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Correlations between Peripheral Blood *Coxiella burnetii* DNA Load, Interleukin-6 Levels, and C-Reactive Protein Levels in Patients with Acute Q Fever

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From 2007 to 2010, the Netherlands experienced the largest reported Q fever outbreak, with >4,000 notified cases. We showed previously that C-reactive protein is the only traditional infection marker reflecting disease activity in acute Q fever. Interleukin-6 is the principal inducer of C-reactive protein. We questioned whether increased C-reactive protein levels in acute Q fever patients coincide with increased interleukin-6 levels and if these levels correlate with the *Coxiella burnetii* DNA load in serum. In addition, we studied their correlation with disease severity, expressed by hospital admission and the development of fatigue. Interleukin-6 and C-reactive protein levels were analyzed in sera from 102 patients diagnosed with seronegative PCR-positive acute Q fever. Significant but weak negative correlations were observed between bacterial DNA loads expressed as cycle threshold values and interleukin-6 and C-reactive protein levels, while a significant moderate-strong positive correlation was present between interleukin-6 and C-reactive protein levels. Furthermore, significantly higher interleukin-6 and C-reactive protein levels were observed in hospitalized acute Q fever patients in comparison to those in nonhospitalized patients, while bacterial DNA loads were the same in the two groups. No marker was prognostic for the development of fatigue. In conclusion, the correlation between interleukin-6 and C-reactive protein levels in acute Q fever patients points to an immune activation pathway in which interleukin-6 induces the production of C-reactive protein. Significant differences in interleukin-6 and C-reactive protein levels between hospitalized and nonhospitalized patients despite identical bacterial DNA loads suggest an important role for host factors in disease presentation. Higher interleukin-6 and C-reactive protein levels seem predictive of more severe disease.

From 2007 to 2010, the Netherlands experienced the largest outbreak of acute Q fever reported to date, with >4,000 notified cases. Q fever is a zoonosis caused by the bacterium *Coxiella burnetii*, which is spread via aerosols generated by infected animals or via animal products (1). The most common clinical manifestation of acute Q fever is nonspecific influenza-like illness. Pneumonia and hepatitis are present in more severe cases (2). Asymptomatic infection occurs in 50 to 60% of patients. Among patients with *C. burnetii*, infection progresses to chronic Q fever in 1 to 5%, months to years after the primary infection. Endocarditis, infected aortic aneurysms, and vascular prostheses are the most common clinical manifestations of chronic Q fever. Among the recognized risk factors for chronic Q fever are valve abnormalities, the presence of vascular prosthesis, aneurysm, pregnancy, renal insufficiency, and older age (3).

Previously, we detected *C. burnetii* DNA in sera from 98% of seronegative acute Q fever patients. Around 2 weeks after the onset of symptoms, circulating *C. burnetii* DNA disappeared from the blood compartment, concomitant with the development of an antibody response (4). In addition, we have shown that C-reactive protein (CRP) is the only traditional infection marker adequately reflecting disease activity in acute Q fever, while procalcitonin levels and white blood cell counts are within the normal range or only marginally increased. Furthermore, CRP was the only marker that significantly differentiated between seronegative inpatients and outpatients with acute Q fever (5). Interleukin-6 (IL-6), the principal inducer of CRP (6), is a multifunctional cytokine produced by various types of cells, including T lymphocytes, B lymphocytes, monocytes, fibroblasts, and endothelial cells. It regulates the immune response, hematopoiesis, the acute-phase response, and inflammation (7). Several studies have looked into a role for IL-6 as a mediator of immunological processes in various presentations of Q fever. Unstimulated peripheral blood mononuclear cells (PBMC) from patients with acute Q fever released IL-6, the levels of which correlated with self-reported sickness symptoms (8). Correspondingly, human monocyte-derived macrophages infected by *C. burnetii* secreted IL-6 (9), indicating that lymphocytes and/or monocytes are involved in the production of IL-6 in patients with acute Q fever. Plasma levels of IL-6 were significantly increased in patients with acute Q fever and patients with Q fever endocarditis over those of healthy controls (10). Furthermore, PBMC of patients with Q fever fatigue syndrome (QFS) showed upregulated IL-6 responses upon stimulation with *C. burnetii* antigens, which correlated with symptom scores (11).

