LETTERS

Chlamydia pneumoniae is frequently detected in the blood after acute lung infection

To the Editors:

The obligate intracellular bacterium *Chlamydia pneumoniae* is a common cause of acute respiratory infections with a worldwide seroprevalence of up to 70% [1]. Bacterial persistence following acute infection in the respiratory tract or in atherosclerotic blood vessels has been suggested to be involved in the pathogenesis of chronic inflammatory diseases, such as chronic obstructive pulmonary disease and atherosclerosis [2, 3]. It has been shown in animal models that after acute lung infection with *C. pneumoniae*, the pathogen is systemically distributed in the blood circulation using blood monocytes as a vector [4, 5]. However, data supporting this hypothesis in humans are still missing. From *in vitro* observations, it is known that *C. pneumoniae* infection of human blood monocytes results in a nonreplicative but viable state of the pathogen, which is refractory to antibiotic treatment [6].

The objective of this study was to analyse whether *C. pneumoniae* systemically disseminates from the lung to the blood in patients with an acute lower respiratory tract infection (LRTI). Patients recovering from acute *C. pneumoniae* lung infection were monitored for the presence of *C. pneumoniae* DNA in the blood during follow-up visits (at 30 and 90 days post infection) and compared with patients after *Streptococcus pneumoniae* infection.

Patients presenting at the emergency room or outpatient departments of the University Hospital Schleswig-Holstein, Campus Luebeck (Luebeck, Germany) with radiographically confirmed nonsevere community-acquired pneumonia (CAP) or severe LRTI with fever, productive cough and positive auscultatory findings were tested during a 3-yr period for the presence of *C. pneumoniae* or *S. pneumoniae* as causative pathogens of the disease. Inclusion criteria were the ability to produce sputum, perform a lung function test and willingness to return for follow-up visits after recovery from acute infection; patients with an immunodeficiency and patients from nursing homes were excluded. A total of 42 patients with confirmed *C. pneumoniae* or *S. pneumoniae* infection (CAP: n=40; acute bronchitis: n=2) were initially recruited. To overcome differences in *C. pneumoniae* and *S. pneumoniae* infection frequencies in acute respiratory infections, recruitment of a *S. pneumoniae* patient followed the recruitment of a *C. pneumoniae* patient. Three patients with *C. pneumoniae* infection were excluded from the analysis because of consent withdrawal or loss of follow-up. Clinical characteristics of the patients were documented on day 0, day 30 (visit one) and day 90 (visit two). Respiratory and blood samples were taken upon admission and at each follow-up visit with additional lung function tests on visit one and two.

Diagnosis of *S. pneumoniae* infection was performed on the basis of culture testing of respiratory samples and blood, as well as the detection of pneumococcal antigen in urine (Binax

NOW[®]; Inverness Medical International, Cranfield, UK). Spontaneous or induced sputum samples (10 min inhalation with 3% NaCl solution) were taken on each visit for detection of *S. pneumoniae* (blood agar culture according to the Clinical and Laboratory Standards Institute (Wayne, PA, USA) recommendations) or *C. pneumoniae* (PCR) infection. Isolation of peripheral blood monocytes (PBMC) from blinded EDTA blood samples and detection of *C. pneumoniae* by PCR were performed as described previously [6]. The study protocol was approved by the ethical committee of the University of Luebeck (Nr: 01-148). All patients were informed of the study purpose and gave written consent. Statistical analysis was performed with the SPSS software (SPSS Inc., Chicago, IL, USA) using the t-test for the analysis of nonpaired, parametrical parameters and the Mann–Whitney U-test for the analysis of nonpaired, nonparametrical parameters. Nominal scaled parameters were analysed using the Chi-squared test.

A total of 39 patients were included in the study. In 18 patients *C. pneumoniae* was found in the sputum as the causative pathogen of an acute respiratory infection, including 16 patients with CAP and two patients with acute bronchitis. 21 patients with *S. pneumoniae* infections fulfilled the criteria for CAP. Patient characteristics did not differ between both groups, except that the patients in the *S. pneumoniae* group were significantly older ($p<0.05$) (table 1). At the time of admission, patients suffering from *S. pneumoniae* infection had comparatively higher systemic inflammatory markers, e.g. the C-reactive protein and the white blood cell count.

