No Correlation between Giant Cell Arteritis and Chlamydia pneumoniae Infection: Investigation of 189 Patients by Standard and Improved PCR Methods†

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A total of 189 temporal artery biopsy samples from giant cell arteritis (GCA) patients were investigated using sensitive PCR targeting Chlamydia pneumoniae. Chlamydial DNA was detected in 17 samples, 11 of which were positive for chlamydial antigens. Our data did not reveal strong evidence that C. pneumoniae plays an important role in the pathogenesis of GCA.

Giant cell arteritis (GCA) is a vasculitis disease affecting medium- to large-sized arteries. Some studies have presented evidence of a coincidence of cyclic fluctuations of Chlamydia pneumoniae epidemics and cases of GCA (3, 13). Conflicting data exist regarding the role of C. pneumoniae in the pathogenesis of GCA. Some authors found a strong correlation between GCA and detection of C. pneumoniae (12, 15), whereas others failed to detect the pathogen (6, 7, 11). The discrepancies among the published data may derive from variations in either the quality or quantity of the DNA used or the specificity and sensitivity of the PCR system employed. To address these and other potential impediments to screening success, the DNA purification method and the PCR screening system used here were optimized for investigation of paraffin-embedded temporal artery biopsy samples. Temporal artery biopsy samples from 224 GCA patients who underwent consecutive biopsy procedures at the University Eye Hospital (Freiburg, Germany) between 1993 and 2006 were screened. Classification criteria for GCA according to the 1990 guidelines of the American College of Rheumatology (ACR) were applied to select the cases. In this manner we identified 115 potential case subjects. A total of 109 patients whose temporal artery biopsy specimens from at least one side gave negative results and whose postbiopsy clinical patterns were not consistent with GCA were used as controls.

For isolation of genomic DNA, a QiAmp DNA Micro kit (Qiagen, Hilden, Germany) was used according to the manufacturer’s instructions. Alternatively, a hot phenol-chloroform DNA isolation method was used as described previously (15). The two methods yielded equal amounts of genomic DNA. With the exception of primers p90n and p91n (5′-ACACTCG/3′-AACGAACTC and 5′-AAGGCCAGGTAAAGGTC-3′, respectively), which were designed with Primer3 software (http://primer3.sourceforge.net), the other primers were as previously described (4, 8–10). A PCR assay targeting the β-globin gene was used to compare the two methods. By the use of primers GH20 and PC04, detection of human genomic DNA in minimal amounts of paraffin-embedded tissue was possible.

Serial dilutions of DNA were used, and β-globin amplification was found to be possible at a dilution of 100 pg of DNA. After successful detection of human β-globin DNA, C. pneumoniae outer PCR (i.e., PCR targeting a larger fragment which can be unambiguously amplified and which contains the smaller intended fragment) was performed. Samples were analyzed using standard PCR targeting the 16S rRNA gene (primers CpnA and CpnB) and Touchdown enzyme time release PCR (TETR-PCR) targeting the 16S-to-23S spacer region (primers CPN-90 and CPN-91) (5, 10, 11, 15) of C. pneumoniae to determine the most sensitive PCR. TETR-PCR allowed amplification of C. pneumoniae fragments (197 bp) smaller than those amplified by the standard PCR method (463 bp) initially used in previous studies (5, 15). Both PCR techniques are recommended by the U.S. Centers for Disease Control and Prevention and the Laboratory Center for Disease Control (Canada) (2). Comparison of the two PCR methods revealed sensitivities of 1 inclusion-forming unit (IFU) (Fig. 1B, lane 5) and 102 IFU (Fig. 1A, lane 3), respectively.

To further increase sensitivity, a nested PCR was applied for the two PCR methods using 10% of the PCR products from the outer PCR with primers pTW50 and pTW51 to amplify the CpnA-CpnB fragment or with primers p90n and p91n to amplify the CPN-90–CPN-95 fragment. As shown in Fig. 2A, lane 4, the sensitivity of the standard PCR increased to a limit of 1 IFU whereas the TETR-PCR was able to detect 0.1 IFU (Fig. 2B, lane 5).

The optimized methods were employed to screen samples from 115 and 109 GCA and control patients, respectively.
Human genomic DNA was detected in 102 of 115 GCA specimens and in 87 of 109 control specimens by the use of β-globin PCR. It has been shown in other studies that it is difficult to amplify long fragments from formalin-fixed and paraffin-embedded tissue (1, 14). This might be one reason underlying the failure to detect chlamydial DNA in paraffin-embedded tissue samples of GCA patients in previous studies (6, 7, 11). Therefore, by using β-globin PCR we were able to identify and exclude 35 of 224 samples from further investigations, preventing false-negative results (15).

