Microbiological Challenges in the Diagnosis of Chronic Q Fever

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Diagnosis of chronic Q fever is difficult. PCR and culture lack sensitivity; hence, diagnosis relies mainly on serologic tests using an immunofluorescence assay (IFA). Optimal phase I IgG cutoff titers are debated but are estimated to be between 1:800 and 1:1,600. In patients with proven, probable, or possible chronic Q fever, we studied phase I IgG antibody titers at the time of positive blood PCR, at diagnosis, and at peak levels during chronic Q fever. We evaluated 200 patients, of whom 93 (46.5%) had proven, 51 (25.5%) had probable, and 56 (28.0%) had possible chronic Q fever. Sixty-five percent of proven cases had positive Coxiella burnetii PCR results for blood, which was associated with high phase I IgG. Median phase I IgG titers at diagnosis and peak titers in patients with proven chronic Q fever were significantly higher than those for patients with probable and possible chronic Q fever. The positive predictive values for proven chronic Q fever, compared to possible chronic Q fever, at titers 1:1,024, 1:2,048, 1:4,096, and ≥1:8,192 were 62.2%, 66.7%, 76.5%, and ≥86.2%, respectively. However, sensitivity dropped to <60% when cutoff titers of ≥1:1,982 were used. Although our study demonstrated a strong association between high phase I IgG titers and proven chronic Q fever, increasing the current diagnostic phase I IgG cutoff to >1:1,024 is not recommended due to increased false-negative findings (sensitivity < 60%) and the high morbidity and mortality of untreated chronic Q fever. Our study emphasizes that serologic results are not diagnostic on their own but should always be interpreted in combination with clinical parameters.

Q fever is caused by Coxiella burnetii, which is a worldwide prevalent intracellular Gram-negative bacterium leading to outbreaks (11, 15). Q fever has both acute and chronic manifestations. Acute Q fever is characterized by a mild, self-limiting influenza-like illness, sometimes complicated by pneumonia or hepatitis (6, 11). In 1 to 5% of all infected patients, chronic Q fever develops, which can occur even years after primary infection (10, 11, 19). Established risk factors that predispose to chronic Q fever are preexistent cardiac valvulopathy, vascular grafts and aneurysms, immunosuppression, and pregnancy (4, 16, 19). The most commonly described forms of chronic Q fever are endocarditis (~75%), vascular infection (~10%), or pregnancy-related disorders (~6%) (16). However, in the Netherlands, half of the chronic Q fever cases consist of patients with infected aneurysms and/or vascular prostheses (2). Untreated chronic disease can lead to severe complications, such as cardiac failure, ruptured aneurysms, or death (11, 12). Between 2007 and 2010, a large outbreak of Q fever occurred in the Netherlands, with more than 4,000 identified cases of acute Q fever and an increasing number of chronic Q fever cases (2, 9, 20, 21). Before complications occur, most patients report no or unspecific symptoms, such as low-grade fever, night sweats, and weight loss (1, 10, 11, 12). In cases of Q fever endocarditis, pathological findings on echocardiogram can be nonspecific or even absent (12, 16).

Due to the nonspecific clinical presentation, diagnosis of chronic Q fever relies on additional microbiological analysis. A positive PCR or culture of C. burnetii in blood or tissue, in the absence of acute Q fever, is diagnostic for chronic Q fever. Nevertheless, in blood, sensitivity is only 50 to 60% for both PCR and culture in patients with chronic Q fever (5, 13). The sensitivity of PCR is reported to diminish when titers of IgG against C. burnetii phase I antigens (phase I IgG) reach 1:25,600 and higher (5). Therefore, serological analysis is pivotal for the diagnosis of chronic Q fever. An avirulent form of C. burnetii (Nine Mile strain phase II) has been produced in vitro that differs antigenically from the infectious agent (Nine Mile phase I) and is useful for serologic diagnostics (15). During acute infection, IgG antibodies against C. burnetii phase II antigens (phase II IgG) are predominantly produced, whereas chronic Q fever is usually characterized by persistent high level of phase I IgG, often in the presence of high phase II IgG titers (11, 14, 15). A phase I IgG cutoff titer of 1:800, which is based on an in-house-developed immunofluorescence assay (IFA), has been proposed and internationally accepted for the serological diagnosis of chronic Q fever (3). In the Netherlands,
confirmed either with positive tests with a cutoff titer of 1:800 or 1:1,024 (6–8, 18).

Recently, the Dutch consensus group for the diagnosis of (chronic) Q fever proposed categorizing patients as having proven, probable, or possible chronic Q fever. This classification ranks the probability of having chronic Q fever based on PCR, serology, clinical parameters, imaging studies, and pathology (see Materials and Methods for details). In a Dutch cohort of proven, probable, and possible chronic Q fever patients, we studied the predictive value of serologic profiles in the diagnosis of chronic Q fever.