To investigate the systemic dissemination of *C. pneumoniae* infection, blood samples for *C. pneumoniae* PCR were taken

TABLE 1 Patient characteristics at the time of admission

	<i>Streptococcus pneumoniae</i>	<i>Chlamydia pneumoniae</i>	p-value
Patients n	21	18	
Age yrs	62.1±3.1	51.0±4.1	0.036*
Males/females n	12/9	12/6	0.542
Smokers %	11 (52.4)	7 (38.9)	0.399
COPD history %	6 (28.6)	2 (11.1)	0.178
CRP mg·L⁻¹	254.47±30.67	115.07±23.60	0.001*
WBC n·nL⁻¹	16.02±0.87	11.79±1.22	0.007*

Data are presented as mean±SEM or n (%), unless otherwise stated. COPD: chronic obstructive pulmonary disease; CRP: C-reactive protein; WBC: white blood cells. *: $p<0.05$.

within 30 and 90 days (visits one and two, respectively) after the infection. PBMC from patients recovering from acute *C. pneumoniae* lung infection were significantly more often positive for chlamydial DNA within 30 days post infection when compared with the *S. pneumoniae* control group (72.7% versus 17%, $p=0.007$) (fig. 1). Detection of *C. pneumoniae* DNA in circulating white blood cells by PCR has previously been shown to be a valuable tool to identify *C. pneumoniae* carriers [7]. Overall prevalence of *C. pneumoniae* DNA positivity has been detected in 18.5% of healthy blood donors and up to 59% in patients suffering from coronary artery disease [8, 9]. Our data indicate that detection frequency of *C. pneumoniae* in the blood increases rapidly (30 days post infection) but is not prolonged (90 days post infection) after acute *C. pneumoniae* lung infection (fig. 1).

Infections with *C. pneumoniae* account for 0.9–13% of CAP worldwide and seem to emerge epidemically over the years [10]. Due to that fact, prospective studies analysing the outcome of patients suffering from acute respiratory *C. pneumoniae* infections over time are arduous to perform and are still missing. Animal models suggest that *C. pneumoniae* may spread from the lung to the blood circulation after initial infection of the lower respiratory tract using blood monocytes as a vector [4]. As *C. pneumoniae* DNA is consistently found in variable proportions of asymptomatic healthy subjects, the clinical relevance of chlamydial persistence in the lung, as well as in blood mononuclear cells, is not fully understood [11]. Thus, *C. pneumoniae* may persist for many months in the lung following acute infection despite long-term courses of tetracycline or doxycycline therapy [10]. In contrast, BLASI *et al.* [12] have shown that in patients with mild-to-moderate chronic bronchitis, the detection of *C. pneumoniae* DNA in PBMC was associated with a higher rate of acute exacerbations. They also showed that sustained clearance of *C. pneumoniae* from the blood following treatment with azithromycin was only achieved in 29% of the patients [12]. This could be due to the low susceptibility of persistent chlamydiae to macrolide therapy [6], but could also indicate systemic dissemination of dead

FIGURE 1. Detection frequency of *Chlamydia pneumoniae* DNA in peripheral blood monocytes (PBMC) from patients after acute respiratory infection with *C. pneumoniae* (■) or *Streptococcus pneumoniae* (▲). PBMC fractions of whole blood samples were significantly more often *C. pneumoniae* DNA positive (PCR) 30 days post infection (visit 1) in patients with acute *C. pneumoniae* infection compared with patients with *S. pneumoniae* infection. #: $p=0.007$.

C. pneumoniae within PBMCs from a chronic reservoir, as intracellular growth and progeny of *C. pneumoniae* is severely impaired in primary monocytes and monocyte-derived macrophages [13].

The limited size of our study precludes firm conclusions regarding the relationship between *C. pneumoniae* dissemination and the clinical course. However, recovery from pneumonia and changes of treatment were not different between patients with or without the detection of *C. pneumoniae* DNA in the blood. In addition, significant changes in lung function or pro-inflammatory cytokine release in the sputum or blood over time were not observed between both groups (data not shown).

In conclusion, systemic dissemination of *C. pneumoniae* from the lung to the blood after acute respiratory infection is observed in 70% of the patients within 30 days post infection. However, the clinical relevance of these findings with respect to disease resolution and the pathogenesis of chronic vascular and pulmonary diseases warrant further investigations.

