Krukenberg Tumour of the Ovary: A Case Report with Light Microscopy, Immunohistochemistry and Electron Microscopy Study

C. FAZZARI1, F. FEDELE1, G. PIZZI1, C. CRISAFULLI1, A. PARISI2 and R.A. CARUSO1

Dipartimento di 1Patologia Umana e di 2Scienze Chirurgiche Generali e Speciali, Policlinico Universitario, Messina, Italy

Abstract. A rare case of a 46-year-old woman with bilateral Krukenberg tumours is reported. Histologically, oedematous ovarian stroma was infiltrated by signet-ring cells arranged singly, in cords or in nests. Immunoreactivity for cytokeratin-7, carcinoembryonic antigen as well as histochemical positivity for mucus demonstrated the epithelial nature of the tumour. The gastric primary site was suggested by the cytoplasmic immunoreactivity for MUC-5AC and by ultrastructural evidence of gastric differentiation in signet-ring cells such as mucous granules with eccentric dense cores and intracellular microcysts, lined by sparse microvilli. Gastric biopsy, performed after pathological diagnosis, revealed a signet-ring cell carcinoma similar to that in the ovaries, confirming the gastric origin of the Krukenberg tumour. Because none of the individual immunohistochemical markers used for tissue identification is both site specific and site sensitive, electron microscopy in combination with immunohistochemistry is a valuable tool for the pathologist in the diagnosis of the tissue origin of a Krukenberg tumour.

Krukenberg tumour, an uncommon metastatic tumour of the ovary, originates in the stomach in the vast majority of cases (1). On occasions, the gastric cancer may be small and remains undetected for several years after oophorectomy (1, 2). Much less frequently, the primary tumour is in the large intestine, breast, gallbladder, uterine cervix, appendix, or urinary bladder (2). Because of the marked proliferation of the ovarian stroma, in some cases the tumours may resemble fibrothecomas on gross examination (3).

In this report, we describe the case of a 46-year-old woman, with bilateral ovarian masses, resembling fibrothecoma, but with a clinically occult gastric cancer. The importance of careful immunohistochemical and electron microscopic study in the diagnosis of the tissue origin of Krukenberg tumour is finally discussed.

Materials and Methods

For light microscopy, the specimens were fixed in 10% formalin for 24 h at room temperature and embedded in paraffin. Sections were stained with haematoxylin-eosin, alcian blue and periodic acid-Schiff (AB-PAS) stains. Additional sections were used for the immunohistochemical stains with the ABC method. Antigen retrieval procedures were carried out in a pressure cooker by boiling the slides in 10 mM citrate buffer (pH 6.0) for 3 min at 1.5 atm pressure. The commercial source of primary antibodies and the antigen retrieval method adopted for each marker are detailed in Table I. Tumor tissue was also obtained in the operating room for immediate fixation in 3% phosphate-buffered glutaraldehyde (pH 7.4) and post-fixed in 1% osmium tetroxide. Semi-thin araldite embedded sections were stained with Giemsa reagent for selection of fields. Thin sections were double-stained with uranyl acetate and lead citrate; they were then examined and photographed in a Zeiss EM 902 electron microscope (Carl Zeiss, Oberkochen, Germany).

Results

A 46-year-old woman, nulliparous, complained of pelvic pain and a round mass, distending the right lower, abdominal wall. Abdominal-pelvic ultrasound examination and computed tomography scan (CT), showed a solid mass in the right ovary (8x6 cm) and in the left ovary (6x4 cm). A total laparo-histerectomy with bilateral salpingo-oophorectomy was performed.

On gross examination, the ovary tumours showed a smooth external surface of a white-greyish colour, with a solid, microcytic and oedematous cut surface resembling a fibrothecoma (Figure 1). Microscopically, both ovaries showed signet-ring tumour cells, single or in nests, with eccentric nuclei and large, pale, vacuolated cytoplasm, surrounded by a stroma, densely fibroblastic or oedematous (Figure 2). AB-PAS stains revealed the presence of mucus in the cytoplasm of signet-ring cells.
Lymphatic invasion was frequently found, particularly at the hilar region. Tumour cells showed cytoplasmic immunoreactivity for CEA, CK-7 and MUC-5 AC (Figure 2). Immunohistochemistry for CA-125, CK-20, chromogranin-A, synaptophysin and vimentin gave negative results.

On electron microscopy, signet-ring cells were filled with mucin granules, heterogeneous in size and electron density (Figures 3 and 4). Some of these granules possessed irregularly-shaped, eccentric, moderately dense cores in the mucous matrix (Figure 3), whereas others had a punctuate substructure. These tumour cells showed no microvilli or other signs of luminal surface specialization and no basal membrane. Signet-ring cells containing microcysts were also observed and appeared aggregated in microglandular structures (Figure 4). Intracellular microcysts were seen as round cytoplasmic cavities, lined by sparse microvilli.

