Chlamydia pneumoniae Antibodies in Severe Essential Hypertension


Abstract—Several studies have implied an association between Chlamydia pneumoniae (C. pneumoniae) and cardiovascular disease. Our study was designed to determine whether this organism is associated with severe essential hypertension in a multiracial British population. Antibodies to C. pneumoniae were measured by microimmunofluorescence in 123 patients with chronic hypertension and 123 control subjects, matched for ethnic origin, age, sex, and smoking habit, admitted to the same hospital with various noncardiovascular, nonpulmonary disorders. Previous infection was defined by IgG 64 to 256, provided that there was no detectable IgM. Multiple regression analyses of matched and unmatched data were used to investigate the influences of antibody levels and potential confounding factors (ethnic origin, age, sex, smoking habit, diabetes mellitus, and social deprivation) on hypertension. A portion of the hypertensive patients underwent echocardiography, estimation of left ventricular mass index, and measurements of fibrinogen, D-dimer, and von Willebrand factor concentrations. Thirty-five percent of hypertensive patients and 17.9% of matched control subjects had antibody titers consistent with previous C. pneumoniae infection. The hypertensive patients differed significantly from their matched control subjects in their level of previous infection, with an odds ratio of 2.5 (95% confidence interval, 1.3 to 4.7). There were no significant differences in antibody levels between patients with left ventricular hypertrophy and those without it. Fibrinogen, D-dimer, and von Willebrand factor concentrations were not significantly associated with antibody levels. These data support an association of C. pneumoniae with severe essential hypertension. They provide no evidence of a predisposition to develop left ventricular hypertrophy in hypertensive patients with C. pneumoniae infection or of associations with hypercoagulability or endothelial dysfunction. (Hypertension. 1998;31:589-594.)

Key Words: hypertension, essential • cardiovascular diseases • infection

Chlamydia pneumoniae was first described in 1986.1 Serological studies indicate that it is one of the most prevalent infectious agents worldwide,2–5 with a wide range of clinical manifestations, including exacerbations of chronic obstructive pulmonary disease and chronic asthma.6 Several groups have demonstrated serological associations with coronary artery disease,7–10 strokes and transient cerebral ischemia,11 and asymptomatic carotid atherosclerosis.12

Our study was designed to test the association of this organism with HT in the multiracial inner-city population of north and west Birmingham, UK, after adjustments for several potential confounding variables. We subjected the HT patients to echocardiography with estimation of LVMI. We also measured concentrations of fibrinogen, fibrin D-dimer, and von Willebrand factor in plasma and searched for associations of these factors with C. pneumoniae antibody levels.

Methods

Study Subjects and Investigations

Approval for this project was obtained from the Hospital Ethical Committee at the City Hospital, Birmingham. Subjects were patients admitted to this hospital via the Emergency Department, all of whom gave informed consent to participation. All patients were initially considered to be eligible: recruitment was prospective and continued at a steady rate, so that those who were eventually included in the HT and control groups were admitted consecutively throughout a 24-month period (March 1993 through March 1995). Exclusion criteria were known or suspected immunodeficiency, hypergammaglobulinaemia, connective tissue disease, and other autoimmune disease.

Patients in the HT group were recruited from outpatients attending the hypertension clinic at Birmingham City Hospital. All had had blood pressures greater than 160/90 on two or more occasions, and were taking antihypertensive medication. Preceding conditions (such as Cushing’s and Conn’s syndromes, pheochromocytoma, and renal artery stenosis) had been excluded, where appropriate. Evidence of end-organ damage (renal failure or proteinuria, hypertensive retinopathy, or other active cardiovascular disease) was not required for recruitment.

Potential control subjects were selected randomly from all admissions to the Emergency Department with acute, nonpulmonary, noncardiovascular disorders, provided that there was no evidence (by the patients’ accounts and from the hospital’s records) of coexisting active cardiac, vascular, or pulmonary disease. Thus, there is no reason to suspect that they were predisposed to acquire C. pneumoniae infection.

At recruitment (on admission in the case of control patients), blood was taken for serology. The HT patients underwent further investigation, namely EKG, echocardiography, and determinations of levels of plasma fibrinogen, fibrin D-dimer, and von Willebrand factor. These investigations, which were ordered by the responsible physi-
cian, were not confined to any part of the study period or to any
discernible subgroup of patients.
LVMI was estimated by echocardiography, using the formulas of
Devereux and the Penn conventions of measurement. Two
echocardiograms were obtained by a single observer, and the coefficient
of variation was <5%. LVMI was defined by LV mass >135 g • m⁻² in
males, or >110 g • m⁻² in females, as estimated by echocardiogra-
y and by EKG using the criteria of Sokolow and Lyon (ie, S in
V5 or V6).12c,12d Two to five milliliters of serum was obtained by
centrifugation within

