Diagnosis of nasopharyngeal tuberculosis by detection of tuberculostearic acid in formalin fixed, paraffin wax embedded tissue biopsy specimens

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SUMMARY The use of gas chromatography and mass spectrometry with selected ion monitoring detected tuberculostearic acid (TBSA) in 10 of 12 formalin fixed, paraffin wax embedded nasopharyngeal and head and neck biopsy specimens from patients with confirmed tuberculosis and carcinoma, and in one of 50 control specimens (giving a sensitivity of 83% and a specificity of 98%). The two false negative cases had very small tissue fragments and the patient with a false positive result may have had pulmonary tuberculosis. Tuberculostearic acid (TBSA) was also detected in nine of 16 specimens from the head and neck region with non-caseating granulomas suspected, but not confirmed, to be tuberculosis. It is concluded that nasopharyngeal tuberculosis is relatively common in Hong Kong and should be considered when biopsy specimens show granulomas. The detection of TBSA in tissue biopsy specimens is a useful, rapid method for the diagnosis of tuberculosis and other mycobacterial infections, and can be conveniently performed within two days on formalin fixed and paraffin wax embedded material.

Tuberculosis and nasopharyngeal carcinoma are both common in Hong Kong. Nasopharyngeal carcinoma has its highest incidence among the Southern Chinese and is the third commonest cause of death from cancer in Hong Kong. Although the incidence of tuberculosis has fallen in recent years, there were still 7432 new cases and 407 deaths notified in 1986. Nasopharyngeal tuberculosis is an uncommon presentation, but five cases were confirmed histologically and 11 were suspected at The Prince of Wales Hospital between 1985 and 1987.

These two conditions may coexist, especially when patients are compromised by radiotherapy or chemotherapy. Biopsy specimens of nasopharyngeal carcinoma without tuberculosis may contain reactive granulomas, and the histological diagnosis of tuberculosis may therefore be difficult in specimens showing early non-caseating granulomas without evidence of acid fast bacilli. As acid fast bacilli are found in only 10% of tuberculosis specimens by direct examination and culture takes several weeks, there is a need for additional rapid and sensitive tests to differentiate granulomas in these two conditions.

Mycobacteria (including Mycobacterium tuberculosis) and some other clinically less important organisms contain tuberculostearic acid as a structural component of their cell walls, and the detection of this substance in clinical specimens may be used for the diagnosis of mycobacterial infection. TBSA can be detected in very low concentrations by gas chromatography and mass spectrometry with selected ion monitoring (GC/MS-SIM), and we have shown that this technique is a sensitive and specific method for the diagnosis of M tuberculosis in sputum and cerebrospinal fluid. We have now investigated the use of TBSA detection in tissue biopsy specimens for the diagnosis of nasopharyngeal tuberculosis. In the present study we analysed formalin fixed, paraffin wax embedded tissue from previously diagnosed cases and controls.

Material and methods

Seventy eight formalin fixed, paraffin wax embedded tissue blocks from the nasopharynx and other areas in the head and neck were analysed retrospectively (table). The sections were stained with haematoxylin and eosin and re-examined by the Ziehl-Neelsen method. Fresh material from some of these specimens had originally been sent for mycobacterial culture and the microbiological results were reviewed. The specimens were then categorised into three groups

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TBSA in tissue biopsy specimens

Detection of tuberculostearic acid in tissue biopsy specimens

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Confirmed tuberculosis:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>5</td>
<td>4</td>
<td>1*</td>
</tr>
<tr>
<td>Larynx</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Vocal cord</td>
<td>2</td>
<td>1</td>
<td>1*</td>
</tr>
<tr>
<td>Cervical node</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>(B) Suspected tuberculosis:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasopharynx with nasophageal carcinoma</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Nasopharynx without nasophageal carcinoma</td>
<td>11</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Larynx</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Vocal cord</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>(C) Controls:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasopharynx with nasophageal carcinoma</td>
<td>34</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>Nasopharynx (normal)</td>
<td>16</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>1</td>
<td>49</td>
</tr>
</tbody>
</table>

*Very little tissue.

Group A (histologically confirmed tuberculosis) comprised 12 specimens with epithelioid granulomas showing acid fast bacilli on microscopy. Only five of these had been cultured and four yielded M tuberculosis. There was no coexisting disease in this group. Group B (suspected tuberculosis) comprised 16 specimens with granulomas but no demonstrable acid fast bacilli. None of these was cultured, and three showed coexisting nasophageal carcinoma. No fungal elements were seen in these specimens. Group C (controls) comprised 50 nasophageal biopsy specimens with no granulomas. Thirty-four were nasophageal carcinomas and 16 were normal histologically. Each specimen consisted of one to three tissue fragments varying from less than 1 mm to 3 mm in diameter. The clinical records of all patients were reviewed for outcome and final diagnosis.

