCR) amplification as previously described [20].

<table>
<thead>
<tr>
<th>Table 1. Definition of Q Fever Endocarditis According to Raoult (2012)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Define criterion</strong></td>
</tr>
<tr>
<td>Positive culture, PCR, or immunochemistry of a cardiac valve</td>
</tr>
<tr>
<td><strong>B. Major criteria</strong></td>
</tr>
<tr>
<td>Microbiology: positive culture or PCR of the blood, an emboli, or</td>
</tr>
<tr>
<td>serology with IgG1 antibody titer ≥6400</td>
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<tr>
<td>Evidence of endocardial involvement:</td>
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<tr>
<td>Echocardiogram positive for IE: oscillating intracardiac mass on valve</td>
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<tr>
<td>or supporting structures, in the path of regurgitant jets or on</td>
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<tr>
<td>implanted material in the absence of an alternative anatomic</td>
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<tr>
<td>explanation; or abscess; or new partial dehiscence of a prosthetic</td>
</tr>
<tr>
<td>valve; or new valvular regurgitation (worsening or changing of</td>
</tr>
<tr>
<td>preexisting murmur is not sufficient)</td>
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<tr>
<td>PET scan displaying a specific valve fixation and mycotic aneurism</td>
</tr>
<tr>
<td><strong>C. Minor criteria</strong></td>
</tr>
<tr>
<td>Predisposing heart condition (known or found on echography)</td>
</tr>
<tr>
<td>Fever, temperature &gt;38°C</td>
</tr>
<tr>
<td>Vascular phenomena, major arterial emboli, septic pulmonary</td>
</tr>
<tr>
<td>infarcts, mycotic aneurysm (observed during PET scan), intracranial</td>
</tr>
<tr>
<td>hemorrhage, conjunctival hemorrhages, and Janeway lesions</td>
</tr>
<tr>
<td>Immunologic phenomena: glomerulonephritis, Osler’s nodes, Roth</td>
</tr>
<tr>
<td>spots, or rheumatoid factor</td>
</tr>
<tr>
<td>Serological evidence: IgG1 antibody titer ≥800 &lt;6400</td>
</tr>
</tbody>
</table>

**Diagnosis definite**

1. 1A criterion
2. 2B criteria
3. 1B criterion and 3C criteria (including 1 microbiological characteristic and a cardiac predisposition)

**Possible diagnosis**

1. 1B criterion and 2C criteria (including 1 microbiological characteristic and a cardiac predisposition)
2. 3C criteria (including 1 microbiological characteristic and a cardiac predisposition)

Source: Raoult [17]. Reprinted with permission from Elsevier.

Abbreviations: IE, infective endocarditis; IgG, immunoglobulin G; PCR, polymerase chain reaction; PET, positron emission tomography.

Case Definitions

Acute Q fever was defined by the association of clinical symptoms (fever and/or hepatitis and/or pneumonia) with serologic criteria for phase II IgG levels ≥200 and phase II IgM levels ≥50 [19], seroconversion, or a positive PCR with no endocarditis. Systematic echocardiography was performed, and antibiotic prophylaxis with doxycycline and hydroxychloroquine for 1 year [3] was recommended for patients with a prosthetic valve, a grade ≥2 valve stenosis or regurgitation [21], a mitral valve prolapse, a bicuspid or other congenital cardiopathy, or a remodeling or thickening valve [22]. Q fever endocarditis was defined according to recently updated criteria [17], including at least 1 microbiological indication and a cardiac predisposition (Table 1).
Detection of Anticardiolipin Antibodies, Antinuclear Antibodies, Rheumatoid Factor, and Lupus Anticoagulant
Sera were analyzed for IgG aCL using previously described methods [23], and levels were expressed in IgG phospholipid-binding units (GPLU) and IgM phospholipid-binding units calculated using dilutions of a positive sample for calibration using values determined by United Kingdom National External Quality Assessment standards. The cutoff value for the presence of IgG aCL was assessed by the method of percentile (99th) using 100 normal control sera and was 22 GPLU. IgG aCL levels were evaluated up to 100 GPLU. We defined 3 levels (low increased, 22–50; moderately increased, 50–90; highly increased, >90 GPLU) of IgG aCL. Antinuclear antibodies were detected by indirect immunofluorescence in HEp-2 cells (Bio-Rad, Marnes-la-Coquette, France). Rheumatoid factor was detected with a positive cutoff value of 20 IU/mL (INODIAG, Signes, France).

