Cytodiagnosis of Bronchogenic Carcinoma and Neuroendocrine Tumor of the Lung by Transthoracic Fine-Needle Aspiration

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To evaluate our experience with the cytodiagnosis of primary lung cancers by transthoracic fine-needle aspiration (TFNA), 106 bronchogenic carcinomas (BC) and 6 neuroendocrine tumors of the lung (NTL) with adequate needle aspirates were reviewed. The cytodiagnostic accuracy rates of BCs were 75.5%, 72%, 100%, 53%, and 50% for bronchogenic adenocarcinomas, squamous-cell carcinomas, small-cell carcinomas, large-cell carcinomas, and mixed carcinomas, respectively. Of the 6 NTLs, 4 typical carcinoid tumors (CT) were correctly diagnosed, 1 atypical CT was wrongly identified as small-cell carcinoma, and 1 large-cell NTL was mistaken for an adenocarcinoma. Diagn. Cytopathol. 2000;23:431–434.

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Key Words: bronchogenic carcinoma; neuroendocrine tumor; carcinoid tumor; transthoracic fine-needle aspiration; cytodiagnostic accuracy; tumor typing

Transthoracic fine-needle aspiration (TFNA) for cytodiagnosis of lung diseases has been used sporadically since 1883, long before radiologic imaging techniques were available.1 Menetrier was the first investigator who diagnosed lung cancer by this technique, in 1886.2 The use of TFNA under fluoroscopic guidance for cytologic evaluation of intrathoracic mass lesions was originally introduced by Dahlgren and Norderström in the 1960s.3 Since that time, this diagnostic procedure has gradually developed, and is now a routine diagnostic procedure. This was, in part, due to advances in radiologic techniques permitting a visual control of biopsy procedures by television fluoroscopy and computed tomography, the refinement of the cyologic criteria of lung cancers, and the availability of pathologists with experience in diagnostic cytology. Over the past 30 yr, this method of clinical investigation proved to be a safe, accurate, and economical diagnostic procedure; and it has gradually gained popularity worldwide.4 5 This paper reports on our experience with the cytodiagnosis of 121 cases of bronchogenic carcinoma (BC) and neuroendocrine tumors of the lung (NTL) by TFNA.

Materials and Methods

To evaluate our experience with the cytodiagnosis of primary lung cancers by TFNA, we reviewed 115 histologically confirmed BCs and 6 NTLs, with cytologic evaluation by the above-mentioned diagnostic modality. Those cases were documented in the files of the Divisions of Anatomic Pathology, Pulmonary Medicine, and Thoracic Surgery at the University of Alberta Hospitals, Edmonton, Alberta, Canada, and in the consultation files of one of us (G.-K.N.) in a 14-yr period ending in December 1997. There were 72 men and 49 women, with ages ranging from 35–88 yr. The tumors in those patients were located in the peripheral or middle zone of the lung, and ranged from 1–4 cm in greatest dimension. In all cases, no endobronchial tumors were detected by bronchoscopy; sputa and cytologic materials obtained by bronchial washing, bronchial brushing, and transbronchial fine-needle aspiration performed during bronchoscopy were all negative for cancer cells. In each case, TFNA was performed under fluoroscopic or computed tomographic guidance, using a 22-gauge, 15-cm-long needle. Four to 10 direct smears were prepared from each needle aspirate (NA). They were either fixed in 95% ethanol...
for staining by the Papanicolaou method and/or air-dried for staining by the Diff-Quik technique. The biopsy needle was rinsed in a vial of 4 ml of a balanced electrolyte solution that was used to make four cytospin smears. In the cases in which tumor typing was difficult, cytochemical staining with periodic acid-Schiff (PAS) and PAS with prior diastase digestion (PASD) was performed on cytospin or direct smears. When a neuroendocrine carcinoma was suspected cytologically, immunocytochemical (IM) staining of the tumor cells with commercial neuron-specific enolase and chromogranin antibodies was performed on ethanol-fixed and Papanicolaou-stained smears without prior destaining, using the avidin biotin complex (ABC) technique.

In all cases, tissue samples from the lung tumors removed by surgery and at autopsy were fixed in formalin and processed for routine histologic examination. Five-micron-thick sections from selected tumor tissue blocks from poorly differentiated nonsmall-cell carcinomas were stained with PAS and with PASD. Selected tissue sections from NTLs were stained with commercial neuron-specific enolase and chromogranin antibodies by the ABC method. Minute fresh tumor tissue samples measuring 1 mm in greatest dimension were fixed in 2% glutaraldehyde and processed for electron microscopic (EM) study, if indicated.

For cytodiagnosis of lung tumors, the cytologic criteria outlined by Koss et al., Johnston and Elson, Tao, and Mackay et al. were used. Tumor typing was based on the predominant cell patterns of NAs, and efforts were made to separate BCs into four major categories: adenocarcinoma, squamous-cell carcinoma, large-cell carcinoma, and small-cell carcinoma. The histologic and ultrastructural diagnoses of lung neoplasms in our series were made according to the criteria defined by Mackay et al. and Colby et al.