MATERIALS AND METHODS

Patients. In order to monitor chronic Q fever cases in the Netherlands after the recent outbreak, a nationwide database has been constructed in which information about all chronic Q fever cases is being collected. Patients are classified as having proven, probable, or possible chronic Q fever according to the recent Dutch consensus guidelines (22). The first group (proven chronic Q fever) consists of patients with chronic Q fever confirmed either with positive C. burnetii PCR on plasma, serum, or tissue or with a phase I IgG of ≥1:1,024 in combination with a proven vascular infection on positron emission tomography (PET), computed tomography (CT), or magnetic resonance imaging (MRI) or endocardial involvement according to the major criteria of the modified Duke criteria. The second group (probable chronic Q fever) concerns patients with phase I IgG of ≥1:1,024 with known risk factors for chronic Q fever, nonmajor valvulopathy according to the modified Duke criteria, suspected nonvascular or noncardial localization of chronic Q fever infection, or aspecific signs of chronic infection. The third group (possible chronic Q fever) consists of patients with solitary phase I IgG of ≥1:1,024, without other indications for probable or proven chronic Q fever (22). The proven chronic Q fever group includes patients with the most definite form of chronic Q fever, in contrast to the possible chronic Q fever group, which represents patients with solitary phase I IgG titers of ≥1:1,024, consistent with chronic Q fever, but without other manifestations of chronic disease. The probable chronic Q fever group includes patients with actual chronic Q fever not (yet) fulfilling the criteria for proven chronic Q fever but also patients without chronic Q fever but with serologic findings consistent with this condition and a known risk factor for chronic Q fever. In the current study, we used information about patients included in this database until September 2011. Patients were included in the analysis when they fulfilled the criteria of proven, probable, or possible chronic Q fever and were aged ≥18 years. Patients for which IFA was not performed or titration was not complete for at least one serological examination were excluded. We also excluded patients who received blood transfusions before determination of serology, because this could have caused significant dilution of the immunoglobulin count.

Microbiological analysis. The routine microbiological work-up for chronic Q fever patients consisted of serology and PCR. Serology and PCR were performed at local laboratories from the different hospitals. Serological analysis was performed using IFA (Focus Diagnostics, Inc., Cypress, CA) on serum samples according to the manufacturer’s instructions. Titration was carried out with dilutions according to a binary scale and a detection cutoff titer of 1:32. PCR for C. burnetii DNA on serum, plasma, and, if available, tissue samples was performed using an in-house assay as previously described. An input volume of 500 μl serum or plasma was used for DNA isolation (17).

Data collection and storage. Information on patient characteristics, imaging results, clinical chemistry, hematology and microbiology results, and antibiotic therapy were collected from the hospital records and were interpreted by two researchers (L. M. Kampschreur and A. M. C. Koop) to avoid information bias. To examine the association of serologic profiles in different subgroups of chronic Q fever (proven, probable, and possible), we evaluated the height of IgG phase I titers at three different time points, which could coincide: the time of chronic Q fever diagnosis, the time of the highest IgG phase I titer per individual (peak value), and the time that PCR for C. burnetii in plasma or serum first became positive. The time of diagnosis was defined as the moment when a patient had clinical evaluation and was classified as having proven, probable, or possible disease according to the outcome of this evaluation. All data were processed and stored anonymously in the SPSS software program, version 18 (SPSS Inc., Chicago, IL).

Statistical methods. We used the binary dilution scales [e.g., 1:1,024 = 1(2)^10], which is therefore 10 on a binary scale, 1,2048 corresponds to 11, etc.) to perform all analyses. We analyzed the distribution of phase I IgG titers in proven chronic Q fever patients with negative or positive C. burnetii PCR in blood. We used logistic regression analysis to estimate the association between the height of phase I IgG titers and a positive PCR in blood. To determine the difference in distribution of phase I IgG titers at the time of diagnosis and at the time of peak titer between patients with proven, probable, and possible chronic Q fever, we used chi-square (χ^2) tests or the Mann-Whitney U test as appropriate. To determine whether IFA can differentiate between “true” chronic Q fever patients and patients with elevated phase I IgG only, we chose to compare patients with proven and possible chronic Q fever, since these two groups represent cases at both ends of the spectrum. We calculated phase I IgG titer test characteristics, sensitivity and specificity, and prediction scores, positive predictive value (PPV) and negative predictive value (NPV), for proven chronic Q fever versus possible chronic Q fever. For this analysis, we chose to evaluate peak titer only, since those, in contrast to titers at the time of diagnosis, were independent of the time of referral to the clinic. To assess the discriminative qualities of the model, a receiver operator characteristics (ROC) curve was constructed, and the area-under-the-ROC curve (AUC) was estimated. In all analyses, the significance level was set at a P value of <0.05.

