Markers of infectious disease emergencies: Focus on patients with community-acquired pneumonia

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Chapter 8

Hypophosphatemia, fever and prolonged length of hospital stay in seronegative PCR positive patients as compared to seropositive patients with early acute Q fever pneumonia

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Biomarkers and clinical signs in seronegative PCR positive patients as compared to seropositive patients with early acute Q fever pneumonia
Abstract

Background: Query fever (Q fever) is a zoonotic infection, caused by the intracellular Gram-negative coccobacillus *Coxiella burnetii*. From 2007 until 2010, a large Q fever outbreak has occurred in the Netherlands. We studied traditional and less common inflammation markers in seronegative and seropositive patients with acute Q fever pneumonia to identify markers that distinguish different disease stages and predict disease severity.

Methods: 443 adult patients presenting at the Emergency Department with community-acquired pneumonia were included in a prospective etiologic study. Patients with acute Q fever pneumonia were identified by PCR and/or serology. Patient characteristics, clinical symptoms, pneumonia severity and inflammation markers were assessed upon presentation. Duration of symptoms, prior therapy and length of hospital stay were retrieved from the hospital information system.

Results: In all, 40 patients with acute Q fever pneumonia were identified. Of these, 29 were seronegative and 11 seropositive at presentation. C-reactive protein (CRP) was the only inflammation marker increased in all seronegative and seropositive patients but no significant difference was observed between groups. In seronegative patients, hypophosphatemia was more common (*P* = 0.01), and length of hospital stay was longer (*P* = 0.02). However, there was no significant difference in pneumonia severity index. Furthermore, phosphate levels were inversely correlated with body temperature (*P* = 0.003).

Conclusions: In acute Q fever pneumonia, CRP is the only traditional inflammation marker adequately reflecting disease activity. Patients with seronegative acute Q fever pneumonia present with hypophosphatemia and have prolonged length of hospital stay when compared to seropositive patients, suggesting an increased disease severity.
Introduction

Query fever (Q fever) is a zoonotic infection, caused by the intracellular Gram-negative coccobacillus *Coxiella burnetii*. The designation of Q fever was made in 1935 by Derrick, after an outbreak of febrile illness in abattoir workers in Queensland. *C. burnetii* can potentially be used as a bioweapon due to its extreme infectivity, which may result in large outbreaks. For this reason, *C. burnetii* is classified as a group B agent by the Centers for Disease Control and Prevention in the USA.

The presentation of Q fever is extremely variable. Infection may lead to asymptomatic seroconversion, acute disease and chronic infection. After exposure, the incubation period may last from 2 to 3 weeks although most patients, 50-60%, remain asymptomatic. In symptomatic patients, the more common presentations of acute Q fever range from a flu-like illness to acute pneumonia and hepatitis. In general, acute Q fever is a self-limiting disease. Symptoms resolve within 2 to 3 weeks, or respond to appropriate antibiotic treatment like doxycycline. Chronic Q fever, which mainly presents as endocarditis or vascular infection, develops in 1 to 5% of patients.

Symptomatic patients with acute Q fever can present in different disease stages. In general, early acute Q fever is characterized by the presence of circulating *C. burnetii* DNA in the absence of circulating antibodies (seronegative stage). Subsequently, IgM antibodies to phase II antigens (IgM-II) appear (seropositive stage) with circulating *C. burnetii* DNA still present in most cases. Thereafter, phase II IgG (IgG-II), phase I IgM (IgM-I) and phase I IgG (IgG-I) antibodies appear with coinciding disappearance of circulating *C. burnetii* DNA allowing for distinguishing time dependent serologic profiles.

