Available online at www.sciencedirect.com
ScienceDirect
Diagnostic Microbiology and Infectious Disease 66 (2010) 385–392
DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
www.elsevier.com/locate/diagmicrobio

Clinical Trial

Chlamydia pneumoniae IgG and IgA antibody titers and prognosis in patients with coronary heart disease: results from the CLARICOR trial

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Received 19 March 2009; accepted 17 November 2009

Abstract

The association observed between coronary heart disease (CHD) and *Chlamydia* (*Chlamydophila*) *pneumoniae* antibodies prompted, during the 1990s, several primary and secondary prevention trials with various antibiotics. In our CLARICOR trial, a randomized placebo-controlled trial in 4372 patients with stable CHD, a brief clarithromycin regimen was followed, unexpectedly, by increased long-term mortality. We now compare *C. pneumoniae* antibody levels at entry with population levels, with the patients' individual histories, and with their subsequent outcomes. IgG antibody levels were somewhat raised, but elevated IgA and IgG titers were unrelated to entry data (including prior acute myocardial infarction), except for an association with smoking and with not using statins. Hazards of mortality and of other outcomes tended to slightly increase with IgA and decrease with IgG titers, but the unfavorable clarithromycin effect was unrelated to antibody levels and remains unexplained. Smoking-related lung disease probably underlies the link between heart disease and increased IgG titers.

Keywords: Coronary heart disease; Clarithromycin; Cardiovascular mortality; *Chlamydia pneumoniae*; *Chlamydia pneumoniae* antibodies

1. Introduction

An association between high stable IgG and IgA *Chlamydia* (*Chlamydophila*) *pneumoniae* antibody titers and chronic coronary heart disease (CHD) as well as acute myocardial infarction (AMI) in men less than 50 years of age was reported in 1988 (Saikku et al., 1988). However,

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doi:10.1016/j.diagmicrobio.2009.11.011

no seroconversions and no *C. pneumoniae* IgM antibodies were demonstrated. The authors concluded that these findings suggested the presence of a chronic *C. pneumoniae* infection in both chronic CHD and AMI. The hypothesis prompted a series of trials that tested secondary prevention of cardiovascular events by treatment with macrolides assumed to be active against *C. pneumoniae* in the vascular wall (Anderson et al., 1999; Gupta et al., 1997; Gurfinkel et al., 1997). The results were promising, and a number of large prevention trials were launched (overviews: Anderson, 2005; Gluud et al., 2008; Hoymans et al., 2007), one of which was the CLARICOR trial (Hansen et al., 2001; Jespersen et al., 2006). Here we randomized 4372 patients with stable CHD with the primary objective of obtaining sufficient data to reliably assess whether brief intervention with a macrolide antibiotic, clarithromycin, reduces the frequency of future coronary events and deaths.

Paradoxically, the results produced a strong suspicion that brief intervention with clarithromycin may cause added cardiovascular deaths in the long run (Gluud et al., 2008; Jespersen et al., 2006). Meanwhile, another source of evidence made the treatable-infection hypothesis lose credence: a randomized trial of the effect of clarithromycin treatment on patients from whom vascular tissue was to be obtained during coronary surgery found no indication of an active *C. pneumoniae* infection and no influence of clarithromycin on *C. pneumoniae* antibody levels (Berg et al., 2005) or on inflammatory markers (Berg et al., 2003). Even so, it is still possible that *C. pneumoniae* antibody titers may be markers for cardiovascular prognosis.

We shall examine the association between *C. pneumoniae* antibody profiles and stable CHD on the basis of the over 4300 patients enrolled in the CLARICOR trial, using also a reference sample of Copenhagen citizens (Schnohr et al., 2001). The prognostic impact of IgG and IgA antibody levels among clarithromycin and placebo receivers is assessed on the basis of outcomes at 2.6 years of follow-up.

2. Methods

2.1. Participants

CLARICOR (Trial registration ClinicalTrials.gov NCT00121550; Hansen et al., 2001; Jespersen et al., 2006) is a randomized, placebo-controlled, blinded, parallel-group multicenter trial in patients with stable CHD. The follow-up through public registers is planned to continue until 2015. Patients were enrolled at 5 university-associated cardiology departments in Copenhagen. The Copenhagen Trial Unit handled all administrative functions, including centralized randomization and communication with blinded outcome assessors.

The city's central hospital database made it possible to identify all patients with a diagnosis of myocardial infarction and/or angina pectoris (International Classification of Diseases, 209–219) during the years 1993 to 1999. Patients were invited for an interview at one of the five units. Inclusion and exclusion criteria have previously been described in detail (Hansen et al., 2001). In particular, the protocol excluded patients receiving certain drugs that might interact unfavorably with clarithromycin.

2.2. Randomization and interventions

Eligible patients underwent a stratified randomization to oral clarithromycin (Klacid Uno®; Abbott, UK) 500 mg once daily for 2 weeks or matching placebo. Between October 1999 and April 2000, we randomized 2172 patients to clarithromycin and 2201 to placebo, but one of the latter was immediately withdrawn as ineligible due to HIV infection. For the present analysis, a further 11 patients who received clarithromycin and 12 who received placebo had to be disregarded due to loss of serum samples or, in the case of a single patient, missing interview data. The analysis therefore comprises 4349 participants (clarithromycin: 1509 males + 652 females = 2161, placebo: 1513 males + 675 females = 2188). The mean (±SD) ages of the males and females were 64.2 (±10.1) and 67.7 (±10.4) years, respectively; 70.8% of the males and 60.8% of the females had a previous AMI diagnosis.

2.3. Follow-up

Follow-up was based on reliable hospital admission and citizen registers (Hansen et al., 2001; Jespersen et al., 2006). A cardiologist event committee received hospital records and death certificates and used a prespecified outcome flowchart form to classify events. In case of death with inadequate information, they classified the cause of death as unknown. Otherwise, they judged death to be cardiovascular unless a noncardiovascular cause was clearly operative. The resulting event records allowed predefined outcomes to be extracted: death, cardiovascular death, and the trial protocol's *primary outcome measure*, which was a composite of all-cause mortality, myocardial infarction, or unstable angina. This formal follow-up procedure was closed in September 2002 after an average of 2.6 years of follow-up (range, 900–1070 days in survivors).

2.4. Healthy reference population

In the Copenhagen City Heart Study of unselected citizens who denied having had ischemic heart disease or stroke (Schnohr et al., 2001), sera from a segment (random time slice) underwent the same antibody tests as the CLARICOR sera. Data on 511 individuals below 85 years of age will be used for comparisons with the CLARICOR data. The mean (±SD) age of the 287 males was 66.1 (±9.6) and that of the 224 females was 69.6 (±9.3) years.

2.5. Serology

Serum samples were obtained at baseline. IgG and IgA *C. pneumoniae* antibodies were determined by a micro-immunofluorescence (MIF) assay (Bennedsen et al., 2002; Dowell et al., 2001) using commercially available reagents: