PREVALENCE AND PERSISTENCE OF *CHLAMYDIA PNEUMONIAE* ANTIBODIES IN PATIENTS UNDERGOING PERCUTANEOUS TRANSLUMINAL CORONARY ANGIOPLASTY (PTCA) AND ASSOCIATIONS WITH CLINICAL DISEASE SEVERITY

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INTRODUCTION

A widespread clinical intervention with proven benefit in the management of symptomatic coronary artery disease is balloon dilation of established atherosclerotic lesions in patients with critical coronary artery stenosis (percutaneous transluminal coronary angioplasty: PTCA). Reocclusion of the dilated artery (restenosis) within 6-12 months following PTCA occurs frequently (30%-50% of procedures), however, and can result in repeated cardiovascular events including death, myocardial infarction, surgery (coronary artery bypass grafting: CABG) or repeat PTCA. The addition of stenting to PTCA, or the substitution of atherectomy for PTCA, can result in fewer short term repeat clinical events. Whether these innovations decrease, or merely delay, the occurrence of restenosis is uncertain.

Chlamydia pneumoniae has been described in atherosclerotic plaques [1] and preliminary treatment results suggest a possible role for this infection in the pathogenesis of atherosclerotic cardiovascular disease [2, 3]. *C. pneumonae* is present in restenotic lesions as well as in native atherosclerotic plaque [4, 5]. It is unknown whether such infection is important to the development of restenosis, which appears to have a different pathophysiologic basis than atheroscleosis [6]. Since the cause(s) of restenosis are unclear [6] it is important to explore a possible association of *C. pneumoniae* infection and restenosis because of potential therapeutic benefits in preventing restenosis.

In this paper we describe (1) the stability over time of *C. pneumoniae* serologic results in patients following PTCA and (2) cross-sectional associations of serologic findings with degrees of clinical severity based on the presence/absence of previous procedures performed for symptomatic coronary artery disease.

METHODS

We are conducting a prospective cohort study of PTCA, with the ultimate goal of determining whether *C. pneumoniae* serologic status at the time of first PTCA is associated with subsequent clinical outcomes. From October 1, 1994 to September 30, 1996 a serologic specimen was obtained from a consecutive series of 374 patients undergoing elective PTCA at a community hospital. Cross-sectional analysis is reported here on results of testing 138 sera from 96 patients enrolled more than once and/or with prior PTCAs.

During PTCA, 7 ml of EDTA-treated blood and 7 ml of untreated blood were collected. Prior to storage, EDTA-treated blood was centrifuged and separated into red cells, buffy coat and plasma fractions (without employing gradient centrifugation). Untreated blood was allowed to clot, separated and the serum fraction was divided into 1 ml aliquots. All specimens were stored at minus 70 degrees C until tested. Some specimens were obtained from heparin-treated patients.

C. pneumoniae-specific IgM, IgG and IgA antibodies were determined by the microimmunofluorescence (MIF) test developed by Wang and Grayston [7], using Kajaani 6 as antigen. Chlamydial immune complexes (CIC) were precipitated as previously described [8]. The L2 strain of *C. trachomatis* was used as a control antigen for IgG, IgA and immune complex testing. Positive titers indicating seroreactivity were defined as IgG≥ 1:32, IgA≥ 1:16 and CIC≥ 1:2. In this study, a combined measure for the presence of "chronic antibody" was taken as IgG≥ 1:128 and/or IgA≥ 1:16 and/or CIC≥ 1:2.

Clinical data pertaining to events preceding the enrollment PTCA were obtained from hospital record review. Patients were classified into three categories representing increasing levels of clinical disease severity: (1) first PTCA on a native vessel, without a prior history of other cardiovascular interventions (Category 1), (2) one or more previous PTCAs on the same native vessel without interventions on other vessels (Category 2) or (3) history of one or more PTCAs on a different vessel and/or CABG (Category 3).

RESULTS

All sera were IgM seronegative, indicating that none of the study patients had an acute primary infection at enrollment.

C. pneumoniae antibody titer stability over time

Multiple sera in 38 patients were obtained a mean of 118 days (4 months) between first and last specimen (Sd 78 days, median 99, range 4-448). Only one patient was IgA seropositive (first and last specimen titers were 1:64). Distributions of IgG and CIC reciprocal titer categories for the first and last specimens for these 38 patients were identical (Table).

