Prevalence of Chlamydia pneumoniae Infection in Squamous Cell Carcinoma of the Head and Neck

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Abstract. On the basis of epidemiological data, an association between Chlamydia pneumoniae (Cp) infection and head and neck cancer might be suggested. The aim of the present study was to detect Cp-DNA within tumour tissue specimens by a two-step polymerase chain reaction. Investigation was planned on the Fleming’s procedure for early termination when initial results were extreme. So, after ten consecutive patients, only one tumour contained Cp-DNA. Hence the prevalence could be regarded as inferior to 60% (2a=b=0.08), the threshold under which a direct role of Cp in head and neck cancer development does not seem to be likely.

Chlamydia pneumoniae (Cp) is a frequent cause of upper respiratory tract infection. In industrialized countries, antibody prevalence begins to increase when children start school. It rises toward old age and suggests that Cp infections are ubiquitous and, since there is no known animal carrier, the human pathogen spread occurs by personal contact (1). Prevalence reaches approximately 60% in early adulthood but may be higher in men, in tobacco smokers and in unemployed people (2).

Studies have suggested a link between Cp and atherosclerosis (3) through an inflammation process (4). Bacterial DNA was detected in atheromatous plaques from coronary arteries at rates that could reach 79% (95%-confidence interval : 61-91%) (5). Chlamydia infections were suspected to play a role in cervical cancer (Chlamydia trachomatis) (6) and also in lung cancer (Cp) (7, 8). As far as we know, the possible involvement of Cp in head and neck cancer has not been examined, despite the way of life of these patients which made them more sensitive to community-acquired infections.

By grouping these observations together, we may wonder whether Cp could not play a role in head and neck cancer development and, indirectly, in the prognosis of the patients whose survival time appears particularly unpredictable (9).

Therefore in this preliminary study we looked for Cp DNA in head and neck tumours with the aim of an early termination of the trial with a threshold of infection prevalence of 60%.

Patients and Methods

Men, younger than 75 years of age, with a performance status > 60 (Karnofsky scale), with a histologically confirmed diagnosis of a stage III-IV squamous cell carcinoma of the head and neck (SCCHN), initially inoperable, were eligible for the study. Furthermore, an expected survival time of at least 8 weeks was required as well as an adequate hematological, renal and hepatic status. Synchronous primary cancers were accepted for study and, in that case, the TNM classification was the one of the more extensive tumour.

Pretreatment assessment included clinical history with past and current cardio-vascular manifestations, physical examination, ECG, echocardiographic measurements, chest radiograph, endoscopy of oesophagus and bronchus and the afore-mentioned biological determinations. Chemotherapy was based upon the Cisplatinum (100mg/m² on D-1)-Fluorouracil (1000mg/m²/d during a 5-day infusion) regimen (10, 11) with hyperhydration.

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Courses were repeated every 3 weeks or as soon as any toxic effects subsided. During each course of chemotherapy, continuous cardiac monitoring was carried out for 24 hours on D-1 and D-4.

Study parameter for Chlamydia pneumoniae. Before chemotherapy, blood samples (2mL) were centrifuged and sera were stored at -20°C. Subsequently they were examined for anti-chlamydial IgG, IgA and IgM antibodies with the microimmunofluorescence (MIF) test according to the method described by Wang and Grayston (12). Sera were tested against Chlamydia psittaci (strain IOL207), Chlamydia trachomatis (strain LB1, serovar E) and Chlamydia psittaci (avian strain Loth). Biopsy provided tumour samples of about 500 mg, – necrosis being cleared off –, which were stored at -80°C before Cp detection by polymerase chain reaction (PCR). Samples were pounded and then the amplifications of the omp1 gene encoding for the major outer membrane protein were carried out both on non-treated pellets and after lysis with proteinase K (200µg/mL). A nested PCR was performed using APNOU and APNOL primers for the first amplification (35 cycles) and APN1 and APN2 primers for the second amplification (35 cycles), as described by Cunningham et al. (13). Five µL of the specimen (in a total volume of 50 µL) was used in the first PCR round ; 3 µL of the PCR products amplified by the outer primers were then transferred to a new 50 µL PCR reaction mix for a second amplification using the inner primers. Amplification products were revealed on 1.6% agarose gel with ethidium bromide. In each assay, positive and negative controls were included. The PCR technique allowed the detection of 1 to 10 Cp bodies per tube of PCR.