Figure 1. Study flowchart. Abbreviations: IgG, immunoglobulin G; IgM, immunoglobulin M.

*Two asymptomatic patients were diagnosed after valve replacement [24]. One was prescribed antibiotic prophylaxis and never developed infective endocarditis. The second patient was lost to follow-up after 2 months, did not have antibiotic prophylaxis, and died 3 years later from cardiac failure.
Statistical Methods
Fisher exact test and the Mann-Whitney U test were used to test for differences between qualitative and continuous data, respectively. A multivariable analysis was performed using a linear regression model with IgG aCL as the dependent variable and including age, sex, and valvulopathy in the analysis. The identification of the discriminating variables for the prediction of endocarditis was assessed using ROC analysis for IgG aCL, serological parameters, rheumatoid factor, and antinuclear antibodies. A dose-dependent relationship was confirmed by a Spearman correlation test. Survival analysis was performed by examining Kaplan-Meier curves with a log-rank test and using a Cox regression model with a forward selection using the likelihood ratio test, incorporating age, sex, and the criteria associated with endocarditis in the literature, or with a P < .2 in univariate analysis. All the tests were 2-sided, and P < .05 was regarded as significant. Subgroup analysis of patients with a valvulopathy with or without antibiotic prophylaxis was planned a priori. The results were reported following the STROBE statement and checklist [25]. The analyses were performed with SPSS version 21.0 (IBM, Paris, France).

RESULTS

Patient Characteristics
During enrollment, 72 acute Q fever patients were included in the study (Figure 1). The mean age was 48 ± 13 years, and 71% (51/72) of the patients were male. No patient was immunocompromised or had a prosthetic heart valve or vascular prosthesis. Control groups without Q fever included 57 healthy blood donors, 100 patients with endocarditis, and 100 patients with a valvulopathy without endocarditis. Thirty-nine patients with Q fever endocarditis were also included. Twenty-one patients currently being treated have been treated for a median of 3.4 months (IQR, 0.5–21 months), and 18 have stopped treatment and were considered cured.

Highly Increased IgG aCL Levels in Acute Q Fever Patients With Valvulopathy
In the control groups without C. burnetii infection, patients with endocarditis had higher IgG aCL levels than blood donors (P < .0001) or patients with uninfected valvulopathy (P < .0001, Table 2, Supplementary Figure 1). No significant difference was observed between patients with valvulopathy and blood donors (P = .72). Patients with acute Q fever had higher IgG aCL levels compared to blood donors (P < .0001), even after stratification for valvulopathy. Among acute Q fever patients, IgG aCL levels were higher in patients with a valvulopathy (P = .005). Using linear regression analysis, male sex (P = .013) and valvulopathy (P = .001), but not age, were independent determinants of higher IgG aCL levels. In 7 patients with a rapid rise in phase I IgG titers, only transeophageal echocardiography (5 cases) or a second transthoracic echocardiogram (TTE; 2 cases) identified the underlying valvulopathy, and all had IgG aCL >80 GPLU at diagnosis. There was no difference between patients with treated or cured Q fever endocarditis and patients with valvulopathy without Q fever (P = .99).