A large ongoing community outbreak of Q fever, that started in the first half of 2007 and lasted until 2010, has been identified in the Netherlands. Previously, we have shown that patients diagnosed in this epidemic with acute Q fever in the seronegative stage present with a marked increase in C-reactive protein (CRP) levels while procalcitonin concentrations and white blood cell (WBC) counts are within the normal range or only marginally increased. Another less common inflammation marker that has been suggested as predictor of severity of illness in patients admitted with bacterial pneumonia is hypophosphatemia. Low serum phosphate levels (<0.8 mmol/L) are common in hospitalized patients. Symptomatic hypophosphatemia generally occurs when serum phosphate level falls below 0.32 mmol/L and may result in musculoskeletal, neurological, cardiopulmonary, haematological and metabolic dysfunction. Low serum phosphate levels are a well-known phenomenon in septic patients and in patients with respiratory illness specifically with bacterial pneumonia.

Major causes of hypophosphatemia are decreased intestinal absorption, internal redistribution of phosphate and increased urinary loss. Elevated body temperature
Biomarkers and clinical signs in seronegative PCR positive patients as compared to seropositive patients with early acute Q fever pneumonia

has also been suggested as cause of hypophosphatemia.\textsuperscript{14} In patients with malaria, serum phosphate was inversely correlated with body temperature, and each 1°C increase in body temperature was associated with a reduction of 0.18 mmol/L in serum phosphate level.\textsuperscript{14}

Here, we set out to study traditional and less common inflammation markers in seronegative and seropositive patients with acute Q fever pneumonia to identify potential markers that distinguish patients in different disease stages and predict disease severity. The identification of inflammation markers that differentiate between serostatus and predict disease severity, may contribute to decisions regarding hospital admission and treatment.

Since the symptoms of acute Q fever (pneumonia) usually resolve within 2 to 3 weeks and antibodies are also detected after 2 to 3 weeks from the onset of the disease\textsuperscript{4}, we believe it is reasonable to hypothesize that symptomatic seronegative patients present with more severe disease when compared to seropositive patients, as symptoms are probably already subsiding in the latter patient group.

We analyzed 40 patients who presented at our Emergency Department with acute Q fever pneumonia and were included in a study on the etiology of community-acquired pneumonia (CAP). This cohort of well-described patients provided the opportunity to compare seronegative and seropositive patients with regards to CRP level, WBC, neutrophil and lymphocyte counts, phosphate level, pneumonia severity index (PSI), arterial blood gas analysis, fever, duration of symptoms, influence of prior antibiotic therapy and length of hospital stay.

\section*{Methods}

\subsection*{Study cohort}
A total number of 443 adult patients (age $\geq$ 18 years) were considered for inclusion in a prospective observational study on the etiology of CAP initiated by the National Institute for Public Health and the Environment that ran from November 2007 until February 2010 at the Emergency Department of the Jeroen Bosch Hospital in ‘s-Hertogenbosch, The Netherlands. CAP was defined as an acute symptomatic infection of the lower respiratory tract, which had developed outside a hospital or nursing home, whereby a new infiltrate was demonstrated on a chest X-ray.\textsuperscript{15} Patient characteristics and clinical symptoms, including body temperature and PSI, were assessed upon presentation at the Emergency Department.\textsuperscript{16} Samples collected at the Emergency Department for microbiological analysis included an acute phase serum sample for serological tests (\textit{Mycoplasma pneumoniae}, \textit{Legionella pneumophila} serogroups 1-6, \textit{Chlamydophila psittaci} and \textit{C. burnetii}) and molecular diagnostic tests (\textit{C. burnetii}), a urine sample for urinary antigen testing (\textit{Streptococcus pneumonia} and \textit{L. pneumophila} serogroup 1),
sputum and blood samples for routine bacterial culture, and sputum and a combined nose and throat swab for molecular diagnostic tests (influenza A virus, including influenza A H1N1 from June 2009 onwards, influenza B virus, respiratory syncytial virus A and B, rhinovirus, enterovirus, parainfluenzavirus 1-4, adenovirus, coronavirus and human metapneumovirus). Microbiological analysis was complete for all patients included. Duration of symptoms, (prior) antibiotic therapy and length of hospital stay were retrieved from the hospital information system. Although the intention in our hospital is to treat patients according to the Dutch Working Party on Antibiotic Policy (SWAB) guidelines, the Q fever outbreak forced us to add empirical antibiotic coverage for C. burnetii in most patients presenting with CAP regardless of SWAB guidelines recommendations. Four weeks after inclusion, a convalescent phase serum sample was obtained for follow-up serological tests. A regional medical ethics committee approved the study protocol and patients entered the study only after informed consent was obtained.