On the basis of histomorphological data obtained on the ovaries, the diagnosis of Krukenberg tumour with probable origin from the stomach was carried out. After pathological diagnosis, gastroscopy revealed a small ulcer about 1 cm in diameter over the greater curvature of the gastric antrum. The histological examination of the gastric biopsy revealed small nests of signet-ring cells infiltrating the gastric submucosa, similar to that seen in the ovarian sections, confirming the gastric origin of the Krukenberg tumour. Total colonoscopy showed no abnormal findings.

A total gastrectomy was performed. Post-operative histological examination of the gastric specimen was reported as signet-ring cell carcinoma of the stomach invading the serosa with clear surgical margins and metastases of five of the 16 lesser curvature lymph nodes. On follow-up, 7 months after surgery, there was no clinical or radiological evidence of recurrence.

**Discussion**

The diagnosis of Krukenberg tumours largely depends on the recognition of their characteristic light microscopic features such as densely fibroblastic and/or oedematous stroma that appears diffusely infiltrated by malignant signet-ring cells arranged singly, in cords or in nests (1). Krukenberg tumours

Table I. Antibodies used in this study.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Clone</th>
<th>Dilution</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUC5AC</td>
<td>CLH2</td>
<td>1/100</td>
<td>NOVOCASTRA Newcastle, UK</td>
</tr>
<tr>
<td>CA125</td>
<td>OC125</td>
<td>1/100</td>
<td></td>
</tr>
<tr>
<td>Chromogranin A</td>
<td>DAK-A3</td>
<td>1/1800</td>
<td></td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>SY38</td>
<td>1/10</td>
<td>DAKO</td>
</tr>
<tr>
<td>Vimentin</td>
<td>V9</td>
<td>1/200</td>
<td>Glostrup, Denmark</td>
</tr>
<tr>
<td>CEA</td>
<td>II-7</td>
<td>1/100</td>
<td></td>
</tr>
<tr>
<td>CK20</td>
<td>Ks 20.8</td>
<td>1/100</td>
<td></td>
</tr>
<tr>
<td>CK7</td>
<td>OV-TL 12/30</td>
<td>1/100</td>
<td></td>
</tr>
</tbody>
</table>

Antigen retrieval was performed using a pressure cooker.
must be distinguished from ovarian tumors that contain signet-ring cells filled with either mucinous or non-mucinous material. Ovarian tumours with signet-ring-containing mucins include primary mucinous carcinomas and mucinous carcinoid tumors (3). Primary mucinous ovarian tumours tend to be more commonly unilateral with a complex papillary pattern. The diagnosis in favour of mucinous carcinoid is confirmed by immunostains for chromogranin and synaptophysin. Ovarian tumors that can contain signet-ring cells filled with non-mucinous material include signet-ring stromal tumor, sclerosing stromal cell tumor and clear cell adenocarcinoma of the ovary. Usually these tumours showed no mucin reactivity to AB-PAS stain nor ultrastructural evidence of mucin granules (4).

Immunohistochemical evaluation may aid in distinguishing primary ovarian carcinomas from metastatic carcinomas. Usually, a CK7/CK20+ or CK7+/CK20+ immunophenotype (CK20 positivity, in particular) favours a metastatic gastrointestinal carcinoma (3). Moreover, positive staining for gastric mucin gene MUC5 AC suggests a metastatic gastric carcinoma over primary ovarian adenocarcinoma (3). Although these immunohistochemical markers assist in narrowing the differential diagnosis of the primary tumour, none of the individual immunohistochemical markers used for tissue identification is both site specific and site sensitive. For this reason, use of multiple immunohistochemical markers is needed to improve the diagnostic accuracy of primary site prediction (5).

In the present study, the electron microscopy showed gastric differentiation in signet-ring cells (6, 7). In fact, some of the mucin granules were characterized by an eccentric, dense core in the mucous matrix, reminiscent of the pyloric gland (mucopeptic) cell granules (7). Other granules had a punctate substructure, similar to that seen in the foveolar cell component of gastric carcinomas (7). Moreover, microcysts, observed in the cytoplasm of some signet-ring cells, are lined sparsely by microvilli, as documented by electron microscopy in some gastric carcinomas (6, 7).
We experienced a patient with bilateral Krukenberg tumour derived from an occult, advanced, gastric carcinoma. Our study suggests that the combination of immunohistochemistry and electron microscopy was very useful in the determination of the gastric origin of Krukenberg tumour.

References