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In this study, we questioned whether increased CRP levels in seronegative acute Q fever patients coincide with increased IL-6 levels and if these levels correlate with the bacterial DNA load in serum. In addition, we studied if these markers correlate with disease severity as expressed by hospital admission.

MATERIALS AND METHODS

Acute Q fever patients. From April to August 2009 at the Department of Medical Microbiology and Infection Control of the Jeroen Bosch Hospital ('s-Hertogenbosch, the Netherlands), we analyzed 102 symptomatic patients diagnosed with acute Q fever by *C. burnetii* PCR before the development of an IgM antibody response (seronegativity).

Seronegativity was defined as the absence in serum samples of IgM antibodies against *C. burnetii* phase II antigens, measured by either immunofluorescence assay (IFA) (Focus Diagnostics, Inc., Cypress, CA, USA) or, from 1 May 2009 forward (12), by enzyme-linked immunosorbent assay (ELISA) (Institut Virion-Serion GmbH, Würzburg, Germany). PCR positivity was defined as the presence of *C. burnetii* DNA in serum samples (cycle threshold [C_T] value, <45) as measured by a previously described in-house real-time PCR targeting the multicopy insertion sequence IS*1111*, which was performed in duplicate (4). The Dutch Q Fever Consensus Group has stated that a positive PCR result in the presence of an appropriate clinical picture is sufficient for the diagnosis of acute Q fever (13). To perform statistical analysis, the mean C_T value from the duplicate PCR results was used for each individual patient. Since real-time PCR C_T values inversely correlate with the logarithm of the quantity of DNA, we considered the C_T value to be a semiquantitative representation of the circulating *C. burnetii* DNA load, with a lower C_T value indicating a higher load. An undetectable outcome of the PCR was presented as a C_T value of 45.0.

An IFA performed on routine follow-up serum samples received at 3, 6, or 12 months after initial diagnosis revealed the presence of IgG antibodies against phase I and/or phase II antigens in 93 patients, confirming the diagnosis of acute Q fever. No follow-up samples were received from the remaining nine patients.

Data on hospital admissions were acquired from the hospital information system of the Jeroen Bosch Hospital and the neighboring Bernhoven Hospital, with locations in Oss and Veghel, both in the Netherlands.

Interleukin-6 and C-reactive protein levels. Acute-phase serum samples stored at −20°C were thawed, and subsequently, IL-6 and CRP levels were analyzed by the high-sensitivity human cytokine kit HSCYTO-60SK (Merck Millipore, Billerica, MA, USA) and the human CRP ELISA kit CYT298 (Merck Millipore), respectively, according to the manufacturer's instructions.

Level of experienced fatigue. Four years after the diagnosis of acute Q fever, all the patients who were still alive, were ≥18 years of age, and from whom a 12-month follow-up sample was received were mailed a questionnaire that covered, among other items, the fatigue subdomain of the Nijmegen Clinical Screening Instrument (NCSI). The NCSI measures health status and covers symptoms, functional impairment, and quality of life as main domains and fatigue as one of eight subdomains. For each subdomain, normative data indicating normal functioning or mild or severe problems were generated (14).

Statistical analysis. Logarithmic transformation of IL-6 and CRP levels and subsequent logistic regression analysis were used to analyze the relationships between C_T values and IL-6 and CRP levels. The levels of parameters among groups were analyzed with the Mann-Whitney U test. All analyses were performed using SPSS Statistics 19.0.0 (SPSS, Inc., Chicago, IL, USA).

Ethics statement. Since the serum samples used in this study were drawn for routine microbiological analysis by order of the treating physician, individual patient consent was not obtained for the analysis of IL-6 and CRP levels. In 2009, the Internal Review Board of the Jeroen Bosch Hospital approved of the anonymous use of discarded blood for validation procedures or research purposes. All patients who donated blood were informed of this possibility and had the right of refusal. The study that included data on the level of experienced fatigue following acute Q fever was approved by the Medical Ethical Committee Tilburg (METOPP Tilburg) and by the Internal Review Board of the Jeroen Bosch Hospital. Each patient signed an informed consent form.