**TBSA analysis by GC/MS-SIM**

Specimens were recovered from paraffin wax embedded tissue blocks by heating at 60°C until the wax melted, and immersing in xylene overnight. Tuberculostearic acid was then extracted by saponification, derivatized by boron trichloride methanolysis, and examined by gas chromatography and mass spectrometry as described previously for sputum and cerebrospinal fluid specimens. The methyl ester of tuberculostearic acid was detected by selected monitoring of ions at the mass:charge (m:e) ratios of 312 and 167. Tissue extract derivatives containing detectable amounts of both ions eluting simultaneously at a retention time of 27.2 minutes were designated positive for tuberculostearic acid. Control specimens of pure paraffin wax and xylene did not contain tuberculostearic acid.

**Results**

Biopsy specimens from 10 of the 12 histologically confirmed tuberculous cases were positive for tuberculostearic acid (table). Four of the positive specimens were from cervical lymph nodes, four from the nasopharynx, and one each from the larynx and vocal cord. One nasophageal biopsy specimen (with nasophageal carcinoma) of the 50 nongranulomatous controls was positive for tuberculostearic acid (table). The sensitivity of the test for the diagnosis of tuberculosis was thus 83% and the specificity 98%, compared with conventional methods. The two false negative cases, however (one each from the nasopharynx and vocal cord), consisted of single fragments of tissue of less than 1.0 mm in diameter. The false positive result was produced by a nasophageal carcinoma biopsy from a patient with a chest x-ray picture which was suspicious of tuberculosis at the time of biopsy. Follow up x-rays at six months and one year were normal, but the patient was not investigated further for tuberculosis.

Nine of 16 specimens showing granulomas but no acid fast bacilli were also positive for tuberculostearic acid; one biopsy specimen was from the larynx and eight from the nasopharynx (two with nasophageal carcinoma) (table). Only four of these nine patients were investigated microbiologically, and acid fast bacilli were seen in a sputum smear from one of them. Seven had multiple cervical lymph nodes also showing epithelioid granulomas (one with acid fast bacilli), four had abnormal chest x-ray pictures, and one had evidence of multiple organ disease compatible with tuberculosis. Four of these nine tuberculostearic acid positive cases were treated for tuberculosis but the others were lost to follow up. Of the seven patients with tuberculostearic acid negative biopsy specimens, one was treated for tuberculosis without investigation and the others were discharged.

**Discussion**

This study has shown that the detection of tuberculostearic acid in formalin fixed, paraffin wax embedded tissue specimens is useful for the rapid diagnosis of tuberculosis (and presumably other mycobacterial) infections. Compared with conventional microscopy and culture, the sensitivity of our test was 83% and specificity 98% (table). The one false positive result was in a patient with a chest shadow who may have had tuberculosis, and specimens from the two false negative cases were less than 1.0 mm in diameter. Larger tissue samples would be more likely to give...
positive results. Furthermore, nine of 16 specimens with granulomas but no detectable acid fast bacilli were also positive for tuberculostearic acid (table). These results suggest that the detection of tuberculostearic acid in tissue biopsy specimens is a more sensitive test for the diagnosis of nasopharyngeal tuberculosis than conventional methods. It is certainly more rapid than culture as analysis of paraffin wax embedded blocks for tuberculostearic acid takes only about two days.

These results also show that in Hong Kong, where tuberculosis is common, granulomatous inflammation in a nasopharyngeal biopsy specimen is suggestive of tuberculosis and that nasopharyngeal tuberculosis may be more common than is usually suspected. During the three years 1985 to 1987, the morbid anatomy department of The Prince of Wales Hospital received 1124 nasopharyngeal biopsy specimens with a clinical suspicion of nasopharyngeal carcinoma. Three hundred and eighty one (34%) of these were nasopharyngeal carcinoma. Granulomas were present in 19 (2%) specimens (three with nasopharyngeal carcinoma), and acid fast bacilli were present in five (all with no nasopharyngeal carcinoma). Three of the 14 biopsy specimens with no acid fast bacilli were interpreted as reactive to nasopharyngeal carcinoma, and 11 were reported as "granulomatous inflammation, tuberculosis cannot be excluded, please work up if clinically indicated". Only five of these 11 non-nasopharyngeal carcinoma cases were further investigated for tuberculosis, but tuberculostearic acid was detected in eight of them (two nasopharyngeal carcinoma, six non-nasopharyngeal carcinoma).

These results suggest that in Hong Kong many patients with nasopharyngeal biopsy specimens showing non-caseating granulomas without acid fast bacilli may have tuberculosis and should be further investigated. In this retrospective study sample size was often suboptimal, not all patients were available for recall or follow up, and few of them were investigated properly for tuberculosis. A prospective study with appropriate clinical, radiological, histological and microbiological analysis is in progress.

The detection of tuberculostearic acid in tissue biopsy specimens can give a rapid confirmation of mycobacterial infection within two days. Furthermore, the analysis can be performed retrospectively on any available formalin fixed or wax embedded material. While this test is particularly valuable in areas where tuberculosis is endemic, it would also be useful for the detection of mycobacteria in lesions from patients with AIDS.

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References


Requests for reprints to: Professor G L French, Department of Microbiology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong.
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