Statistics. The study was designed on the basis of the Fleming’s procedure for early termination when initial results were extreme (14). With an α and β risk, respectively, equal to 0.04 and 0.08, a maximum of 25 consecutive tumours should be examined in a two-stage approach: after the first ten patients, if there are fewer than 3 or more than 7 tumours with a Cp infection, we may conclude for a prevalence, respectively, inferior to 60% or ≥ 60%; 15 more patients would have to be recruited only when positive observations were between 3 and 7.

Results

From April to October 1998, 10 consecutive patients entered the study. Tumour sites, TNM classification and serum determinations of IgG against Cp are detailed in Table I. Only one tumour among the first ten samples revealed Cp-DNA materials (Table I-patient VI). Serum analyses also showed for this patient Cp-IgG and -IgA titres, respectively, as high as 256 and 24. For patient III, the IgG serum level of 128 was associated with combined cross-reactions against Chlamydia trachomatis and C. psittaci (IgG titres = 16). Cp-IgA (serum titre = 12) was also detected in patient I.

Clinical variables and the response rate to treatment were quite similar to those obtained in previous studies (Table I) (11, 15). Toxicity was moderate since no grade 3-4 toxicity was observed except for a grade 3 non-documented infection (patient II), occurring without leucopenia and treated by non-Cp-specific antibiotic. Tobacco abuse reached a median of 30 pack-years (range : 10-63), whereas median alcohol consumption was 55g/day (18-90). In this way, patient VI was an average consumer of alcohol (50g/day) and tobacco (30 pack-years). By comparing the small group of individuals with Cp-IgG versus those without antibodies, the age at

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Tumour site</th>
<th>TNM</th>
<th>Cp-IgG</th>
<th>CR</th>
<th>ST</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>63</td>
<td>Oral Cavity (OC)</td>
<td>T4 N0 M0</td>
<td>16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Y</td>
<td>25.8 →&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>62</td>
<td>Oral Cavity</td>
<td>T4 N0 M0</td>
<td>16</td>
<td>Y</td>
<td>25.6 →</td>
</tr>
<tr>
<td>III</td>
<td>52</td>
<td>Hypopharynx (HP)</td>
<td>T3 N0 M0</td>
<td>128&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Y</td>
<td>24.9 →</td>
</tr>
<tr>
<td>IV</td>
<td>59</td>
<td>Hypopharynx</td>
<td>T3 N1 M0</td>
<td>-&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Y</td>
<td>22.5 +&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>V</td>
<td>47</td>
<td>Oropharynx (OP)</td>
<td>T2 N1 M0</td>
<td>-</td>
<td>Y</td>
<td>22.2 →</td>
</tr>
<tr>
<td>VI</td>
<td>43</td>
<td>OP + OC</td>
<td>T4 N0 M0</td>
<td>256&lt;sup&gt;d&lt;/sup&gt;</td>
<td>N</td>
<td>22.4 →</td>
</tr>
<tr>
<td>VII</td>
<td>57</td>
<td>OP + HP</td>
<td>T2 N2a M0</td>
<td>-</td>
<td>N</td>
<td>20.9&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>VIII</td>
<td>48</td>
<td>Oropharynx</td>
<td>T4 N2b M0</td>
<td>-</td>
<td>N</td>
<td>12.8&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>IX</td>
<td>51</td>
<td>Pharyngolarynx</td>
<td>T3 N0 M0</td>
<td>16</td>
<td>Y</td>
<td>20.4 →</td>
</tr>
<tr>
<td>X</td>
<td>65</td>
<td>Oral Cavity</td>
<td>T4 N3M1</td>
<td>16</td>
<td>N</td>
<td>15.3&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1CR (Y – N): complete response (Yes-No) after the whole therapeutic regimen including surgery for patients I-III and IX ; 2ST: survival time (months) ; 3→ for patient still alive and + for those who died;