Q fever diagnostics
Real time PCR for C. burnetii DNA, targeting the multicopy IS1111 insertion element, was performed on acute phase serum samples as described. Serologic diagnosis of Q fever was made by immunofluorescence assay for IgM-II, IgG-II, IgM-I and IgG-I antibodies, according to the manufacturer’s instructions (IFA; Focus Diagnostics, Inc., Cypress, CA, USA). Titers ≥ 1:32 were considered positive. For reasons of efficiency, an enzyme-linked immunosorbent assay (ELISA) measuring IgM-II antibodies (Institut Virion\Serion GmbH, Würzburg, Germany) was introduced in May 2009 as serologic screening assay. The IgM-II screening ELISA was performed according to the manufacturer’s instructions on a DSX automated ELISA processing system (Dynex Technologies, Chantilly, VA, USA). From that time onwards, IFA was performed only in case of a positive or doubtful result in the IgM-II screening ELISA. The diagnosis acute Q fever was made according to current guidelines recently published by the Dutch Society for Microbiology and the National Institute for Public Health and the Environment. Diagnosis of acute Q fever was made through either demonstration of circulating C. burnetii DNA by real time PCR or by seroconversion of antibodies to phase II and/or phase I antigens.

Inflammation markers and blood gas analysis
CRP and phosphate levels were measured with a fully automated enzyme-linked immuno-assay using an Aeroset 2.0 analyzer (Abbott Diagnostics, Santa Clara, California, USA). WBC, neutrophil and lymphocyte counts were determined on a Sysmex XE-2100 hematology analyzer (Sysmex Corporation, Kobe, Japan). Arterial blood gas analysis was performed on a Rapidlab 1265 analyzer (Siemens Healthcare, Erlangen, Germany). All samples were analyzed upon presentation at the Emergency Department and data were retrieved from the hospital information system.
Statistical Analysis.
Correlation coefficients were calculated to assess the direction and magnitude of association between variables. Additionally, chi-square tests were performed to explore associations. With regard to the continuous variables, we firstly judged for fit to the normal distribution by using stem-and-leaf plots and quantile-quantile (QQ) plots. As our data did not follow a normal distribution, Mann-Whitney U tests were performed for comparison of variables in different groups. A $P$-value of less than 0.05 was considered to indicate statistical significance. All reported $P$-values are two sided. Statistical analyses were performed using PASW 18.0 for Windows (SPSS Inc., Chicago, IL).

Results

Patients with acute Q fever
In all, 40 patients with CAP resulting from acute Q fever were identified in this study. Of these, 29 patients were seronegative at time of presentation (seronegative group). Of the remaining 11 patients, 8 presented with solitary IgM-II antibodies, while 3 presented with IgM-II and IgG-II antibodies (seropositive group). In the overall group of 40 patients, all but two seropositive patients had a positive serum PCR in the acute phase. In follow-up serum samples, all patients in the overall group showed seroconversion of antibodies against phase I and/or phase II antigens.