Due to its ability to process a shorter fragment length and its higher sensitivity, we used the TETR-PCR. A total of 3 of 102 GCA specimens and 2 of 87 control specimens were positive for Chlamydia pneumoniae DNA by outer PCR. When the nested PCR was applied, an additional nine and three samples from GCA and control patients, respectively, were shown to be positive for C. pneumoniae DNA. Sequencing of all 17 nested PCR amplicons identified the obtained fragments as specific for C. pneumoniae. These findings contradict another report in the literature: Regan and coworkers reported in 2002 that none of the investigated temporal arteries from 90 GCA patients were positive for Chlamydia pneumoniae DNA. As shown in Table 1, none of the clinical parameters for C. pneumoniae DNA were statistically different between the GCA and control patients. In addition, no statistical differences were detected when we compared the ACR-positive and the ACR-negative patients with respect to C. pneumoniae DNA detection (10.9% versus 8.3%; P = 0.45). As shown in Table 1, none of the clinical parameters for C. pneumoniae PCR results was not statistically significant (P = 0.45). As shown in Table 1, none of the clinical parameters for C. pneumoniae PCR results was not statistically significant (P = 0.45). As shown in Table 1, none of the clinical parameters for C. pneumoniae PCR results was not statistically significant (P = 0.45).

![Image](http://jcm.asm.org/)

**FIG. 1.** Outer PCR detection of C. pneumoniae in paraffin-embedded biopsy samples. (A) Standard PCR amplification products of the target 16S rRNA gene generated using primers CpnA and CpnB; (B) TETR-PCR amplification products of the target 16S–23S spacer rRNA gene generated using primers CPN-90 and CPN-91. Lanes M, DNA molecular weight marker VIII (Roche). Lanes 1 to 9 of both panels show the results obtained with C. pneumoniae DNA template containing 10⁴ to 10⁻³ IFUs. Lane 10, positive control; lane 11, negative control (without template). Data are representative of the results of three experiments.

![Image](http://jcm.asm.org/)

**FIG. 2.** Nested PCR detection of C. pneumoniae in paraffin-embedded biopsy samples. (A) Standard PCR amplification products of the target 16S rRNA gene generated using primers pTW50 and pTW51; (B) TETR-PCR amplification products of the target 16S–23S spacer rRNA gene generated using primers p90n and p91n. CpnA-CpnB and CPN-90-CPN-91 amplified PCR products (10%) were used as a template for primer pair pTW50 and pTW51 and primer pair p90n and p91n, respectively, Lanes M, DNA molecular weight marker VIII (Roche). Lanes 1 to 7 of both panels contain Chlamydia pneumoniae DNA outer products obtained with 10⁴ to 10⁻³ IFUs. Lane 8, negative control (without template). Data are representative of the results of three experiments.

<table>
<thead>
<tr>
<th>Patient group parameter</th>
<th>Test result for C. pneumoniae DNA</th>
<th>p&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>Positive</td>
<td>70.5</td>
</tr>
<tr>
<td>Female (%)</td>
<td>Negative</td>
<td>69.5</td>
</tr>
<tr>
<td>Headache (%)</td>
<td>Positive</td>
<td>64.3</td>
</tr>
<tr>
<td>Jaw claudication (%)</td>
<td>Negative</td>
<td>75.9</td>
</tr>
<tr>
<td>Fever (%)</td>
<td>Positive</td>
<td>35.7</td>
</tr>
<tr>
<td>Weight loss (%)</td>
<td>Negative</td>
<td>41.7</td>
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<tr>
<td>AION (%)</td>
<td>Positive</td>
<td>28.6</td>
</tr>
<tr>
<td>CAO (%)</td>
<td>Negative</td>
<td>28.6</td>
</tr>
<tr>
<td>CRP (mg/liter)</td>
<td>Positive</td>
<td>9.11</td>
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<tr>
<td>ESR (mm/h)</td>
<td>Negative</td>
<td>61.7</td>
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<tr>
<td>Fibrinogen (mg/dl)</td>
<td>Positive</td>
<td>680</td>
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<tr>
<td>ACR criteria (%)</td>
<td>Negative</td>
<td>78.6</td>
</tr>
<tr>
<td>Positive biopsy result (%)</td>
<td>Positive</td>
<td>64.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> AION, anterior ischemic optic neuropathy; CAO, conscious, alert, oriented; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

<sup>b</sup> P values achieved were corrected according to the Bonferroni method. P < 0.05, statistically significant; P > 0.05, not statistically significant.
Germany). Paraffin-embedded lung sections of C. pneumoniae-infected C57BL/6 mice served as positive controls. Of 12 C. pneumoniae DNA-positive GCA specimens, 7 were positive for C. pneumoniae antigen. However, C. pneumoniae antigen was detected in four of five control specimens. A possible reason for the failure to detect C. pneumoniae antigen in six specimens might have been the patchy distribution of vascular lesions in GCA (9).

In conclusion, we show here that optimal DNA purification and sensitive PCR methods in combination with a second, independent method are key factors for the successful detection of rare targets in small paraffin-embedded tissue samples. Moreover, the lack of correlation between C. pneumoniae DNA-positive and -negative patients for all clinical parameters and ACR criteria in our study suggests that C. pneumoniae is no more than an innocent bystander in the artery tissue.

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REFERENCES