### Table 2. Comparison of Immunoglobulin G Anticardiolipin Levels According to Patient Category

<table>
<thead>
<tr>
<th>Patient Category</th>
<th>Mean ± SD (GPLU)</th>
<th>Negative Samples (&lt;22 GPLU)</th>
<th>Positive Samples (&gt;22 GPLU)</th>
<th>Positive Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>No evidence of Coxiella burnetii infection</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Blood donors (n = 57)</td>
<td>4.66 ± 3.34</td>
<td>57/57 (100%)</td>
<td>0/57 (0%)</td>
<td>0/57 (0%)</td>
</tr>
<tr>
<td>Valvulopathy (n = 100)</td>
<td>5.47 ± 5.37</td>
<td>98/100 (98%)</td>
<td>2/100 (2%)</td>
<td>0/100 (0%)</td>
</tr>
<tr>
<td>Endocarditis (n = 100)</td>
<td>16.31 ± 22.90</td>
<td>83/100 (83%)</td>
<td>17/100 (17%)</td>
<td>10/100 (10%)</td>
</tr>
<tr>
<td>Acute Q fever (n = 72)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Acute Q fever without valvulopathy (n = 41)</td>
<td>35.52 ± 36.71</td>
<td>21/41 (51%)</td>
<td>20/41 (49%)</td>
<td>10/41 (24%)</td>
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<tr>
<td>Acute Q fever with valvulopathy (n = 31)</td>
<td>64.92 ± 40.42</td>
<td>10/31 (32%)</td>
<td>21/31 (68%)</td>
<td>2/31 (6%)</td>
</tr>
<tr>
<td>Q fever endocarditis (n = 39)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Q fever endocarditis under treatment (n = 21)</td>
<td>5.24 ± 5.97</td>
<td>20/21 (95%)</td>
<td>1/21 (5%)</td>
<td>1/21 (5%)</td>
</tr>
<tr>
<td>Cured Q fever endocarditis (n = 18)</td>
<td>5.27 ± 3.71</td>
<td>18/18 (100%)</td>
<td>0/18 (0%)</td>
<td>0/18 (0%)</td>
</tr>
</tbody>
</table>

Overall, 41 of 72 patients with acute Q fever were positive for immunoglobulin G anticardiolipin (57%). Abbreviations: GPLU, immunoglobulin G phospholipid-binding units; SD, standard deviation.
Follow-up After Acute Q Fever
The median duration of follow-up was 925 days (IQR, 546–1401). One patient died after 3 years from metastatic colon cancer. None of the 41 patients without significant valvulopathy progressed to endocarditis. Of the 31 patients with significant valvulopathy, 18 completed antibioprophylaxy with no progression to endocarditis, and 13 patients without complete antibioprophylaxy progressed to endocarditis (Supplementary Table 1). Consequently, the inclusion of both these covariates in the regression analyses is not justified. The reasons for the lack of antibiotic prophylaxis were a delayed expert opinion (n = 4), a negative first TTE (n = 3), and poor compliance (n = 3). In 1 patient, the diagnosis of acute Q fever was delayed and 2 patients had an extremely rapid evolution. All 13 patients who progressed to endocarditis showed a good outcome on treatment and none of these patients underwent surgery. The IgG aCL levels peaked at the time of acute Q fever diagnosis and subsequently gradually decreased in all patients (Supplementary Figure 2).

Dose-Dependent Relationship Between IgG aCL at Acute Q Fever Diagnosis and a Predictive Positive Value for Q Fever Endocarditis
The presence of IgG aCL was significantly associated with progression to endocarditis (odds ratio [OR], 5.69 [95% confidence interval {CI}, 1.16–27.9], P = .029). Highly increased IgG aCL
levels were found in 11 of 13 endocarditis patients, whereas IgG aCL levels were negative in the 2 remaining patients (Supplementary Table 1). In ROC analysis, the area under the curve (AUC) was 0.78 (95% CI, .64–.92; \( P = .001 \); Figure 2). The optimal obtained threshold was 92 GPLU and corresponded to a sensitivity of 84%, a specificity of 76%, a positive predictive value (PPV) of 44%, a negative predictive value of 96%, and an accuracy of 78%; 11 of 25 patients with IgG aCL levels >90 GPLU progressed to endocarditis, compared with 2 of 47 without this criterion (OR, 17.7 [95% CI, 3.49–89.5], \( P < .001 \)).

A significant correlation was found between a PPV and IgG aCL levels (\( \rho = 0.98, P < .001 \); Supplementary Figure 3).