Inflammation markers in seronegative and seropositive patients
All patients in both groups presented with an increased CRP level (normal range $< 6$ mg/L). There were no significant differences in CRP level, WBC and lymphocyte counts between both groups. A significant difference was found in the neutrophil count (normal range 1.5-7.5 x 10^9/L) with values of 7.6 ± 2.2 (mean ± SD) vs. 6.1 ± 1.1 x 10^9/L in the seronegative and seropositive groups ($P = 0.03$). Phosphate level was significantly lower in the seronegative group compared to the seropositive group (0.7 ± 0.2 vs. 0.9 ± 0.2 mmol/L, $P = 0.01$). Furthermore, hypophosphatemia (normal range 0.8-1.4 mmol/L) was significantly more common in seronegative patients when compared to seropositive patients (62.1% (n = 18) vs. 18.2% (n = 2), $P = 0.01$). In the seronegative patients, a statistically significant negative correlation was observed between phosphate level and body temperature ($R = -0.539$, $P = 0.003$). This correlation remained significant in the overall group ($R = -0.513$, $P = 0.01$), although no significant correlation was observed in the seropositive group ($R = -0.387$, $P > 0.20$). Table 1 summarizes levels of inflammation markers in the seronegative and seropositive group.
Table 1. Comparison of Inflammation Markers and Clinical Parameters in Seronegative and Seropositive Patients with Acute Q Fever Pneumonia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Seronegative group (n = 29)</th>
<th>Seropositive group (n=11)</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>20,0 (69)</td>
<td>9,0 (82)</td>
<td>0,69</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54,0 ± 16,3</td>
<td>53,4 ± 14,4</td>
<td>0,81</td>
</tr>
<tr>
<td>Body temperature (°C)</td>
<td>38,8 ± 1,1</td>
<td>38,5 ± 1,1</td>
<td>0,28</td>
</tr>
<tr>
<td>Pneumonia severity index</td>
<td>63,5 ± 32,3</td>
<td>56,6 ± 19,8</td>
<td>0,77</td>
</tr>
<tr>
<td>Length of hospital stay (days)*</td>
<td>7,7 ± 4,2</td>
<td>4,9 ± 1,6</td>
<td>0,02**</td>
</tr>
<tr>
<td>Duration of symptoms</td>
<td>3,7 ± 1,9</td>
<td>4,6 ± 1,7</td>
<td>0,18</td>
</tr>
<tr>
<td>Prior antibiotic therapy</td>
<td>9,0 (32.1)</td>
<td>5,0 (45.5)</td>
<td>0,44</td>
</tr>
<tr>
<td>Absolute neutrophil count (10e9/L)</td>
<td>7,6 ± 2,2</td>
<td>6,1 ± 1,1</td>
<td>0,03**</td>
</tr>
<tr>
<td>Absolute lymphocyte count (10e9/L)</td>
<td>1,1 ± 0,5</td>
<td>1,2 ± 0,5</td>
<td>0,39</td>
</tr>
<tr>
<td>Phosphate level (mmol/L)</td>
<td>0,7 ± 0,2</td>
<td>0,9 ± 0,2</td>
<td>0,01**</td>
</tr>
<tr>
<td>pH</td>
<td>7,4 ± 0,0</td>
<td>7,5 ± 0,1</td>
<td>0,64</td>
</tr>
<tr>
<td>pCO2 (mmHg)</td>
<td>33,1 ± 5,3</td>
<td>32,8 ± 4,5</td>
<td>0,80</td>
</tr>
<tr>
<td>Bicarbonate (mmol/L)</td>
<td>24,5 ± 3,1</td>
<td>24,9 ± 2,6</td>
<td>0,59</td>
</tr>
</tbody>
</table>

Data are number (%) of subjects for gender and prior antibiotic therapy and mean ± SD for all other values.

* Length of hospital stay was applicable in 30 patients, including 21 seronegative patients.

** Significant P < 0.05.

Clinical parameters in seronegative and seropositive patients.

There were no significant differences in gender, body temperature, duration of symptoms, prior antibiotic therapy and PSI score at admission between both groups. Although there was no significant difference, the mean body temperature was elevated in both the seronegative (38,8 ± 1,1° C) and seropositive (38,5 ± 1,1° C) group. The mean PSI score classified patients in both groups in the low risk class II (PSI score 51-70). On admission, there was no significant difference in empirical antibiotic treatment between both groups. Shortly after admission, all patients were treated with an appropriate antibiotic covering C. burnetii (doxycycline, moxifloxacin or ciprofloxacin). Mean time to appropriate antibiotic treatment was 0.6 days in the seronegative group compared to 0.4 days in the seropositive group (P = 0.91). In the group of seronegative patients, 72.4% (n = 21) required hospital admission compared to 81.8% (n = 9) in the seropositive group (P = 0.70). Length of hospital stay was significantly longer in
seronegative patients with a duration of 7.7 ± 4.2 days as compared to 4.9 ± 1.6 days in seropositive patients ($P = 0.02$). Table 1 summarizes levels of clinical parameters in the seronegative and seropositive groups.

