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CASE STUDY

Detection of *Chlamydia pneumoniae* in unexplained pulmonary hypertension

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Detection of Chlamydia pneumoniae *in unexplained pulmonary hypertension. D. Theegarten, O. Anhenn, S. Aretz, M. Maass, G. Mogilevski. ©ERS Journals Ltd 2002.*

ABSTRACT: The pathogenesis of primary pulmonary hypertension is still unclear. The case of a 68-yr-old female patient who complained of recurrent dizzy spells and collapses over a period of 6 weeks and died of global cardiac failure is presented.

Autopsy revealed severe pulmonary hypertension, slight chronic bronchitis, and bronchiolitis as well as intra-alveolar accumulation of macrophages. Chlamydiae were detected within the pulmonary arteries and in intramural and intra-alveolar macrophages by immunofluorescence, confocal laser scanning microscopy, scanning and transmission electron microscopy. Nested-polymerase chain reaction (PCR) and nonradioactive deoxyribonucleic acid (DNA) hybridization of PCR products from pulmonary arteries revealed *Chlamydia pneumoniae* DNA.

***Chlamydia pneumoniae* has already been detected in atherosclerosis and in pulmonary emphysema. It can induce proliferation of smooth muscle cells. *Chlamydia pneumoniae* might be relevant in aggravation of primary pulmonary hypertension and might perhaps be a trigger factor in some cases.**

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Primary pulmonary hypertension is a rare disease of unknown pathogenesis [1, 2]. Pathology reveals ectasia and pulmonary sclerosis of the large pulmonary arteries. In the smaller arteries intima fibrosis, media hypertrophy, plexiform lesions, and necrotizing arteriitis are seen. Pulmonary arteries in advanced primary pulmonary hypertension present with atherosclerotic lesions. *Chlamydia pneumoniae* has been detected in atherosclerotic plaques by electron microscopy, immunohistochemistry, polymerase chain reaction (PCR), and culture [3, 4]. *C. pneumoniae* is a known agent of respiratory infections and associated with asthma, chronic obstructive pulmonary disease and emphysema [5]. Possible *C. pneumoniae* colonization of the pulmonary arteries in pulmonary hypertension has to be investigated.

Case report

A 68-yr-old female was admitted to hospital with a history of recurrent dizzy spells for a period of 6 weeks. Attacks were accompanied by collapses, dyspnoea, perspiration, and sometimes vomitus. Acute global cardiac failure developed (New York Heart Association Stage IV). Ultrasonography revealed pulmonary hypertension, a cor pulmonale, and a reduced ejection fraction of the left ventricle (30% predicted). In spite of catecholamine therapy and respiratory ventilation the patient died within hours from cardiogenic shock.

Autopsy was performed, tissue for light and electron microscopy was fixed in 3.5% formaldehyde. Material for scanning electron microscopy was dried by the critical-point method and sputtered with gold after mounting. For transmission electron microscopy tissue was embedded in epon after postfixation with osmium tetroxide. Specimens were first viewed using semithin cuts and stained with basic fuchsin and methylene blue. Blocks of adequate quality were chosen for further investigations. For immunofluorescence, specimens were fixed in methanol acetone (1:1) and triple stained with: 1) genus specific rabbit antiserum (Biodesign, Dunn Labortechnik Ansbach, Germany; dilution 1:500) against Chlamydia lipopolysaccharide; 2) monoclonal antibodies from mouse against vimentin or CD68 (DAKO, Hamburg, Germany; dilution 1:50/1:20); and 3) 4′,6-Diamidino-2-phenylindol (DAPI, Sigma, Deisenhofen, Germany; dilution 1:10,000). Alexa Fluor 594 ($F(ab')_2$-fragments of goat anti-rabbit, dilution 1:200) and Alexa Fluor 488 ($F(ab')_2$-fragments of goat anti-mouse, dilution 1:200) were used as secondary antibodies (Molecular Probes Europe, Leiden, the Netherlands).

Parts of the large pulmonary arteries were cut out, homogenized and used for PCR. Nested-PCR and nonradioactive deoxyribonucleic acid (DNA)

hybridization of PCR products for detection of *C. pneumoniae* DNA was carried out using a protocol previously evaluated for arterial tissue [4].

Results

Autopsy revealed severe ectasia and sclerosis of the large pulmonary arteries. Microscopically, foam-cell aggregates and lymphocytes were seen (fig. 1a). In the smaller pulmonary arteries intimal fibrosis, media hypertrophy, and plexiform lesions are found in variable extents (Grade IV according to HEATH and EDWARDS [6], fig. 1b and c). Further chronic dyscrine bronchitis, slightly focal chronic inflammation of the terminal and respiratory bronchioles (fig. 1c), and intra-alveolar accumulation of macrophages were seen. Besides nonsevere atherosclerosis of the coronary arteries, no signs of primary cardiac disease could be detected. The right ventricle showed severe hypertrophy. The liver revealed chronic congestion and siderosis.