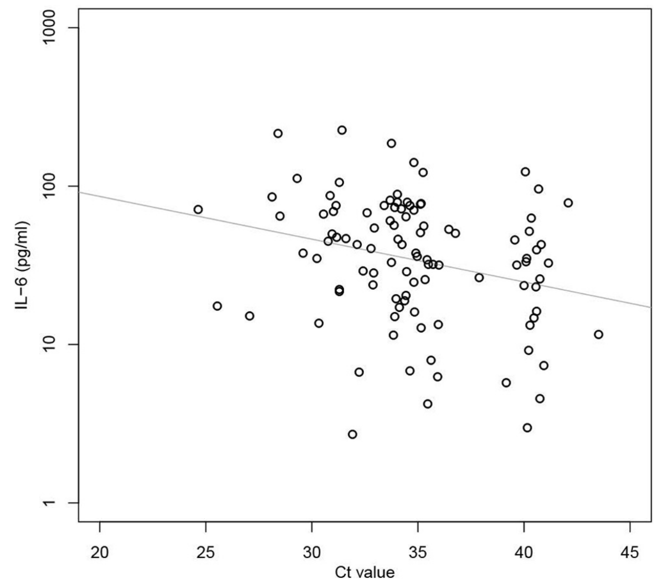

FIG 1 *Coxiella burnetii* DNA load expressed as real-time PCR cycle threshold (C_T) values and interleukin-6 levels in 102 seronegative PCR-positive acute Q fever patients (Spearman's rank coefficient, −0.283; $P = 0.004$).

RESULTS

Of the 102 patients diagnosed with seronegative PCR-positive acute Q fever from April to August 2009, 66 were male (64.7%). The mean (± standard deviation) age was 48 ± 16 years (range, 17 to 85 years). Twenty-four patients were hospitalized because of acute Q fever (23.5%), while the other patients were treated at home. The number of days between the onset of disease, which was known for 69 (67.6%) patients, and referral of serum for Q fever diagnostics was 5 ± 3 days (range, 1 to 14 days).

The C_T values in seronegative PCR-positive acute Q fever patients were 34.9 ± 3.9 (range, 24.7 to 43.5). In all but one patient, at least one of the duplicate PCR results yielded a C_T value of <40.0. The IL-6 levels were 49 ± 41 pg/ml (range, 3 to 226 pg/ml). Increased IL-6 levels (defined as levels of ≥9 pg/ml) were measured in 92 patients (90.2%). The CRP levels were 157 ± 95 mg/liter (range, 2 to 506 mg/liter). Increased CRP levels (defined as levels of ≥4 pg/ml) were found in 101 patients (99.0%).

Figure 1 shows the relation between C_T values and IL-6 levels in seronegative PCR-positive acute Q fever patients. Using Spearman's rank correlation coefficient, a significant negative correlation between C_T values and IL-6 levels was found ($r_s = -0.283$; $P = 0.004$). Figure 2 illustrates the relation between IL-6 levels and CRP levels. Spearman's rank correlation coefficient revealed a significant moderate-strong positive correlation between IL-6 levels and CRP levels ($r_s = 0.641$; $P < 0.001$). Figure 3 shows the relation between C_T values and CRP levels for which Spearman's rank correlation coefficient revealed a significant negative correlation ($r_s = -0.229$; $P = 0.021$).

An analysis by the Mann-Whitney U test revealed higher levels of IL-6 ($P = 0.026$) and CRP ($P = 0.025$) in the 24 hospitalized

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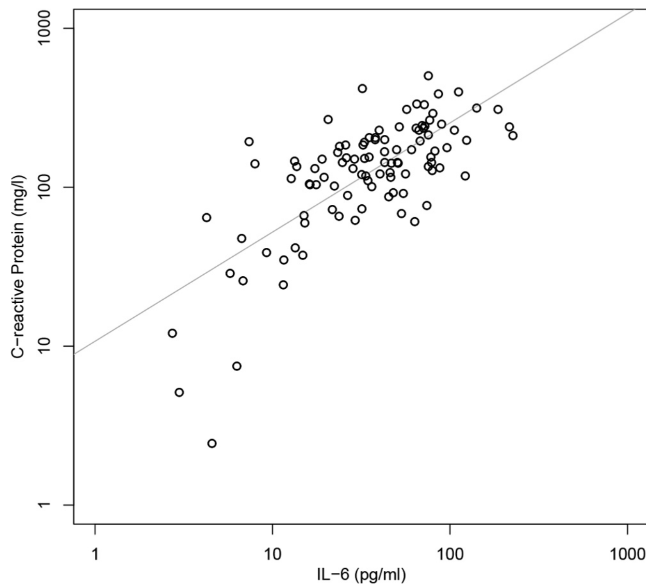