Serological Testing
Two to five milliliters of serum was obtained by centrifugation within
6 hours of venipuncture and stored at ~20°C until analysis. Each blood
sample was labeled only with a serial number; thus, the investigator
was blind to all patient data at the time of testing, and remained so
until statistical analysis of the results.
Sera were tested by one investigator using Maxiscreen Chlamydia
MIF slides (IO International Ltd) and fluorescence-conjugated anti-
human immunoglobulins. Only an even pattern of elementary body
fluorescence was regarded as positive. In every batch of slides tested,
two control serum preparations known to be positive for this organism
and two negative control samples were each applied to two slides. All
sera were screened at a dilution of 1:8; thereafter, positive sera were
tested at dilutions of 1:8 to 1:1024.
Because HT patients were not acutely ill when bled, we did not expect
to find evidence of acute C. pneumoniae infection, and we were
primarily interested in the frequency of previous infection. Titers
indicating previous infection without recrudescence were presumed
to be IgG 64 to 256, provided that IgM could not be detected. Titers
of IgG ≥512 or IgM ≥8 were considered to indicate acute infection
or reinfection just before entry into the study. Clearly, acute infections
should have no bearing on long-standing hypertension, but because
they could mask serological evidence of previous infection, patients
with such titers were considered separately from previously infected
and noninfected patients in the analysis of results.
Rheumatoid factor was assayed in patients in whom connective
tissue diseases were suspected on clinical grounds, and whom we
suspected had connective tissue diseases on clinical grounds, and whom we
further tested for the presence of rheumatoid factor. Where
this test was positive, in 2 HT patients and 24 control subjects,
the finding of IgM antibodies was discounted for the diagnosis
of acute C. pneumoniae infection.
Acute infection was diagnosed solely on the basis of IgM
titers in 5 HT patients and 30 control subjects, of whom 5 HT
patients and 22 control subjects would otherwise have been
defined by their IgG titers as previously infected. Two HT
patients and 11 control subjects were older than 60 years, and
therefore were within the age group that has been associated with
suspected rheumatoid factor production. Of these, 2 HT patients and 1 control subject subsequently took part in the

Analysis of Data
After recruitment was complete, we used a computer program to
match each of the HT patients with one of the much larger number
Demographic Characteristics of HT and Control Groups (Matched)

<table>
<thead>
<tr>
<th>Demographic Characteristics</th>
<th>HT (n=123)</th>
<th>Controls (n=123)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnic origin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>67 (54.5%)</td>
<td>67 (54.5%)</td>
</tr>
<tr>
<td>Afro-Caribbean</td>
<td>38 (30.9%)</td>
<td>38 (30.9%)</td>
</tr>
<tr>
<td>Asian</td>
<td>18 (14.6%)</td>
<td>18 (14.6%)</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>23-77</td>
<td>18-86</td>
</tr>
<tr>
<td>Mean</td>
<td>55</td>
<td>54.7</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>13.9</td>
<td>16.0</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>58 (47.2%)</td>
<td>58 (47.2%)</td>
</tr>
<tr>
<td>Female</td>
<td>65 (52.8%)</td>
<td>65 (52.8%)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>25 (20.3%)</td>
<td>25 (20.3%)</td>
</tr>
<tr>
<td>Previous (&gt;3 months)</td>
<td>24 (19.5%)</td>
<td>24 (19.5%)</td>
</tr>
<tr>
<td>Never</td>
<td>74 (60.2%)</td>
<td>74 (60.2%)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin-dependent</td>
<td>0</td>
<td>3 (2.4%)</td>
</tr>
<tr>
<td>Non-insulin-dependent</td>
<td>2 (1.6%)</td>
<td>6 (4.9%)</td>
</tr>
<tr>
<td>Diabetes, type unknown</td>
<td>3 (2.4%)</td>
<td>8 (6.5%)</td>
</tr>
<tr>
<td>Not diabetic</td>
<td>118 (95.9%)</td>
<td>106 (86.2%)</td>
</tr>
<tr>
<td>Townsend score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>6.6</td>
<td>4.0</td>
</tr>
<tr>
<td>Minimum</td>
<td>4.4</td>
<td>-4.8</td>
</tr>
<tr>
<td>Maximum</td>
<td>9.8</td>
<td>9.8</td>
</tr>
</tbody>
</table>

matched analysis. There were therefore 3 patients in the matched analysis who might conceivably have been identified wrongly as having acute infection.

The presence or absence of LVH was ascertained by echocardiography and EKG in 127 (94.1%) of the HT patients. Plasma fibrinogen, fibrin D-dimer, and von Willebrand factor were measured in 118 (87.4%), 115 (85.2%), and 120 (88.9%) of the HT patients, respectively.