Ethical consideration. The design of the database and the use of the collected information for analysis and scientific publications were approved by the Medical Research Ethics Committee of the University Medical Center Utrecht in Utrecht, the Netherlands.

RESULTS

In total, 200 patients were included in our study. Ninety-three patients (46.5%) had proven chronic Q fever, 51 patients (25.5%) had probable chronic Q fever, and 56 patients (28.0%) had possible chronic Q fever. In all, 138 patients (69.0%) were male, 69 males had proven chronic Q fever (74.2% of proven chronic Q fever cases), 33 males had probable chronic Q fever (64.7%), and 36 males had possible chronic Q fever (64.3%). Mean age at diagnosis was 64.4 years, with a standard deviation (SD) of ± 14.5 years (proven, 69.0 ± 12.4 years; probable, 63.1 ± 14.1 years; possible, 57.3 ± 14.5 years).

Of the 93 patients with proven chronic Q fever, 52 patients (55.9%) had a positive C. burnetii PCR result for blood only (plasma or serum), 10 (10.8%) for tissue only, and 13 (14.0%) for both blood and tissue. For 18 patients (19.4%), C. burnetii DNA could not be detected in either blood or tissue, but these cases were defined as proven chronic Q fever based on evidence of endovascular or endocardial infection.

Figure 1 demonstrates the distribution of phase I IgG titers at the time of a positive or negative C. burnetii PCR for blood, irrespective of the outcome of PCR for tissue, in 87 proven chronic Q fever patients (for 4 patients, no PCR analysis of blood was performed; for two patients, phase I IgG was not available at the time of PCR analysis). Logistic regression analysis demonstrated a significant rise in the probability of positive PCR in the case of each
increasing phase I IgG titer, using a binary dilution scale (odds ratio [OR], 1.35; 95% confidence interval [CI], 1.02 to 1.77; \(P = 0.033\)).

Peak IFA values were available for all 200 patients. Titers at the time of diagnosis were available for 196 patients: for one proven chronic Q fever patient, no IFA titration at time of diagnosis was performed, and for three patients, blood and fluid transfusion was administered before blood sampling for microbiological analysis. In 93 cases (46.5%), the IFA result at the time of diagnosis was at the same time as the peak IFA value.

A box plot presenting the distribution of phase I IgG titers at the time of diagnosis and time of peak titers among patients with proven, probable, and possible chronic Q fever cases. The black bars indicate the median IgG titers. Titers on the y axis are represented on a binary scale. Median phase I IgG titers were significantly higher (\(P < 0.01\)) than 1:2,048 and 1:4,096, respectively, for both time points for possible chronic Q fever.

**DISCUSSION**

We have shown that the height of phase I IgG titers is strongly associated with the chance of proven chronic Q fever in patients with a serological suspicion of chronic Q fever. This relation was most clear when peak phase I IgG titers were used. IFA titers of 1:8,192 had a very high PPV (85%) for proven chronic Q fever, thereby urging the need for an extensive search for an infectious focus and a low threshold for the initiation of antibiotic treatment for patients presenting with such titers. On the other hand, the sensitivity using this cutoff titer is less than 60%, leading to unacceptably high percentages of missed cases. Cutoff titers of 1:1,024, 1:2,048, and 1:4,096 of phase I IgG have high sensitivities and specificities of cutoff titers of IgG antibodies against *Coxiella burnetii* phase I antigens compared to possible chronic Q fever at the time of peak I IgG titers. Table 1 displays the test characteristics and prediction scores for the different cutoff levels of peak phase I IgG titers when proven chronic Q fever cases are compared with possible chronic Q fever cases. The positive predictive value (PPV) for proven chronic Q fever ranged from 62.2% to 94.3% at titers of 1:1,024 to \(\geq 1:16,384\). Test characteristics of titers at the time of diagnosis and peak titers are displayed in a ROC curve in Fig. 3. The AUC was 0.78 (95% CI, 0.70 to 0.85) for phase I IgG titers at the time of diagnosis and 0.79 (95% CI, 0.72 to 0.86) for phase I IgG peak titers for differentiating proven chronic Q fever from possible chronic Q fever.