Results
In 9 cases the NAs were scanty in cellularity and showed no cancer cells, and they were eliminated from this review. One hundred and six BCs and 6 NTLs with adequate cell samples and histologic confirmation formed the basis of this study. There were no cases with false-positive or false-negative cytodiagnoses in our series. The pathological data of those 112 BCs and NTLs are summarized in Tables I and II.

Bronchogenic Carcinomas
Among the four major histologic types of bronchogenic carcinomas, the cytohistologic correlation rate was 75.5% for adenocarcinomas (AC) including 6 bronchioalveolar-cell carcinomas, 72% for squamous-cell carcinomas (SQCC), 100% for small-cell carcinomas (SCC), and 53% for large-cell carcinomas (LCC). Of the 2 cases of mixed carcinoma, SCC, and non-SCC, only one case was correctly diagnosed (Table I). Among the 57 cases of bronchogenic AC, 14 poorly differentiated tumors were mistaken to be poorly differentiated SQCCs or LCCs. Seven poorly differentiated SQCCs were wrongly diagnosed as ACs in 3, as LCCs in 2, and as SCC in 2 cases. The NAs of the last two cases in this group revealed loosely clustered small tumor cells with hyperchromatic nuclei and linear basophilic nuclear debris. The classic nuclear molding seen in cytologic materials from small-cell carcinoma is not present (Papanicolaou stain, ×400).

Table I. Cytohistologic Correlation of 106 Bronchogenic Carcinomas

<table>
<thead>
<tr>
<th>Histodiagnosis</th>
<th>AC</th>
<th>SQCC</th>
<th>SCC</th>
<th>LCC</th>
<th>Mixed CA</th>
<th>CTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC (57)</td>
<td>43</td>
<td>4</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>75.5%</td>
</tr>
<tr>
<td>SQCC (25)</td>
<td>3</td>
<td>18</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>72.0%</td>
</tr>
<tr>
<td>SCC (5)</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>100.0%</td>
</tr>
<tr>
<td>LCC (17)</td>
<td>6</td>
<td>2</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>53.0%</td>
</tr>
<tr>
<td>Mixed CA (2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>50.0%</td>
</tr>
</tbody>
</table>

*a Number in parentheses is number of tumors. AC, adenocarcinoma; SQCC, squamous-cell carcinoma; SCC, small-cell carcinoma; LCC, large-cell carcinoma; Mixed CA, mixed carcinoma; CTR, correct tumor typing rate.

Table II. Cytohistologic Correlation of Six Neuroendocrine Tumors of the Lung

<table>
<thead>
<tr>
<th>Histodiagnosis</th>
<th>Typical CT</th>
<th>Atypical CT</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical CT (4)</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Atypical CT (1)</td>
<td>0</td>
<td>0</td>
<td>SCC (1)</td>
</tr>
<tr>
<td>Large-cell neuroendocrine tumor (1)</td>
<td>0</td>
<td>0</td>
<td>AC (1)</td>
</tr>
</tbody>
</table>

*a Number in parentheses is number of tumors. CT, carcinoid tumor; SCC, small-cell carcinoma; AC, adenocarcinoma.

Fig. 1. Needle aspirate of squamous-cell carcinoma, small-cell type, showing tumor cells with ink-dark nuclei, scant cytoplasm, and abundant basophilic linear nuclear debris. The classic nuclear molding seen in cytologic materials from small-cell carcinoma is not present (Papanicolaou stain, ×400).
Neuroendocrine Tumors

Of the 6 peripheral NTLs, 4 typical carcinoid tumors were correctly identified (Table II). In one case of atypical carcinoid tumor the tumor cells were more pleomorphic, nuclear molding was present in a few small tumor cell clusters, and a small amount of basophilic nuclear debris was also noted. The cytologic findings in this case were consistent with a SCC (Fig. 2). In the case of large-cell NTL, single and loosely clustered polygonal malignant cells with a moderate amount of granular cytoplasm, round nuclei, and prominent nucleoli were present, suggesting a well-differentiated AC (Fig. 3). Histologic sections of the tumor revealed a large-cell NTL consisting of polygonal tumor cells arranged predominantly in trabecular patterns (Fig. 4). The tumor cells stained positively with NSE and chromogranin antibodies, and showed intracytoplasmic membrane-bound and dense-core neurosecretory granules and poorly formed cell junctions by EM examination, confirming the light microscopic diagnosis of large-cell NTL.

The correct tumor typing rate of NTLs in our series was 67%.

Discussion

Bronchogenic carcinomas are the most common primary lung cancers, with about 180,000 new cases expected to be diagnosed in the United States in 1999.11 In our institution, about 200 new cases of BC have been diagnosed annually in the past decade, and over 90% of them were successfully diagnosed cytologically by bronchial washing, bronchial brushing, transbronchial needle aspiration biopsy,12 and/or bite biopsy of the tumors; and less than 10% of them required TFNA for cytdiagnosis.