**Discussion**

Early acute Q fever is characterized by a seronegative stage in which circulating *C. burnetii* DNA is present in the absence of circulating antibodies. Subsequently, in the seropositive stage, IgM-II antibodies appear, initially with circulating *C. burnetii* DNA still present in most cases. We have previously shown that patients with acute Q fever in the seronegative stage present with a marked increase in CRP levels while procalcitonin concentrations and WBC counts are within normal range or only marginally increased. Here, we expand on that work by comparing inflammation markers and clinical parameters in seronegative and seropositive patients with acute Q fever pneumonia. In both the seronegative and seropositive group increased CRP levels were found. However, there was no significant difference in CRP level between both groups, which is consistent with the absence of a difference in clinical severity of pneumonia as assessed by the PSI. In both groups, the WBC count was within the normal range as previously shown in seronegative patients. A marginally increased neutrophil count was observed in seronegative patients, which reached statistical significance compared to seropositive patients. Acute phase lymphocyte counts are known to be decreased in patients presenting with CAP. Consistent with our observations regarding CRP level and PSI, we did not find a significant difference between both groups in lymphocyte counts. In both groups, however, lymphocyte counts were in the lower range of normal values. Overall, these observations show that CRP level is the only traditional inflammation marker reflecting disease activity during seronegative as well as seropositive acute Q fever.

Hypophosphatemia is often observed in acute infections and has been suggested as a predictor of the severity of illness in patients admitted to the hospital with bacterial pneumonia. We found a significant difference between phosphate levels in the seronegative and seropositive group. Mean phosphate level was below the lower limit of normal in the seronegative group only and hypophosphatemia was significantly more common in this group. This difference did not coincide with a difference in pneumonia severity between groups as expressed in the PSI score. However, the overall group consisted of 40 patients and a larger study needs to be performed to establish a definitive relation between phosphate level and pneumonia severity in acute Q fever pneumonia.

Seronegative patients had a significantly longer length of hospital stay which indicates that admission early in the course of acute Q fever pneumonia does not
coincide with quick discharge despite timely initiation of antibiotic treatment. Since hypophosphatemia was more common in the seronegative group, this supports a previous observation that hospital stay was twice as long in hypophosphatemic patients with respiratory illness as compared to normophosphatemic patients.\(^{12}\)

Elevated body temperature may be one of the causes of hypophosphatemia in patients with acute Q fever pneumonia. Likewise, fever has been proposed as one of the causes of hypophosphatemia in malaria patients in whom a significant negative correlation was found between phosphate level and fever.\(^{14}\) Previous observations suggest a causal relation between hyperthermia and hypophosphatemia in patients undergoing total-body hyperthermia as an adjunct to the treatment of solid malignant tumours. Hyperthermia appears to decrease renal tubular reabsorptive capacity, which in turn leads to an increased clearance and fractional excretion of phosphate. This effect is reversible with normalisation of phosphate levels after cessation of the hyperthermia.\(^{19,20}\) Another mechanism for this phenomenon may be hyperthermia-induced hyperventilation resulting in respiratory alkalosis, a pathophysiologic state known to increase redistribution of phosphate into the cells.\(^{19}\) Our data confirmed a statistically significant negative correlation between phosphate level and body temperature in acute Q fever pneumonia.

In sum, our present study extends on our previous observation that CRP level is the only traditional inflammation marker adequately reflecting disease activity in acute Q fever. Here, we demonstrate that patients with seronegative acute Q fever pneumonia present with hypophosphatemia when compared to seropositive patients. Furthermore, we observed an inverse correlation between phosphate level and body temperature. In addition, seronegativity coincided with a longer hospital stay. This may suggest increased disease severity and duration in seronegative patients compared to seropositive patients. However, a larger cohort needs to be studied to establish a definitive relation between serostatus, phosphate level and pneumonia severity in acute Q fever pneumonia.
References