**TABLE 1** Sensitivities and specificities of cutoff titers of IgG antibodies against *Coxiella burnetii* phase I antigens for proven chronic Q fever compared to possible chronic Q fever at the time of peak I IgG titers

| IgG phase I titer | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|------------------|----------------|----------------|---------|---------|
| 1:1,024          | 97.8           | ND             | 62.2    | ND      |
| 1:2,048          | 94.6           | 21.4           | 66.7    | 70.6    |
| 1:4,096          | 80.6           | 58.9           | 76.5    | 64.7    |
| 1:8,192          | 60.2           | 83.9           | 86.2    | 55.9    |
| 1:16,384         | 47.3           | 94.6           | 93.6    | 52.0    |
| \(\geq 1:32,768\) | 25.8           | 96.4           | 94.4    | 47.8    |

\(\text{PPV, positive predictive value; NPV, negative predictive value; ND, not determinable.}\)
for diagnosis of proven chronic Q fever (97.8%, 94.6%, and 80.6%, respectively) but also a low PPV (62.2%, 66.7%, and 76.5%, respectively) (Table 1). With our data, test characteristics of phase I IgG titers of <1:1,024 could not be defined, since we used a phase I IgG cutoff titer of ≥1:1,024 for the diagnosis of suspected chronic Q fever. However, there were three PCR-positive proven chronic Q fever patients with a phase I IgG titer of 1:512 at the time of diagnosis. In one of these patients, this could tentatively be explained by the use of several immunosuppressive agents impairing the serological response to C. burnetii. In the other patients, we found no explanation. This illustrates that a phase I IgG titer of <1:1,024 does not exclude chronic Q fever, presumably especially in immunocompromised hosts.

We confirmed that C. burnetii PCR with blood samples does not exhibit enough sensitivity for the diagnosis of chronic Q fever, as illustrated by the fact that no circulating C. burnetii DNA was detectable in 69.9% of proven chronic Q fever cases, the most definite cases of chronic Q fever. In contrast to the report of Fenollar et al. (5), we were not able to demonstrate that sensitivity of C. burnetii PCR on blood samples is negatively influenced by phase I IgG titers. Instead, we found a positive association between the height of the phase I IgG titer and the likelihood of a positive PCR in blood samples. The discriminative performance of IgG titers for differentiation between proven and possible chronic Q fever (areas under the ROC curve of 0.78 for titers at the time of diagnosis and 0.79 for peak titers) suggests that serology alone is no more than a reasonable predictor to identify chronic Q fever patients. In our opinion, increasing the currently used cutoff titers of phase I IgG to >1:1,024 or >1:800 (depending on which IFA test is used) is not preferable because of the unacceptably high number of undetected cases and the high morbidity and mortality of untreated chronic Q fever. We recommend maintaining a cutoff phase I IgG of 1:1,024 (in a case where the IFA from Focus Diagnostics, Inc., is used) as a screening tool for chronic Q fever. To prevent unnecessary long-term antibiotic treatment, it is therefore important to define accompanying clinical parameters to secure the diagnosis of chronic Q fever. An attempt to define these clinical parameters was formulated in the recent Dutch classification for the diagnosis of chronic Q fever (22).

Our study has several weaknesses. First, our patients were classified in recently defined subgroups, namely, proven, probable, and possible chronic Q fever. Confirmation of this classification is still lacking. In particular, patients classified as having probable chronic Q fever might include both patients with actual chronic Q fever not (yet) fulfilling the criteria for proven chronic Q fever and patients with a high phase I IgG titer who happen to have a known risk factor for chronic Q fever in the absence of the disease. Because of this heterogeneity, we chose to focus on the difference between the groups of proven and possible chronic Q fever patients, since these two groups represent both ends of the spectrum of probability of actual chronic Q fever. Second, in the beginning of the Dutch Q fever epidemic, diagnostic tests were not yet executed following a standard diagnostic work-up. Therefore, particularly for patients classified as possible chronic Q fever cases, further radiological investigations are sometimes lacking. As a consequence, this might have underestimated the number of proven or probable chronic Q fever patients. On the other hand, most untreated possible chronic Q fever cases have shown a spontaneous decline of phase I IgG to <1:1,024, indicating that indeed no chronic Q fever infection was present in these patients. Third, the Dutch classification for the diagnosis of chronic Q fever includes only patients with phase I IgG titers of ≥1:1,024 and/or a positive C. burnetii PCR result. Therefore, no analysis of titers beneath the cutoff of 1:1,024 was performed. This would be interesting, however, since we found three patients with PCR-positive proven chronic Q fever who presented with a phase I IgG titer of 1:512.

In conclusion, we demonstrated that high phase I IgG titers are strongly associated with proven chronic Q fever, especially if titers exceeded 1:4,096. We confirmed the low sensitivity of PCR analysis of blood for diagnosis of chronic Q fever, but in contrast to previous reports, we found a positive association between heights of phase I IgG and positive blood PCR results. Due to the low sensitivity of high phase I IgG titers and high morbidity and mortality of untreated chronic Q fever, we would not advocate increasing cutoff values of phase I IgG titers for the diagnosis of chronic Q fever. Above all, this study emphasizes that serology is not a diagnostic tool on its own but should be interpreted with regard to the clinical background.

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