Matching was achieved, on the basis of the criteria stated above, between 123 HT patients and 123 control subjects (Table). The following examination of C. pneumoniae antibody levels in hypertension relates only to the matched analysis.

C. pneumoniae and Hypertension

Of the matched HT patients, 43 (35.0%) had serological evidence of previous infection, 9 (7.3%) acute (re)infection, and 71 (57.7%) no infection. Of the matched control subjects, 22 (17.9%) had evidence of previous infection, 11 (8.9%) acute (re)infection, and 90 (73.2%) none.

Regression analysis suggested associations of HT with previous C. pneumoniae infection (OR, 2 · 5; 95% confidence interval [CI], 1.3 to 4.7) but not with acute (re)infection (OR, 1.0; 95% CI, 0.4 to 2.6). Among matched HT patients, the distribution of the three possible serological outcomes—acute (re)infection, previous infection, and none— differed from that among matched control subjects with a probability of P<.05, but this difference was entirely due to levels of previous infection. If we had omitted the 3 matched patients over 60 years of age, not tested for rheumatoid factor, in whom we detected acute C. pneumoniae infection, it would not have altered the lack of association between hypertension and acute (re)infection.

This analysis revealed no evidence of statistical interactions of ethnic origin, age, sex, or smoking habit with the C. pneumoniae–HT association. The analysis of unmatched groups was in agreement with the matched analysis, and it revealed no evidence of interactions of diabetes mellitus or Townsend score with the C. pneumoniae–HT association.

C. pneumoniae and LVH in Hypertension

Among 127 of the HT patients, EKG and echocardiography showed LVH in 92 and were normal in 35. Mean LVMI measurements were 173.5 and 124.1 g · m⁻², respectively. Serological results indicated previous infection, acute (re)infection, and no infection in 34.8%, 5.4%, and 59.8% of patients with LVH, and 31.4%, 2.9%, and 65.7% of those with no LVH. By χ² analysis, there was no significant variation between the two categories in their distributions of previous infection, acute (re)infection, and no infection.

Fibrinogen, Fibrin D-Dimer, and von Willebrand Factor Levels

In HT patients with serological evidence of previous infection, acute (re)infection, and no infection (after adjustment by regression analysis for variations in ethnic origin, age, sex, and smoking habit), mean plasma concentrations of fibrinogen were 10.9 (95% CI, 9.4 to 12.6), 10.9 (8.8 to 12.9), and 11.5 (10.0 to 12.9) μmol/L; of fibrin D-dimer, 273 (184 to 405), 253 (150 to 424), and 228 (156 to 333) ng/dL; and of von Willebrand factor, 120 (110 to 131), 120 (101 to 140), and 125 (115 to 135) IU/dL, respectively. There were thus no significant differences in these variables between HT patients with previous infection, acute (re)infection, and no infection.

Discussion

C. pneumoniae and Hypertension

Hypertension exemplifies probably better than any other disorder the complexity of polygenic disease. In 95% of cases (so-called “essential hypertension”), no single cause can be identified; although factors such as consumption of alcohol and caffeine, salt intake, smoking, obesity, and physical inactivity may clearly contribute to increasing blood pressure. The notion that infections may predispose to hypertension is not new. For example, such a role has been proposed for Helicobacter pylori: of 33 patients in one urban general practice with unequivoval H. pylori gastritis, 42% had sustained hypertension compared with 12% of dyspeptic patients without H. pylori. Schreiber et al (1992) reported that intravenous injections of either live or heat-killed group B β-hemolytic streptococci in newborn lambs caused significant dose-dependent increases in systemic vascular resistance and mean systemic arterial pressure and that these effects were partly blocked by leukotriene D4 receptor antagonists, suggesting that leukotrienes might mediate hypertension in this infection. This is one of several immunologic changes that might be relevant to the infectious
origin of essential hypertension. Furthermore, chronic chlamydial infections have a marked propensity to cause fibrosis (as seen, for example, in the cicatricial scarring of the cornea that characterizes trachoma and in fibrosis of the Fallopian tubes in pelvic inflammatory disease due to C. trachomatis). It is therefore reasonable to speculate that C. pneumoniae within vascular endothelial cells might, by a similar process, lead to an increase in vascular resistance.

There are wide variations in the prevalence and incidence of hypertension in different parts of the world. Both in the United Kingdom and United States, it is more common among black people than in the white population. C. pneumoniae antibodies have been associated with Afro-Caribbean origin, raising the possibility that a genetic predisposition to this infection may contribute to the development of hypertension.

