Peroperative frozen section analysis of TTF-1 antigen expression

S Camilleri-Broet, M Alifano, M Morcos, E Comperat, P Magdeleinat, B Marmey, T J Molina, J-F Régnard, J Audouin

Background: The assessment of thyroid transcription factor 1 (TTF-1) expression is a useful way to investigate the origin of lung adenocarcinomas or large cell carcinomas when dealing with a solitary lung nodule in a patient with a history of extrathoracic cancer. However, if immunohistochemical analysis has not been performed before surgery, a peroperative frozen section may be insufficient to distinguish between a primary pulmonary tumour and a metastatic tumour.

Aims: To develop a technique for the rapid assessment of TTF-1 expression that could improve the ability of frozen section peroperative histological diagnosis to answer such questions.

Methods: A rapid immunohistochemical technique (lasting 30 minutes) to assess the expression of TTF-1 was developed and tested.

Results: Among the 45 interpretable cases, results of frozen section immunohistochemistry were similar to those found by the standard immunohistochemical technique for the expression of TTF-1.

Conclusions: This technique enables TTF-1 to be analysed peroperatively, but further prospective studies are needed to assess its usefulness in routine practice.

Material and Methods

Material

Fifty consecutive cases received in the laboratory for peroperative frozen section examination were analysed (table 1). Fresh tissue was slow frozen, cut into 4 μm thick sections in a cryostat, and routinely stained with Mayer’s haematoxylin. Stained nuclei were counterstained with Gruenwald Giemsa. The lung is one of the most frequent sites of metastasis for extrathoracic tumours. Among tumours that can metastasise to the lung, some adenocarcinomas (such as colon and renal cell tumours) are of special interest because of the potential benefit of surgery in terms of a cure. Although the most common presentation is multiple nodules, solitary metastasis does occur. In this case, the distinction between solitary metastasis and primary lung cancer remains the major task, because it carries different therapeutic options (lobectomy for primary lung cancer, wedge resection for metastatic lesions). The differential diagnosis is based on clinical (smoking habit, stage, and clinical history of the previous tumour), radiographic, and histological features. In the case of adenocarcinomas or large cell carcinomas, the distinction can be difficult, even at classic histological examination in such cases, positivity for thyroid transcription factor 1 (TTF1; a protein normally expressed in thyroid and lung parenchyma) is thought to indicate the primary lung origin of the tumour. In the case of adenocarcinomas or large cell carcinomas, the distinction can be difficult, even at classic histological examination.

RESULTS (TABLE 1)

The final pathological diagnoses were: primary lung carcinoma, 34 cases; lung metastasis, 11 cases; malignant mesothelioma, two cases; and non-neoplastic lesion, three cases. Five cases could not be interpreted because of either the absence of neoplastic cell proliferation (three) or non-valid negativity of the tumour cells in the absence of an internal positive control (two). One of this last group was a TTF-1 expressing lung adenocarcinoma. The second was a TTF-1 negative renal clear cell carcinoma metastasis.

Figures 1 and 2 show typical staining patterns for negative and positive specimens, respectively, stained using the peroperative frozen section method.

Among the remaining 45 cases, 23 were positive for TTF-1 on both techniques (peroperative frozen and classic paraffin wax embedded material). The sections were pretreated in a microwave, incubated for two hours with the anti-TTF-1 antibody, and staining was visualised by the standard SABC method. Classic immunohistochemistry was interpreted without knowledge of the peroperative frozen section TTF-1 results.

Abbreviations: SABC, streptavidin–biotin–peroxidase; TTF-1, thyroid transcription factor 1
wax embedded tissue). All of the positive cases except for one were primary lung carcinomas, namely: 17 of 17 adenocarcinomas, two of four large cell carcinomas, three of four neuroendocrine tumours (two large cell neuroendocrine carcinomas and one typical carcinoid tumour). In contrast, there was no expression of TTF-1 in the eight squamous cell carcinomas.

One metastatic colonic carcinoma was positive for TTF-1 on both the peroperative frozen and classic paraffin wax embedded slides. The diagnoses of pulmonary metastatic lesions were established on history and histological features of well differentiated gland forming adenocarcinoma with characteristic colonic crypts and a CK7+/CK20− profile.

The intensity of staining for TTF-1 was similar in peroperative frozen and classic paraffin wax embedded tissues. In some cases, wide areas of necrosis were the cause of non-specific staining.

**DISCUSSION**

Our method of assessment of TTF-1 expression on peroperative frozen sections is rapid and sensitive. We found that this

<table>
<thead>
<tr>
<th>Number</th>
<th>Peroperative TTF-1 positive</th>
<th>Paraffin wax embedded tissue TTF-1 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary lung cancer</td>
<td>34</td>
<td>17*</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Neuroendocrine tumour</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Metastases</td>
<td>11</td>
<td>1</td>
</tr>
<tr>
<td>Colonic</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Kidney</td>
<td>2</td>
<td>0*</td>
</tr>
<tr>
<td>Breast</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Malignant mesothelioma</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>No tumour</td>
<td>3</td>
<td>ND</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>23</td>
</tr>
</tbody>
</table>

*ND, not done; TTF-1, thyroid transcription factor 1.

**Take home messages**

- The results of immunohistochemistry for thyroid transcription factor 1 (TTF-1) expression were similar for peroperative frozen sections and standard paraffin wax embedded material.
- This technique enables TTF-1 to be analysed, but further prospective studies are needed to assess its usefulness in routine practice.

Peroperative TTF-1 staining must be interpreted taking into account other diagnostic elements (history, radiological aspect) and the histological subtype. TTF-1 is negative in most squamous cell carcinomas, but it is well known that the absence of TTF-1 expression does not exclude a primary lung origin of an adenocarcinoma or a large cell carcinoma. With respect to specificity, our data confirm the good (but not perfect) performance of TTF-1 expression, in agreement with other studies dealing with the classic method of detection. However, further studies need to be performed to answer the question of the usefulness of this technique in the frozen section diagnosis process, and whether such a technique would have an influence on the attitude of the surgeon intraoperatively, when dealing with the differential diagnosis between primary lung or metastatic tumours.

**ACKNOWLEDGEMENTS**

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REFERENCES


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