

DETECTION OF *CHLAMYDIA PNEUMONIAE* IN ABDOMINAL AORTIC ANEURYSM SPECIMENS FROM PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) AND ASTHMA: PILOT RESULTS

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INTRODUCTION

Chlamydia pneumoniae is an established cause for acute respiratory tract infections, and a growing body of evidence also implicates chronic *C. pneumoniae* infection in the pathogenesis of a number of chronic cardiopulmonary conditions including the asthma-chronic bronchitis-COPD complex and atherosclerosis [1]. Animal studies suggest that the lung serves as the portal of entry for systemic infection by *C. pneumoniae* [2]. Since several previous epidemiologic studies have linked *C. pneumoniae*-associated lung diseases (chronic bronchitis and asthma) with clinical atherosclerosis [3-5], we sought evidence for *C. pneumoniae* in atherosclerotic tissue of patients, some of whom had a clinical diagnosis of COPD and/or asthma.

METHODS

Formalin-fixed, paraffin-embedded material obtained from patients undergoing elective abdominal aortic aneurysm repair at a community hospital were selected by convenience sampling from the database of pathologic specimens. All tested specimens were verified as containing atheromatous material by one of the investigators (C.K.C.).

For polymerase chain reaction (PCR) testing, DNA was extracted from formalin fixed tissues by the Chelex boiling method. Briefly, sections were boiled in a 5% suspension of Chelex 100 chelating resin (Sigma Chemical Co., St. Louis, MO) in sterile water. The resulting supernatants were extracted with pheno/chloroform and precipitated with ethanol by standard methods. PCR was done using the HL-1 - HR-1 primer sets and confirmation of presumptive positives was done by immunochemiluminescence as previously described [6].

Immunocytochemistry (ICC) staining was performed by the avidin:biotinylated enzyme complex using the chlamydia genus-specific monoclonal antibody (CF-2) as described previously [7]. Normal mouse ascitic fluid was used as a control antibody. Hematoxylin was used as counterstain.

Hospital medical records for all tested patients were reviewed for smoking history, clinical COPD and/or asthma. For one patient reported in detail (see *Case report*, below), outpatient medical records and results of serologic testing for *C. pneumoniae* by the microimmunofluorescence (MIF) test were also available.

RESULTS

Abdominal atheroma material from 15 patients (average age 69, 14 males) was tested by PCR and ICC. Six of 15 patients had a history of COPD; two of these 6 were treated with inhaled bronchodilators and steroids for asthma-related symptoms. One patient was positive by PCR and an additional 5 patients were positive by ICC (40% total positive). All 5 patients who were ICC positive were noted to have staining within foam cells. Clinical characteristics of the positive and negative patients are presented in the Table.

Table. Clinical characteristics of patients with and without evidence for *C. pneumoniae* in aortic aneurysm plaque

	PCR/ICC results	
	positive (n=6)	negative (n=9)
Age, mean (range)	70 (64-80)	69 (58-83)
Smoking status		
current*	2	4
former	1	1
never	2	2
not recorded	1	2
COPD	3** (50)	3 (33)

* smoking within the past 12 months

** an additional patient had chronic sinusitis

Case Report

A 75 year old male was diagnosed with chronic asthmatic bronchitis on the basis of chest tightness and wheezing relieved by administration of bronchodilators and inhaled steroids, accompanied by a chronic productive cough. Pulmonary function testing revealed a pre-bronchodilator functional vital capacity (FVC) of 103% predicted but an FEV1 of only 58% predicted; after bronchodilator, there was a 15% increase in FVC and a 12% increase in FEV1, substantiating the diagnosis of reversible airway obstruction. *Chlamydia pneumoniae* MIF serologic testing revealed an IgG antibody titer of 1:1024 without an IgM response. After a 6 week course of therapy with oral azithromycin (one gram weekly) his chronic cough and sputum production gradually resolved but pulmonary function testing revealed persistent fixed obstruction. Tissue obtained from a previous aortic aneurysm repair was positive for *C. pneumoniae* by PCR but not by ICC.

DISCUSSION

Previous studies have shown that *C. pneumoniae* is detectable in aortic aneurysm atheromata [8, 9]. This study provides further evidence that *C. pneumoniae* infects abdominal aneurysms and can be detected within foam cells. The observation that immunohistochemical staining identified *C. pneumoniae* within foam cells, a hallmark of the atherosclerotic process, is also in agreement with previous work [7]. The clinical sequelae of aortic aneurysm are an

increasing problem with major public health consequences [10]. Whether *C. pneumoniae* infection is causal in the development of aneurysms requires further investigation.

Evidence linking *C. pneumoniae* with asthma [11] and COPD [12] is primarily seroepidemiologic, although sputum PCR positivity has also been reported in COPD [13]. To our knowledge, this is the first report of identification of *C. pneumoniae* in vascular tissue of a patient with chronic asthmatic bronchitis associated with serologic evidence suggesting chronic *C. pneumoniae* infection. The diagnoses of chronic asthmatic bronchitis and atherosclerosis could have been coincidental, as COPD and atherosclerosis share smoking as a common risk factor. However, smoking is also a risk factor for *C. pneumoniae* infection [14] and it has been suggested that *C. pneumoniae* infection itself may be a common risk factor for COPD and atherosclerosis [3].

Asthma and chronic bronchitis/COPD have been associated specifically with clinical atherosclerosis in a number of previous reports [3-5, 15, 16]. Coronary heart disease mortality (but not all cause mortality) [15] and nonfatal myocardial infarction [16] are associated with a rapid decline in FEV1 and increased levels of serum IgE, respectively, both of which are well known attributes of asthma. Symptoms of chronic bronchitis have also been associated with the risk of coronary disease in a Finnish population-based study [3]. In a Swedish population-based study, steroid-dependent asthma was also associated with death from heart disease but not with death from cancer [4]. In a prospective population-based study of cardiovascular risk factors in the elderly, univariate associations between asthma and cardiovascular disease were found that disappeared in multivariate analyses controlling for multiple risk factors including smoking and chronic bronchitis [5].

In future studies it would be worthwhile investigating whether *C. pneumoniae* is detectable in both lung and vascular tissue of patients with both COPD and atherosclerosis, as treatment might favorably affect multiple conditions associated with infection. Similar studies in asthma patients are also suggested by the results of this pilot project.

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