Chlamydial bacteriophage: No role in acute coronary events?

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BACKGROUND: A relationship between *Chlamydia pneumoniae* infection and acute coronary syndromes has not been consistently found in published studies. It has been hypothesized that a bacteriophage-infected subset of *C. pneumoniae* may be uniquely equipped to promote atherosclerosis and acute coronary syndromes through the expression of phage genes.

METHODS: The authors performed a pilot case-control study of acute coronary events. Case and control subjects were characterized demographically and according to recognized coronary risk factors. These subjects also provided serum for the detection of antibody to the elementary bodies of *C. pneumoniae* and antibody to the Vp1 protein coded by the phage. Bivariate and multivariate comparisons were performed using statistics appropriate for paired analyses.

RESULTS: Antibodies to *C. pneumoniae*, Vp1 protein or both were not associated with acute coronary events by bivariate or multivariate analysis. As expected, case subjects were significantly more likely to have hypertension, hypercholesterolemia or diabetes mellitus.

CONCLUSION: The present study adds to a growing body of literature that does not support the hypothesized relationship between *C. pneumoniae* (or a phage-infected subset of *C. pneumoniae*) and acute coronary syndromes.

Key Words: Acute coronary syndromes; Bacteriophage; Case-control study; *Chlamydia pneumoniae*

Bactériophage de *Chlamydia*: aucun rôle dans les syndromes coronariens aigus?

CONTEXTE: On n’a pas toujours réussi à établir un lien entre les infections à *Chlamydia pneumoniae* et les syndromes coronariens aigus (SCA) dans les études publiées. Des chercheurs ont donc émis l’hypothèse selon laquelle un sous-groupe de *C. pneumoniae* infecté par un bactériophage serait le seul à pouvoir favoriser l’athérosclérose et les SCA par l’expression de gènes phagiques.

MÉTHODE: Les auteurs ont mené une étude pilote de type cas/témoins sur des SCA. Ils ont caractérisé les témoins et les malades en fonction de données démographiques et des facteurs de risque de maladie coronarienne reconnus. Une recherche d’anticorps contre les corps d’inclusion de *C. pneumoniae* et contre la protéine Vp1 codée par le phage a aussi été effectuée à partir de prélèvements sanguins. Enfin, des comparaisons à deux et à plusieurs variables ont été établies à l’aide de méthodes statistiques, adaptées aux analyses appariées.

RÉSULTATS: Aucun lien n’a été établi entre les anticorps contre *C. pneumoniae*, la protéine Vp1 ou les deux, et les SCA, selon les analyses bi- et multivariées. Comme prévu, les sujets malades connaissaient un risque passablement plus élevé d’hypertension, d’hypercholestérolémie ou de diabète sucré.

CONCLUSION: La présente étude vient étoffer le faisceau d’arguments de plus en plus large, infirmant l’hypothèse selon laquelle il y aurait un lien entre *C. pneumoniae* (ou un sous-groupe de *C. pneumoniae* infecté par un phage) et les SCA.
aneurysm (AAA) found that the relationship between seropositivity for phage together with \textit{C. pneumoniae} and case status was strong (OR 13.9, 95% CI 1.1 to 173). This has led to the fascinating conjecture that phage-encoded genes may be linked to ACS. A link is plausible in that this newly described phage is highly homologous to another phage from \textit{Chlamydia psittaci}. The latter phage has been shown to alter the \textit{chlamydial} developmental pathway and produce abnormally large reticulate bodies (12). These forms lyse late in the \textit{chlamydial} developmental cycle, compromising the integrity of hyperinfected HeLa cells in vitro. Hypothetically, the lysis of abnormal reticulate bodies within host cells could release heat shock protein, lipopolysaccharide or other moieties to directly induce inflammation.

We describe a population-based, case-control study to test the hypothesis that seropositivity for the PhiCpn1 phage is more prevalent among persons presenting to the hospital with ACS than among age- and sex-matched control subjects.

**METHODS**

A prospective case-control study of ACS was performed. Case subjects consisted of incident patients newly admitted to Vancouver General Hospital (Vancouver, British Columbia) with first-episode myocardial infarction (chest pain of more than 20 min in duration, with diagnostic electrocardiogram changes and elevation in creatine kinase MB and/or troponin I) or unstable angina (chest pain with characteristic electrocardiogram changes but no Q-wave formation or elevation in creatine kinase MB and/or troponin I). Control subjects were individually matched to case subjects for age, sex and neighbourhood of residence by random selection from a list of eligible clients obtained from the British Columbia Medical Services Plan client registry. Matched subjects were interviewed as closely as possible to the time of the ACS event.