FIG 2 Interleukin-6 and C-reactive protein levels in 102 seronegative PCR-positive acute Q fever patients (Spearman's rank coefficient, 0.641; $P < 0.001$).

acute Q fever patients than those in the 78 patients who were not admitted. Normal-range levels of either IL-6 only ($n = 9$) or both IL-6 and CRP ($n = 1$) were observed in nonhospitalized patients only. No differences in gender, age, or C_T values were observed between the two groups (Table 1).

Four years after the diagnosis of acute Q fever, 83 out of 102 patients had previously submitted a 12-month follow-up sample, were still alive, and were ≥18 years old. As for one patient, the address was unknown, and 82 patients were mailed the NCSI questionnaire that covered the fatigue subdomain. Among the 58 (70.7%) respondents, a normal outcome for the fatigue subdomain was found in 24 patients (41.4%), while an abnormal outcome (combining mild and severe problems) was obtained from 34 patients (58.6%). Analysis by the Mann-Whitney U test revealed no differences in C_T values and IL-6 and CRP levels during acute Q fever between patients with a normal outcome and those with an abnormal outcome for the fatigue subdomain 4 years after their diagnosis of acute Q fever (data not shown).

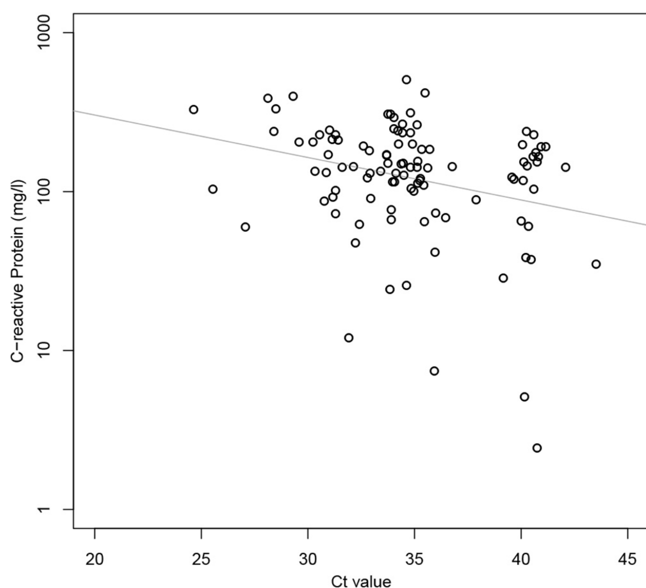

FIG 3 *Coxiella burnetii* DNA load expressed as real-time PCR cycle threshold (C_T) values and C-reactive protein levels in 102 seronegative PCR-positive acute Q fever patients (Spearman's rank coefficient, −0.229; $P = 0.021$).

TABLE 1 Patient characteristics, *Coxiella burnetii* DNA loads in sera expressed as cycle threshold values, and levels of circulating infection markers in seronegative PCR-positive acute Q fever patients

Characteristic	Hospital admission: Yes ($n = 24$)	No ($n = 78$)	P^a
Gender (% male)	67	62	0.650
Age (yr)[b]	52 ± 20	48 ± 14	0.230
Cycle threshold value[b]	34.7 ± 3.8	34.9 ± 3.9	0.444
Interleukin-6 level (pg/ml)[b]	65 ± 52	44 ± 36	0.026
C-reactive protein level (mg/liter)[b]	196 ± 101	145 ± 90	0.025

[a] Mann-Whitney U test.
[b] Values are means ± standard deviations.

DISCUSSION

We previously showed that CRP levels, unlike levels of procalcitonin and white blood cell counts, adequately reflect disease activity in acute Q fever patients (5). Here, we show that IL-6 levels are also increased in the majority (91.2%) of acute Q fever patients. Furthermore, we reveal a significant moderate-strong positive correlation between IL-6 levels and CRP levels in acute Q fever patients. These findings are illustrative of the immune activation pathway in which IL-6 induces the production of CRP during the acute-phase response (6).