<sup>a</sup>associated with Cp-IgA (titre: 12); <sup>b</sup>with cross reaction against Chlamydia trachomatis and C. psittaci (IgG titres = 16); <sup>c</sup>-: for negative results ;

<sup>d</sup>the only patient with a Cp-DNA detected in the tumour sample and whose serum Cp-IgA titre was determined at 24.
initiation of smoking was lower for the former (13 y ± 3) than the latter 19 y ± 3.5 ; \( p=0.02 \); average ± SD). Except for 2 cases with hypertension (patients II & VIII), no patient had a significant past history of cardiovascular disease. Initial ECG showed right bundle branch block in one patient (I) and for two others left ventricular hypertrophy (VI & IX). For both the last patients, echocardiography displayed impaired diastolic relaxation whereas in patient X it revealed light mitral regurgitation. The median value of left ventricular ejection fraction was measured at 66%, with extreme ranges of 58 and 76% (patient VI : 59%). During chemotherapy no significant clinical and ECG alterations could be detected, particularly for the 24-cardiac monitoring on D-1 and D-4, except for two minor manifestations of sinusal tachycardia.

Discussion

This kind of study, the results of which are rapidly available, may be recommended as an initial sounding out before a more extensive programme of study. According to the Fleming's procedure, the study reaches the conclusion that SCCHN Cp infestation is no greater than, or equal to, 60% with a high statistical power, despite few patients. However the statement is based upon three conditions: (i) the sensititivity of Cp detection is similar to that reported in the literature, (ii) a 60% rate of Cp-DNA in tumours is relevant to the working hypothesis and, finally, (iii) a small ten-tumour "sampling" is representative of advanced SCCHN, at least for our recruitment.

As previously defined, the sensititivity of PCR is greater than, or similar to, that reported in the literature (see Patients and Methods). Furthermore when a microorganism, such as Cp or Helicobacter, is suspected of being involved in the pathogenesis of either atherosclerosis or gastric adenocarcinoma, the proportion of tissue specimens with bacterial DNA is reportedly greater than 60% (5), though lower in more recently published results (16, 17). Finally, it is worth keeping in mind that the tumour sampling avoided the necrotic material which was dissected whereas the mononuclear blood cells infiltrating the tumour could be the vehicle of Cp (18, 19). However the rate of Cp seropositivity appeared as rather modest in our patients. Particularly, levels of positive specific IgA antibody concerned only two patients (2/10; 95%-confidence interval : 2.5-55.6%) who could be suspected of chronic infection. The proportion of 2/10 appears significantly inferior to that reported by Lindholt (20) for patients with a high risk of aneurysm progression in whom it reached 83% (95% confidence interval : 74-93). Finally, we displayed the well-known link between smoking and Cp infection since the patients with Cp-IgG had started to smoke younger than their counterparts without IgG (2).

The unbiased sampling of the small group of ten patients remains the last point to be discussed. With this aim in view, ten patients with advanced SCCHN were recruited consecutively. Their pretherapeutic criteria, the therapeutic efficacy and toxicity matched the ones observed in patients we treated previously (9, 11, 15). On looking at Table I, there are no bacteriological or serologic criteria which seem to be associated with therapeutic efficacy, toxicity and survival times. Finally, we did not observe any major cardiac anomalies before and during treatment in spite of the chemotherapy regimen including Fluorouracil.

Conclusively, there is no clear evidence for Cp infection playing a direct role in the development of SCCHN as well as in the prognosis and the therapeutic drug toxicity; the Fleming’s procedure, which may be regarded as a random sampling with early termination, deserves to be used in an initial phase in a strategy of trials.

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References


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