IgG aCL Is an Earlier and Better Predictive Variable Than C. burnetii Serology, Rheumatoid Factor, and Antinuclear Antibodies for Progression to Endocarditis

IgG aCL levels were a better predictor for progression to endocarditis than anti-Coxiella burnetii serology at diagnosis (Figure 2 and Supplementary Table 2). Notably, IgG aCL levels correlated with phase II IgM (Spearman \( \rho = 0.32, P = .007 \)) at diagnosis, but this parameter was less predictive (AUC, 0.68 [95% CI, .502–.85], \( P = .045 \)). Other serological parameters were not discriminating. A level of IgG aCL >90 GPLU was associated with an accuracy of 80% at diagnosis, which increased to 90% when patients who had received antibiotic prophylaxis were excluded. This optimal accuracy occurred considerably earlier than the maximal accuracy of 81% for phase I IgG levels \( \geq 800 \) (72% after the exclusion of patients receiving antibiotic prophylaxis) obtained at 4 months (Figure 3). Conversely, antinuclear antibodies and rheumatoid factors were not predictive of progression to endocarditis (Supplementary Table 2 and Supplementary Figure 4).

High IgG aCL Levels Are an Independent and Specific Predictor of Q Fever Endocarditis

Survival analysis using a log-rank test determined that age >40 years (\( P = .028 \)), phase II IgM >3200 (\( P = .004 \)), a positive IgG aCL test (\( P = .026 \)), and high levels of IgG aCL (>90 GPLU; \( P < .0001 \)) were linked with progression to endocarditis, and this conclusion was confirmed by examination of Kaplan-Meier curves (Figure 4). A Cox regression model including age, sex,
phase II IgM >3200, and IgG aCL >90 GPLU demonstrated that highly increased levels of IgG aCL (adjusted hazard ratio [AHR], 12.95 [95% CI, 2.85–58.95], \( P = .001 \)) and high levels of phase II IgM (AHR, 6.59 [95% CI, 1.37–31.62], \( P = .018 \)) were the only independent predictors of progression to endocarditis.

**DISCUSSION**

More than half of patients with acute Q fever had positive IgG aCL tests, and one-third had highly increased IgG aCL levels. For the first time, we found higher levels of IgG aCL in acute Q fever patients with a valvulopathy; the positive predictive value was high for progression to endocarditis, as 1 of every 2 patients with highly increased IgG aCL levels without antibiotic prophylaxis progressed to endocarditis. IgG aCL determination at diagnosis, which also correlated with phase II IgM, was an earlier and better predictor than specific C. burnetii serology for progression to endocarditis.

The determination of IgG aCL levels, achievable using the same serum as that used for Q fever serology, could be a useful diagnostic biomarker for valvulopathy, as these levels are highly elevated when valvulopathy is diagnosed only after a second echocardiography. IgG aCL determination would also be a quicker and cheaper predictive marker than echocardiography. The accuracy of this biomarker varied with time, as IgG aCL appeared as an early prognostic factor, whereas phase I IgG, which is more accurate after the third month, is not a prognostic but a diagnostic marker of endocarditis \([17, 26]\). According to our results, no patient <40 years of age without known valvulopathy and with IgG aCL levels <75 GPLU progressed to endocarditis and therefore did not require echocardiography but simply a serological follow-up at 3 and 6 months (Figure 5). These recommendations for young patients correspond to those for the US Navy \([27]\). In all patients >40 years of age, transthoracic echocardiography should be performed because of the increased prevalence of valvulopathy and Q fever endocarditis. In such patients without significant valvulopathy on initial transthoracic echocardiography, but with raised IgG aCL levels (>75 GPLU), a transesophageal echocardiography should be performed to rule out an undiagnosed valvulopathy.

In summary, IgG aCL determination at acute Q fever diagnosis has been identified here as an early, discriminating, and predictive biomarker of progression to Q fever endocarditis. This biomarker could play a critical role in the early management of acute Q fever and prevention of Q fever endocarditis and thus should be performed in all patients upon acute Q fever diagnosis.

**Supplementary Data**

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyrighted. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

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**Author contributions.** D. R., the director of the center, supervised the study’s design, conduct, and reporting. M. M. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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