Presence of *Chlamydiales* DNA in samples negative by broad-range bacterial 16S rRNA PCRs: new insights into chlamydial pathogenic role

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Abstract

Since routine eubacterial 16S rRNA PCR does not amplify members of the *Chlamydiales* order, we tested all samples received in our laboratory during a 10 months period using a pan-Chlamydiales real-time PCR. 3 of 107 samples (2.8%) revealed to be positive, suggesting a role of some *Chlamydiales* in the pathogenesis of chronic bronchial stenosis or bronchial stenosis superinfection and as agents of orthopaedic prosthesis infections.

Keywords: Broad-range bacterial 16S rRNA PCR, *Chlamydiales*, *Chlamydia*-related bacteria, molecular diagnosis, pan-*Chlamydiales* PCR

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Introduction

Numerous syndromes of a suspected infectious origin remain of unknown microbial aetiology even after investigation by a thorough microbial diagnostic assessment that includes the use of broad-range bacterial PCR targeting the 16S rRNA encoding gene on normally sterile samples. However, this technique fails to detect bacteria from the *Chlamydiales* order because of the highly different *Chlamydiales* 16S rRNA sequences compared to bacteria from other orders. We thus wondered whether members of the *Chlamydiales* order may be implicated in the occurrence of cryptogenic infections.

In this study, we performed a pan-*Chlamydiales* PCR [1] on all samples received in our laboratory between 11 May 2012 and 11 March 2013 and all samples that tested negative by 16S rRNA PCR analysis. A total of 107 samples from 69 patients were investigated by our homemade pan-*Chlamydiales* PCR in duplicate, as previously described [1]. Overall, three samples from two patients were positive. DNA sequencing was not successful in any of these samples, probably because of the low amount of targeted DNA. (DNA was detected between a range of 36.2 to 44.7 cycle threshold (C_T), corresponding to 3160 and 8 copies of DNA/ml respectively).

The first patient, a 60-year-old woman who had undergone several knee joint replacements and who had metabolic syndrome, was admitted to the emergency ward with fever, dyspnoea and an increasing systemic inflammatory response syndrome. Joint infection was documented by a knee arthrocentesis revealing a high amount of polymorphonuclear cells. However, routine cultures and 16S rRNA PCR were all negative. A two-step revision of the knee prosthesis was performed. The spacer was loaded with tobramycin and vancomycin and kept for 1 month. Concomitantly, the patient received a treatment with intravenous amoxicillin/clavulanic acid, which was switched to an oral therapy of rifampicin and doxycycline for 3 months because of the high suspicion of prosthesis intracellular bacterial infection due to the negative cultures and broad-range bacterial 16S rRNA PCRs. Interestingly, the pan-*Chlamydiales* PCR was positive for two samples from this patient; one sample was taken from the prosthesis, and the other was a sample of synovial fluid obtained 3 weeks after spacer implantation. The infection could have been of nosocomial origin because the last prosthesis replacement was performed 7 months before the patient's presentation to the hospital. *Chlamydia* spp. could have been the cause of the infection even though these bacteria are more likely to cause reactive arthritis [2,3].

The second patient, a 41-year-old man with no relevant medical history, experienced flulike symptoms while he was in South Africa. Two months later, back in Switzerland, he developed two episodes of bronchopneumonia with fever, cough, dyspnoea, chest pain and a radiologic infiltrate that was located at the left superior lobe. A bronchoscopy was performed after the second episode of bronchopneumonia, revealing a stenosis of a segmental bronchi. All the cultures

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remained sterile, whereas both the 16S rRNA PCR and the broad-range mycobacterial PCRs [4] were negative on all tested lower respiratory tract samples. In addition, urinary antigens for *Streptococcus pneumoniae* and *Legionella pneumophila* also tested negative. The bronchial biopsy sample showed signs of chronic inflammation and fibrosis without any signs of neoplasia. The patient had a good clinical outcome with a treatment of amoxicillin/clavulanate (16 days) and clarithromycin (17 days) and after stenting the bronchi with a metallic stent. The pan-*Chlamydiales* PCR performed on the bronchial biopsy sample was positive.

Our work suggests a role for *Chlamydiales* in orthopaedic prosthesis infections and in the pathogenesis of chronic bronchial stenosis or bronchial stenosis superinfection. This relies on the absence of any alternative identified pathogen despite multiple in-depth investigations, a favourable outcome after beginning empirical therapy with antibiotics with intrinsic activity against most pathogens of the *Chlamydiales* order (i.e. doxycycline, rifampicin and clarithromycin) and the plausibility that the infectious syndromes were caused by intracellular bacteria. In that regard, several *Chlamydiaceae* and *Chlamydia*-related bacteria have already been associated with pulmonary infections [5–7].

The value of this work is limited by the absence of identification of the aetiological agent at the species level. However, this work shows the added value of pan-*Chlamydiales* PCR in such cases of infections of unknown aetiology and suggests a role of some member of the *Chlamydiales* order as an agent of prosthetic infection and in the pathogenesis of bronchial stenosis or bronchial stenosis superinfection. Further studies should be done in order to precisely identify the *Chlamydiales* species involved in these syndromes.

Conflict of Interest

None declared.

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