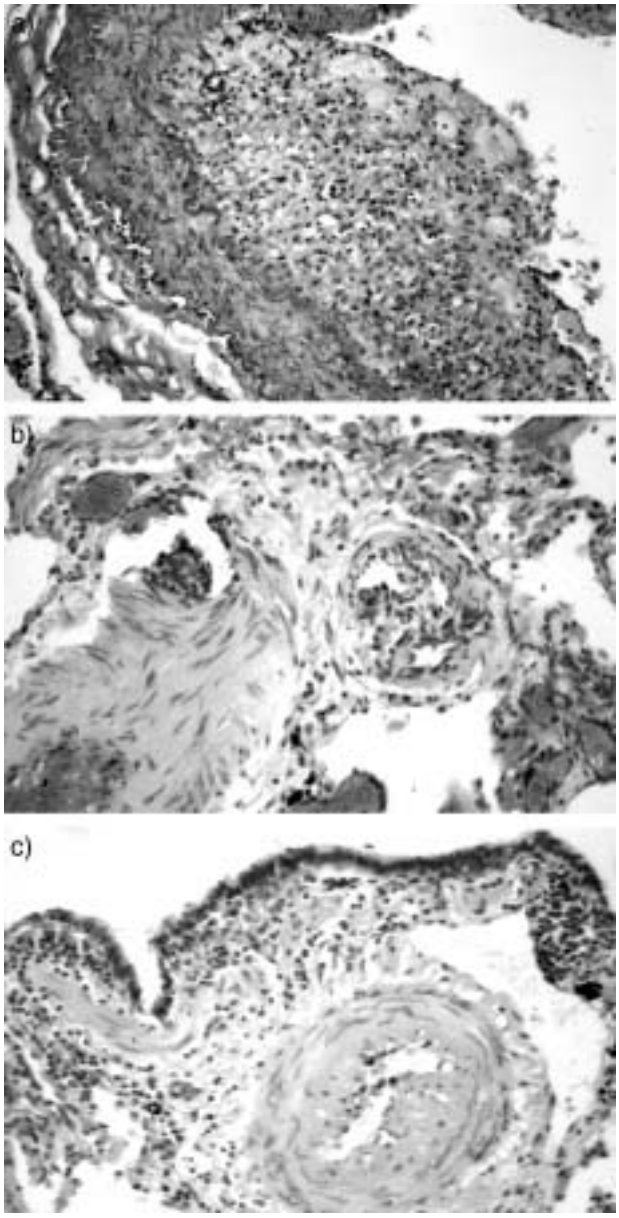

Fig. 1.–Histology. a) In the large pulmonary arteries foam cell aggregates and a few lymphocytes were dominant (Elastica van Gieson stained, original magnification ×100). b) Media hypertrophy, intima fibrosis, and rarely plexiform lesions were found (haematoxylin and eosin stained, original magnification ×100). c) Sometimes slight chronic inflammation was seen in the respiratory bronchioles (haematoxylin and eosin stained, original magnification ×100).

Immunofluorescence and confocal laser scanning microscopy with genus-specific antibodies and antiserum against *Chlamydia spp.* revealed spots on the endothelium and within the thickened intima and hypertrophic media of the large and small pulmonary arteries. Bacteria were seen in smooth muscle cells (fig. 2a), and in the intramural and the intra-alveolar macrophages. 4′,6-diamino-2-phenylindol (DAPI) spots were found in similar distribution.

Scanning electron microscopy shows 0.5–0.8 μm spherical bodies in the destruction areas of the thickened intima of the arterial walls (fig. 2b). In transmission electron microscopy these bodies possess a contrast-rich outer lipid bilayer membrane. In the centre, different granular and dense structures were seen. The diameter of the bodies varied between 0.2–0.8 μm. Spherical bodies were found lying in small groups and sometimes in vacuoles. Bodies could be detected within myofibroblasts in the large and small arteries and the adjacent lung tissue (fig. 2c).

Nested PCR and nonradioactive DNA hybridization of PCR products from the homogenized pulmonary arteries showed a strongly positive reaction for *C. pneumoniae* (128 bp product). The German *C. pneumoniae* respiratory isolate MUL-1 was used as a control.

Discussion

C. pneumoniae has been detected in atherosclerosis and in pulmonary emphysema [3–5]. Although long-time persistence is a paradigm in pathogenesis of chlamydial infections [7], the role of *C. pneumoniae* in these diseases is still under debate. Interpretations rank from a causal role in ultrachronic infection to an innocent bystander phenomenon.