The cytodiagnosis of BCs by TFNA, in general, is relatively straightforward, as malignant epithelial cells are readily identifiable in representative or adequate cell samples. The cytologic manifestations of different types of BCs in sputum, in bronchial washing and brushing, and in NA are essentially similar.7 However, the NA contains a larger number of cancer cells and tumor tissue fragments because it samples the tumor tissue directly.7 The TFNA of lung cancers as reported in the literature has shown sensitivity rates in the range of 75–95%, and specificity rates of 99% or more.7

The cytologic tumor typing of well- and moderately differentiated ACs or SQCCs is usually not problematic, as aspirated tumor cells commonly show classic cellular arrangements and glandular or squamous cytoplasmic features. However, cytologic typing of poorly differentiated ACs or SQCCs is challenging, as tumor cells may show equivocal cytoplasmic differentiation toward a squamous or glandular cell line. As shown in Table I, the accuracy rates of tumor typing for

Fig. 2. Needle aspirate of atypical carcinoid tumor, showing loosely aggregated tumor cells with scant cytoplasm, slightly pleomorphic nuclei, nuclear molding, and a small amount of basophilic nuclear debris (Papanicolaou stain, ×400).

Fig. 3. Needle aspirate of large-cell neuroendocrine tumor of the lung, showing single and loosely aggregated large, polygonal malignant epithelial cells with ill-defined, granular cytoplasm, oval nuclei, and prominent nucleoli, mimicking cells derived from an adenocarcinoma (Diff-Quik stain, ×400).

Fig. 4. Histology of a large-cell neuroendocrine tumor of the lung, showing polygonal cells with prominent nucleoli in a trabecular pattern (hematoxylin-eosin stain, ×250).
bronchogenic ACs, SQCCs, SCCs, and LCCs in our series were 75.5%, 72%, 100%, and 53%, respectively; and these were within the ranges reported by other series: 60–96% for ACs, 70–88% for SQCCs, 17.9–71% for LCCs, and 81.7–100% for SCCs. The wide ranges of accuracy rates of BCs may be, in part, explained by the fact that up to 66% of these cancers are heterogenous and display two or more histologic patterns. Therefore, a high cythistologic correlation cannot be expected. If the BCs in our series were classified cythologically only into two main categories of SCC and non-SCC, the cythistologic correlation rate would be 99%.

The cythologic typing of BCs suffers interobserver and intraobserver variations. In one study, an intraobserver variation in interpretation of cell type of 15% and an interobserver variation of 20% were noted. For histologictyping of BCs, an intraobserver variability of 2–20%, and an interobserver variation as high as 53% for poorly differentiated SQCCs, 58% for poorly differentiated ACs, and 90% for LCCs, were reported. This variability indicates that the histologic diagnosis of BC does not necessarily constitute the gold standard for the cytdiagnosis of these tumors, and sometimes the cythology technique is more accurate for tumor typing. Our experience with bronchogenic SCCs confirmed the above-mentioned statement: on several occasions in which a bronchial brushing and a small bronchial bite biopsy of tumors were taken during bronchoscopy, the tumor tissue fragments were difficult to interpret because of crushing artifactual changes, and the cell samples showed typical cythologic patterns of SCCs.

The cythologic manifestations of typical carcinoid tumors in materials obtained by TFNA and bronchial brushing are essentially similar and characteristic for these tumors. However, an atypical carcinoid tumor may yield cells mimicking those of a SCC. In this situation, IM and EM studies of aspirated tumor cells are not helpful to the cythologic differential diagnosis, as cells derived from those two tumors share common IM and ultrastructural features. The difficulty is compounded by the fact that SCCs often show mediastinal lymph node and distant metastases when the tumors are diagnosed, and that regional metastasis and distant metastasis may be present in 40–48% and 20%, respectively, of patients with atypical carcinoid tumors at presentation. Thus, the clinical and imaging data of the patient are also not of diagnostic help in this case, and histologic examination of the lung tumor is the only means to solve this diagnostic dilemma.

The cytdiagnosis of a large-cell NTL is also challenging, as its cythologic criteria have not been well-defined. Therefore, IM and/or EM studies of aspirated tumor cells are needed to demonstrate their neuroendocrine features before a diagnosis of large-cell NTL can be made. It should be borne in mind that a nonsmall-cell BC may show rare neurosecretory granules by EM study and may stain weakly and focally positively with NSE and/or chromogranin antibodies. Therefore, a histologic diagnosis of a large-cell NTL can only be made, if acinar and/or trabecular patterns and evidence of neuroendocrine differentiation of the tumor cells are present. As in cases of BC, the histologic diagnosis of NTL also suffers interobserver variations. In a recent study conducted by Travis et al., 40 cases of NTLs including SCCs were circulated among 5 experienced lung pathologists. Unanimous agreement occurred in only 70% of SCCs, 58% of typical carcinoid tumors, 50% of atypical carcinoid tumors, and 40% of large-cell NTLs; a majority diagnosis was achieved in 90%, 92%, 75%, and 50% of the above-mentioned histologic subtypes of NTLs, respectively.

References