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Support Statement: This work was supported by German Federal Ministry of Education and Research (Bundesministerium für Bildung und Forschung), Competence Network CAPNETZ (TP A6) and the European Initiative to Fight Chlamydial Infections by Unbiased Genomics (ECIBUG) within the ERA-NET PathoGenoMics framework (BMBF).

Statement of Interest: None declared.

Acknowledgements: We gratefully thank U. Knuppertz and H. Richartz (Medical Clinic III, University Hospital Schleswig-Holstein, Campus Lübeck, Germany) for excellent technical support.

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DOI: 10.1183/09031936.00102310

Antisynthetase syndrome positive for anti-threonyl-tRNA synthetase (anti-PL7) antibodies

To the Editors:

Antisynthetase syndrome (ASS) is characterised by an inflammatory myositis associated with interstitial lung disease (ILD) and antisynthetase antibodies. Other symptoms, including arthritis, Raynaud's phenomenon and mechanic's hands, are also associated with ASS. Several antisynthetase antibodies [1] have been described, with anti-Jo1 being the most common. Little is known about the clinical manifestations of ASS associated with anti-threonyl-tRNA synthetase antibodies (anti-PL7) [2–4], most probably because anti-PL7 antibodies are particularly rare (5% of myositis [5]) and have not been routinely researched in the past.

We conducted this retrospective multicentric study to describe the clinical, radiographic and biological manifestations of ASS in 12 patients.

This retrospective study was conducted in five university hospitals. The patients were followed from 2000–2010 in the Depts of Pneumology (n=7) and Internal Medicine (n=5). 16 anti-PL7 positive patients were identified due to immunological laboratory databases. To exclude false-positive patients, we only included patients who successively tested positive for anti-PL7 twice (n=13): the three patients tested only once did not disclose any symptoms in accordance with ASS. We excluded one patient for whom clinical data were missing. The 12 included patients tested positive for anti-PL7 antibodies at least twice by immunodot (immuno-DOT D-tek; Diasorin, Antony, France) and/or Western blot using protein extracts from Hep2 cells (n=7 and n=5, respectively). They presented with one or more ASS symptoms, including ILD and/or myositis. A search for anti-DNA and anti-extractable nuclear antigen antibodies was systematically performed, whereas rheumatoid factor and anti-citrullinated peptide were available in four and two patients, respectively.

The characterisation of the ILD pattern was made by several radiologists who were all experienced in ILD and were based on the American Thoracic Society/European Respiratory Society 2002 consensus [6].

Pulmonary hypertension was suspected by echocardiography when systolic pulmonary arterial pressure was >40 mmHg.

For the follow-up, we defined pulmonary aggravation/improvement as an increase/decrease in dyspnoea, according to New York Heart Association (NYHA) stages and/or deteriorating/improving pulmonary function tests (>10% decrease/increase in carbon monoxide transfer factor and/or total lung capacity between the first and the last tests).

This study was approved by hospital local ethical committees. The patients of this study are anonymously reported and in accordance with the French law, a patient consent was required.

The mean age at the first sign of clinical symptoms was 56.3 yrs (range 31–71 yrs). Three patients had interrupted smoking habits; none had known toxic environmental exposure or any family history of ILD.

All patients presented with ILD at the onset of the disease. Muscular symptoms occurred in half of the patients. Fever and other symptoms are detailed in table 1.

11 patients complained of mild (NYHA II, n=5) to severe (NYHA III–IV, n=6) dyspnoea as an initial symptom of ILD. Dyspnoea was chronic (n=7) or acute (n=4), and could be associated with dry cough (n=7) and clubbing (n=2). Examination revealed fine crackles in all patients. ILD was confirmed by high-resolution computed tomography (CT) scans in all cases. Based on this CT scan, nine out of 12 patients exhibited nonspecific interstitial pneumonia (NSIP) pattern with bibasal mild fibrosis, including ground-glass and intralobular reticulation. Traction bronchiectasia were initially described in four of these patients. A pattern of organising pneumonia (OP) was present in a further two cases, with subpleural and peribronchovascular condensations. Ground-glass opacities were associated with bronchial wall thickening and mosaic pattern in the remaining case, in accordance with obliterative bronchiolitis (OB).

Bronchoalveolar lavage (BAL) revealed alveolitis with an increased number of neutrophils (five out of eight patients) or lymphocytes (three out of eight patients). Lung biopsy was