The presented case has to be classified as primary pulmonary hypertension. Histologically, foam cells and lymphocytes were seen in the large pulmonary arteries, indicating an inflammatory reaction. *C. pneumoniae* was detected by scanning electron microscopy, transmission electron microscopy, immunofluorescence, and nested PCR. *C. pneumoniae* was found in the arterial walls as well as in intra-alveolar accumulated macrophages. This means that bacterial colonization of the lung and the pulmonary vessels, as well as an inflammatory reaction, exist side by side. Primary pulmonary hypertension seems to require a permissive genotype, a susceptible phenotype (*e.g.* endothelial dysfunction) and, in many cases, an exogenous trigger [1, 2]. Endothelial cell-derived

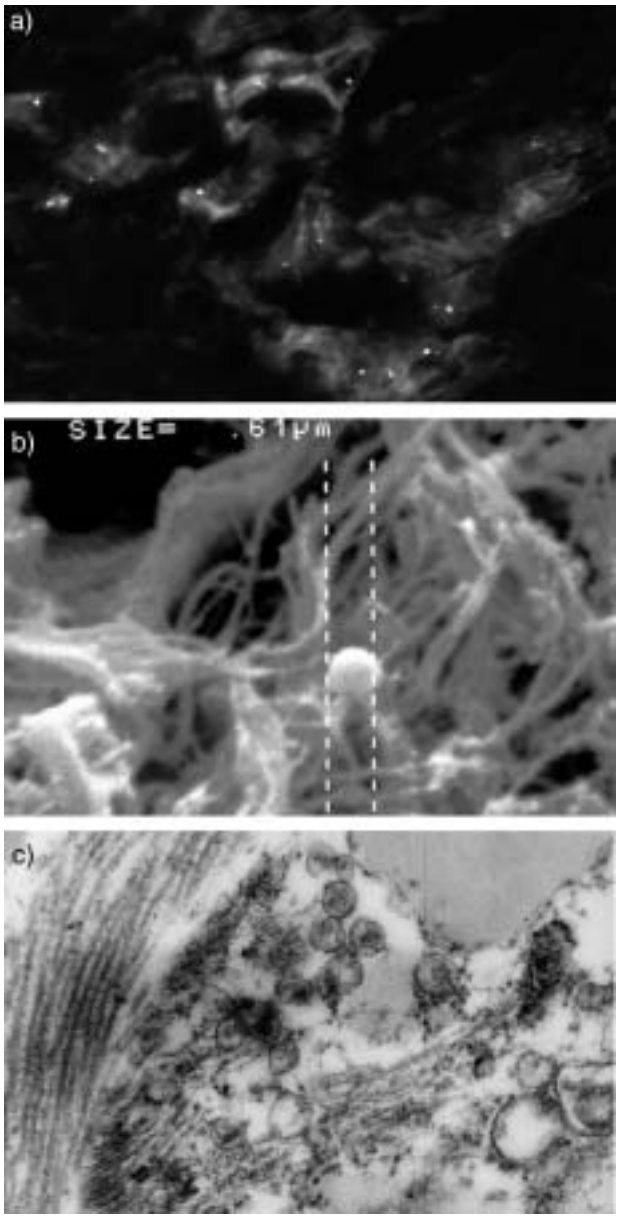

Fig. 2. – Immunofluorescence and electron microscopy. a) Confocal laser scanning microscopy with antiserum against *Chlamydia spp*. shows bright spots within the hypertrophic media of small arteries demonstrating the positive immunofluorescence reaction to *Chlamydia spp*. (double staining with actin (pale staining), objective magnification ×50). b) Spherical bodies with a diameter of 0.61 µm were seen in lytic areas of the thickened intima, macrophages were found nearby (scanning electron microscopy, original magnification ×10,000). c) These had a double membrane, were lying in small groups, and were sometimes detected within myofibroblasts (transmission electron microscopy, original magnification ×20,000).

soluble factors are known to induce proliferation of smooth muscle cells in *C. pneumoniae* infection [8]. *C. pneumoniae* infection could therefore aggravate media hypertrophy and perhaps be an exogenous trigger in the beginning of the disease. Under this hypothesis, primary respiratory infection of the bronchioli with consecutive persistence and/or reinfection has to be assumed. Affection of the pulmonary vessels has to be interpreted as a secondary step. Liver cirrhosis and human immunodeficiency virus infection also increase the risk of getting primary pulmonary hypertension and other infections.

Larger collectives of patients with primary pulmonary hypertension have to be examined morphologically and microbiologically for *Chlamydia pneumoniae*. Investigations must be carried out to reveal the true role of *Chlamydia pneumoniae* in these cases.

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