Early acute Q fever is characterized by the presence of *C. burnetii* DNA in serum, which disappears from the blood compartment around 2 weeks after the onset of symptoms as the serological response develops (4). Here, we studied a selected cohort of patients with seronegative PCR-positive acute Q fever, for whom the mean number of days between the onset of disease and referral of serum for Q fever diagnostics was 5 days (range, 1 to 14 to days). Since real-time PCR C_T values inversely correlate with the logarithm of the quantity of DNA, we considered the C_T value to be a semiquantitative representation of circulating *C. burnetii* DNA load, with a lower C_T value indicating a higher load. The weak but significant correlations between C_T values and IL-6 and CRP levels ($r_s = -0.283$ and -0.229, respectively) suggest that bacterial DNA load in itself is not a major determinant controlling the production of IL-6 and CRP during acute Q fever. Likewise, in another study, a weak or no correlation was observed between circulating HIV-1 RNA and IL-6 levels, indicating that viral load is not a major driver of IL-6 in chronic HIV-1 disease (15). In contrast, in *Streptococcus pneumoniae* bacteremia patients, a significant moderate-strong correlation was observed between bacterial DNA load and CRP levels (16). In this context, however, it has to be noted that CRP is a protein specifically discovered as a substance present in acute-phase sera from pneumonia patients reacting with pneumococcal C-polysaccharide (17).

As we confirm our previous observation that CRP levels were higher in hospitalized acute Q fever patients than in patients who were not admitted, we show, in accordance, that IL-6 levels are also higher in hospitalized patients, while normal-range levels of IL-6 are

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observed only in nonhospitalized patients. This indicates that higher levels of these markers are predictive of more severe disease. In agreement, the predictive value of IL-6 and CRP levels was demonstrated in a recent study by Jekarl et al., which showed higher levels in nonsurvivors of sepsis than in those in the survivor group. Furthermore, IL-6 rapidly declined in survivors, making it a suitable candidate for monitoring the effectiveness of antibiotic treatment (18). In this context, it would be of interest to measure bacterial DNA load and CRP and IL-6 levels in sequential samples from acute Q fever patients.

Despite significant differences in the levels of IL-6 and CRP, we found the same bacterial DNA loads in hospitalized and nonhospitalized patients with C_T values of 34.7 and 34.9, respectively, representing approximately 30 circulating *C. burnetii* genome equivalents per milliliter. This suggests that host factors might be of more importance for differences in disease severity among patients with acute Q fever than for bacterial load. This is further supported by the weak correlations between C_T values and IL-6 and CRP levels. In this context, it has been shown that the presence of the T allele of the gamma interferon +874T/A single nucleotide polymorphism (SNP) has a significant association with a high-severity acute sickness response in acute Q fever patients. This SNP in the first intron of the gamma interferon gene is known to modulate gene expression (19). This exemplifies the role of host factors, besides bacterial factors, in determining the severity of disease presentation during acute Q fever.

We found 58.6% of patients with mild or severe fatigue problems 4 years after their diagnosis of acute Q fever. This figure is remarkably close to the 58.9% of abnormal fatigue among 515 acute Q fever patients from the 2007 and 2008 cohorts of the 2007–2010 Dutch Q fever outbreak 1 year after diagnosis. Other publications also reported high rates of protracted fatigue 5 to 10 years after *C. burnetii* infection (14). It has been shown that PBMC of QFS patients display upregulated IL-6 responses upon stimulation with *C. burnetii* antigens (11). Yet, we detected no differences in IL-6 levels, or in DNA loads or CRP levels, during acute Q fever between patients with a normal outcome and those with an abnormal outcome for the fatigue subdomain 4 years after their diagnosis of acute Q fever. This does not support the hypothesis that IL-6 levels during acute Q fever are prognostic for subsequent development of long-term fatigue.

In conclusion, we show a moderate-strong correlation between IL-6 and CRP levels in acute Q fever patients indicative of an immune activation pathway in which IL-6 induces the production of CRP during the acute-phase response. Despite significant differences in the levels of IL-6 and CRP, we found the same bacterial DNA loads in hospitalized and nonhospitalized patients, suggesting an important role for host factors in disease presentation among patients with acute Q fever.

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