Table 1. Identical titer category distributions for first and last serum taken a mean of 4 months apart in 38 patients

IgG	no (%)	CIC	no (%)
negative	13 (34.2)	negative	26 (68.4)
32	10 (26.3)	2	8 (21.1)
64	3 (7.9)	4-6	2 (5.3)
128-256	8 (21.1)	8	2 (5.3)
512	4 (10.5)		

For IgG, one patient became negative (32 to negative) and one became positive (negative to 32). There were no other significant (greater than one-fold titer) changes between the patients' first and last specimens. For CIC, two patients became negative (2 to negative) and two became positive (negative to 2). One

patient had a three-fold titer increase (2 to 6) and another had a single-fold decline (4 to 2).

C. pneumoniae antibodies and clinical severity

Of 95 patients with clinical data available, 20 underwent their first PTCA and had no history of prior interventions (Category 1), 35 had a history of one or more previous PTCAs on the same vessel without a history of other interventions (Category 2) and 40 had an additional history of multivessel PTCAs (n=15) or CABG (n=25) (Category 3). Increasing clinical severity category was associated with increasing prevalence of IgG≥1:128, IgA≥1:16 and CIC≥2 titer categories measured at initial enrollment (Table 2).

Table 2. Antibody prevalence at initial enrollment for 95 patients undergoing an elective PTCA, no.(% of clinical category)

Serologic category	Clinical severity category* 1 (n=20)	2 (n=35)	3 (n=40)	P-trend
a) IgG≥128	2 (10)	8 (23)	14 (35)	.032
b) IgA≥16	0 (0)	0 (0)	8 (20)	.002
c) CIC≥2	3 (15)	8 (23)	16 (40)	.030
a or b or c	4 (20)	10 (29)	23 (58)	.002

*see Methods for descriptions of the categories

After controlling for age, sex and current smoking by logistic regression, the odds ratio (OR) for association of chronic antibody (a or b or c in Table 2) with Category 2 was 1.3 (95%CI, .70-2.6) and with Category 3 was 2.4 (95% CI, 1.3-4.7) using Category 1 as reference (logistic test of trend in the ORs, P=.005).

DISCUSSION

This pilot study found that the *C. pneumoniae* serologic profile remained fairly stable a mean of 4 months post-PTCA in a group of 38 patients enrolled more than once in a study of patients undergoing PTCA at a community hospital (Table 1). With one exception, all titer changes were only one-fold and mostly in borderline titer categories. The exception was one patient who had a three-fold titer increase in immune complexes (from 2 to 6) but this did not affect classification as seropositive. Changes over time in serologic status, indicating possible changes in exposure, could affect the power of prospective cohort studies to predict outcomes months or years later, if only initial serologic results are available for analysis. Stability of titer categories in this patient group suggests that initial serologic results may be acceptable for use, at least when assessing relatively short-term outcomes.

This study also found that *C. pneumoniae* antibodies were related to the clinical severity of coronary artery disease as measured by increasing prevalence of previous procedures performed on the same or additional vessels (Table 2). Significant trends with increasing clinical severity categories were found for IgG, IgA and CIC antibody titer categories measured separately and combined in a

single measure of chronic antibody (IgG≥1:128 and/or IgA≥1:16 and/or CIC≥1:2). Antibodies were most frequent in patients with a history of interventions for multivessel disease (Category 3). Differences were also noted between patients with initial single-vessel intervention (Category 1) and patients with recurrent interventions in the same vessel (Category 2), suggesting that *C. pneumoniae* antibodies may be associated with restenosis. This possibility will be investigated in the larger prospective cohort study. Contrasts between Categories 1 and 2 may be underestimated because Category 1 patients in this report were selected on the basis that multiple sera were available, i.e., they returned for additional PTCA(s) after the initial intervention.

Although the pathogenesis of restenosis may differ from that of atherosclerosis, it is possible that *C. pneumoniae* infection could play a role in both processes. Because restenosis following PTCA remains a significant clinical problem, further investigations into poor clinical outcomes following PTCA are warranted. Furthermore, the "dose-response" association of *C. pneumoniae* antibodies and clinical severity categories argues for a causal association of infection and atherosclerosis.

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