In a large Finnish study that showed a clear association of high titers of chlamydial IgG and IgA antibodies with chronic coronary artery disease and acute myocardial infarction, there was no correlation with other risk factors for these conditions, including hypertension. However, this lack of correlation may not be very significant, unless the research is focused on severe and sustained hypertension, and predisposing conditions (such as Cushing’s and Conn’s syndromes, pheochromocytoma, and renal artery stenosis) are excluded. The authors of the Finnish study did not specify the criteria for diagnosing hypertension in their report, and we suggest that our study of chronic severe hypertensive patients should have a higher probability of detecting such an association.

It is noteworthy that we found substantial levels of acute and previous infection in control subjects as well as HT patients, reflecting the high incidence of C. pneumoniae infection in our community. Nevertheless, the results of our study support an association between chronic severe essential hypertension and C. pneumoniae infection. We did not grade the HT patients according to the severity of their hypertension, but we found no significant differences in C. pneumoniae antibody levels between patients with and those without echocardiographic evidence of LVH. We suggest that several factors may interact to produce this complication of hypertension.

Strategies to take into account potential confounding variables are essential in studies of C. pneumoniae antibodies, which have been associated with Afro-Caribbean origin, increasing age, male sex, and smoking. In this study, we found no noteworthy interactions (ie, effect modification) by these factors with the association between C. pneumoniae and hypertension. Subjects in the HT and control groups also differed in some other demographic characteristics whose influences on the risk of C. pneumoniae infection were unknown (Table). Using unmatched data, we found no interactions of diabetes mellitus or Townsend score with the association between C. pneumoniae and hypertension, although we believe that larger studies would be required to draw firm conclusions about these factors.

Fibrinogen, fibrin D-dimer, and von Willebrand factor may be regarded as markers for intravascular thrombogenesis and fibrin turnover, and for endothelial damage, respectively. Marked elevation of plasma fibrinogen has been reported in patients with severe hypertension; and in such patients, fibrinogen concentration is an independent predictor of blood pressure. Infections also lead to increases in prothrombotic factors, particularly fibrinogen, fibrin D-dimer (a fragment of cross-linked fibrin, hence a marker of thrombosis), and anti-cardiolipin antibodies. However, fibrinogen, fibrin D-dimer, and von Willebrand factor were not significantly associated with the presence of C. pneumoniae antibodies in our study.

Serological Testing

Various techniques are available to detect C. pneumoniae antibodies. The best and most widely used is the MIF assay, which is time-consuming and subject to some operator variation but is sensitive and species-specific and reliably detects IgG, IgM, and IgA. The kit that we used has been used in several studies, and has a performance similar to other MIF assays.

IgG titers $\geq$256 may persist for many months and have generally been accepted (in a single serum specimen) as evidence of previous infection, provided that there is no rise in IgM antibodies. Titers $\geq$512 are generally regarded as indicating acute infection or reinfection. Many authors have used these serological criteria and a large study combining serology with an examination of pharyngeal swabs by polymerase chain reaction has provided evidence in support of them. We chose to reject IgG titers $<46$ to minimize the probability of false-positive results.

IgM is generally considered to signify acute primary infection. A threshold titer of $\geq$16 has been proposed, with titers of 8 indicating “probable acute infection,” but we found no difference in the proportions of patients with IgM antibodies at these two titers in a large pilot study, and therefore saw no merit in drawing a distinction between such low levels of antibody production provided that only an even distribution of definite elementary body fluorescence was accepted as positive. It has been suggested that rheumatoid factor may make the measurement of IgM antibodies unreliable, particularly in elderly patients. We therefore excluded from this study all patients known or thought likely to have connective tissue disease or other autoimmune disease, and measured rheumatoid factor in a further 138 cases in which such diseases were considered less probable, discounting IgM antibodies in the 26 cases in which it was present. As demonstrated above, ignoring IgM titers from the remaining matched patients over 60 years of age (3 patients) would not have significantly altered the relationships of HT to acute and chronic C. pneumoniae infections.

Limitations of this Study

In the HT group, the presence or absence of LVH was ascertained in 94.1% of patients. There appears to be no association of C. pneumoniae infection with the development of LVH. Plasma fibrinogen, fibrin D-dimer, and von Willebrand factor levels were measured in fewer than 90% of the HT patients; in future studies, it will be important to measure these levels in larger numbers of patients, both with and without serological evidence of C. pneumoniae infection.

No attempt was made to control for travel history, ethanol consumption, or diet in this study. Although the last two
factors may influence the probability of systemic hypertension, we do not perceive them to be important factors in our population of severely hypertensive patients. We believe that our strategy for preventing interference by rheumatoid factor in IgM measurement has allowed us to make a valid interpretation of the results. However, in the future we would recommend the routine absorption of sera with anti-
human IgG, as advocated by Verkooyen et al.19

Conclusions

These data support the associations of chronic severe hyper-
tension with previous C. pneumoniae infection. We believe that more research to elucidate the mechanism(s) of this association is warranted.

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