Consenting case and control subjects contributed information on demographics and risk factors, including smoking, hypertension, hypercholesterolemia, diabetes and family history. Subjects also contributed serum for testing. Detection of antibody to the elementary bodies of \textit{C. pneumoniae} and antibody to the Vp1 protein coded by the phage was performed by ELISA as described elsewhere (11).

Data were entered into a Microsoft Access database (Microsoft Corporation, USA), and analyses were conducted using SAS software (version 8.2, SAS Institute Inc, USA). Statistics appropriate for matched pair analyses were used. Univariate analysis was performed using paired Student’s \(t\) tests for continuous variables and McNemar’s \(\chi^2\) test for categorical variables. A forward stepwise procedure was applied to a conditional logistic regression model to control for the frequency of specific risk factors, body mass index and serological findings is presented in Table 1.

As expected, case subjects were significantly more likely to have hypertension, hypercholesterolemia or diabetes mellitus.

They also more frequently reported a family history of cardiovascular disease (\(P=0.06\)) and had a body mass index significantly higher than control subjects.

The serology of control subjects tended to be more frequently reactive for \textit{C. pneumoniae} than that of case subjects (\(P=0.07\)) – a trend opposite to that hypothesized. There was no difference between case and control subjects in the distribution of reactivity to chlamydiaphage protein or to the presence of antibodies both to \textit{C. pneumoniae} and chlamydiaphage protein.

Multivariate analysis indicated that the significant predictors of ACS were family history, hypercholesterolemia and body mass index. Parameter estimates for these variables did not change if the antibody to \textit{C. pneumoniae} or to chlamydiaphage were retained in the model.

**DISCUSSION**

In the present study, we tested the hypothesis that seropositivity for PhiCpn1 phage was more prevalent among persons presenting to hospital with ACS than age-, sex- and neighbourhood-matched control subjects. We used incident cases of ACS and standardized methods of data collection. We found that control subjects were less likely to be infected than cases. Therefore, the present study does not support the hypothesis that prior infection by chlamydiaphage-containing \textit{C. pneumoniae} is associated with ACS.

There are several possible explanations as to why there was no association found in the present study (in contrast with the association found in a previous study of AAA (11)). First, the present study had power of over 80% to find an odds ratio of three or greater but less power to find a smaller association should one have existed. However, because the trend was in the opposite direction to that hypothesized, we do not feel that this limits our conclusions. Second, it is plausible that there could be a different biological relationship between phage-infected \textit{C. pneumoniae} and coronary artery atherosclerosis than might apply in larger vessel disease. Third, the findings in the original study of AAAs may have occurred by chance (11).

**TABLE 1**

**Characteristics of case and control subjects**

<table>
<thead>
<tr>
<th>Case subjects (n=41)</th>
<th>Control subjects (n=41)</th>
<th>Bivariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family history, %</td>
<td>24 18</td>
<td>(P=0.06; ) HR=3.0</td>
<td>(P=0.05)</td>
</tr>
<tr>
<td>Ever smoked, %</td>
<td>23 25</td>
<td>(P=0.67; ) HR=0.83</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>26 14</td>
<td>(P=0.02; ) HR=3.4</td>
<td>NS</td>
</tr>
<tr>
<td>High cholesterol, %</td>
<td>25 7</td>
<td>(P=0.004; ) HR=19</td>
<td>(P=0.02)</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>12 4</td>
<td>(P=0.05; ) HR=3.7</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2) (median)</td>
<td>27.5 24.4</td>
<td>(P=0.004)</td>
<td>(P=0.05)</td>
</tr>
<tr>
<td>Chlamydia \textit{pneumoniae}, %</td>
<td>29 36</td>
<td>(P=0.07; ) HR=0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Elementary body antibody, %</td>
<td>25 25</td>
<td>(P=1.0; ) HR=1.0</td>
<td>NS</td>
</tr>
<tr>
<td>Both Chlamydia \textit{pneumoniae} and phage antibody</td>
<td>17 22</td>
<td>(P=0.32; ) HR=0.67</td>
<td>NS</td>
</tr>
</tbody>
</table>

*HR* Hazard ratio; *NS* Not significant
As a preliminary test of the hypothesis regarding coronary artery disease, it was economical to employ a case-control methodology. However, it would be valuable to test this and other hypotheses in ongoing or future cohort studies.

The present study contributes to a growing body of recent evidence which indicates a low or nonexistent association between prior infection with \textit{C pneumoniae} and cardiac events.

The root causes of inflammation, as it contributes to atherosclerosis, require further study.

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\textbf{REFERENCES}
