Ovarian small cell carcinoma of pulmonary type appearing in ante-mortem ascites: An autopsy case and review of the literature

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Abstract
Ovarian small cell carcinoma of pulmonary type (OSCCPT) is an extremely rare and aggressive disease. The diagnostic significance of cytology of ascites for OSCCPT, however, has not been shown so far. Here, we report the diagnosis of this carcinoma in an autopsy case with ante-mortem cytology of ascites. A 75 year-old woman was detected with bilateral ovarian cancer by radiological imaging. Although operation was planned, massive ascites was discovered a few weeks later. Ascites was removed with abdominocentesis, which cytologically diagnosed presence of carcinoma, suspicious of adenocarcinoma. A few days later, she died. From autopsy samples, we diagnosed this case as bilateral OSCCPT, showing neuroendocrine differentiation by immunohistochemistry. We reviewed ante-mortem cytology of ascites and found scattered small atypical cells. Immunocytochemical study of the cell block of the ascites showed neuroendocrine differentiation of the atypical cells in an identical manner as the autopsy specimens. Since small atypical cells of OSCCPT often exist with other histological tumor components, careful screening of all cells on the preparation is advisable to accurately diagnose OSCCPT by cytology of ascites.

Keywords:
Ovarian small cell carcinoma of pulmonary type; Immunohistochemistry; Cytology; Ascites; Prognosis

1. Introduction
Ovarian small cell carcinoma (OSCC) is a rare tumor with neuroendocrine differentiation and has been classified into two types: hypercalcemic type (OSCCHT) and pulmonary type (OSCCPT). OSCCPT predominantly occurs in young women with hypercalcemia [1,2]. OSCCPT typically occurs in postmenopausal women and histologically resembles pulmonary small cell carcinoma (PSCC) [1,3]. OSCCPT is an extremely rare and aggressive disease. As far as we know, only 25 cases including a study of 11 cases and some case reports have been reported in the English literature (Table 1) [3–16]. In the literature, cytological study has been reported...
in only one case with urine cytology, but cytology of ascites has not been shown so far [13]. Here, we report an autopsy case of OSCCPT with ante-mortem cytological and immunocytochemical analyses of ascites.

### 2. Clinical history

A 75-year-old woman (gravida 2, para 2) consulted the digestive department of our hospital with lower abdominal and back pain late in May 2013. Her past history included appendicitis in her teens, intraductal papillary mucinous neoplasm of the pancreas, hypertension and diabetes mellitus. No family history of benign or malignant tumor was indicated. She smoked 10 cigarettes a day for 23 years. Tumor markers were high: serum carcinoembryonic antigen (CEA) level was 74.5 ng/ml (normal < 5.0) and serum carbohydrate antigen 125 (CA125) level was 1055 U/ml (normal < 37). No hypercalcemia was found: serum calcemic level was 7.9 mg/dl (normal: 8.2–10.2) and the corrected calcemic level based on the serum albumin level was 8.9 mg/dl. Computed tomography (CT) and magnetic resonance imaging (MRI) revealed bilateral ovarian tumors. She was referred to the department of gynecology in the middle of June 2013. MRI findings suggested stage IIIc ovarian cancer. Although operation was planned for the beginning of July, two weeks later, massive ascites was discovered with significant inflammatory findings: white blood cell count was 23500/μl and serum C-reactive protein (CRP) level was 37.5 mg/dl, thus, she was admitted to the hospital for an emergency. Ascites was removed with abdominocentesis, which cytologically diagnosed presence of carcinoma, suspicious of adenocarcinoma. Her general status subsequently deteriorated rapidly with decrease of blood pressure, and a few days later (2 months after her initial consultation), she died.

### 2.1. Cytological findings

In the ante-mortem cytology of ascites, atypical cells with large and eccentric nuclei were seen on the side of the preparation where the smear examination started, and some of them were multinuclear with bizarre morphology. Since some of them formed piled-up structures, we diagnosed this case as...
cancer, suspicious of adenocarcinoma, in consideration of the predominant histological type in ascites. However, upon review of the cytology, we infrequently found small cohesive clusters and solitary small atypical cells which were admixed with reactive mesothelial cells and histiocytes in the center of the preparation. The small cohesive clusters showed cord-like arrangement, prominent nuclear molding and engulfment. The small atypical cells had granular chromatin and showed high nuclear-cytoplasmic ratio. They were suggestive of small cell carcinoma (Fig. 1).

2.2. Autopsy findings

Systemic autopsy except the brain was performed about 8 hours after death. Initial laparotomy revealed 2450 ml bloody ascites with multiple milky-whitish solid masses, up to 1 cm in diameter, in the peritoneum. Her bilateral ovaries were replaced by 5 cm-sized milky-whitish solid masses with hemorrhage and necrosis. Histologically, in the bilateral ovarian masses, atypical cells proliferated diffusely with alveolar pattern mediated by thin fibrous stroma and, in some parts, they had a cord-like appearance (Fig. 2). The atypical cells were small in size and had a high nuclear-cytoplasmic ratio. Their nuclei showed increased chromatin. The large bizarre cells were occasionally intermingled with the small atypical cells (Fig. 2). Immunohistochemically, the small atypical cells showed neuroendocrine differentiation: positivity for CD56, chromogranin A and synaptophysin (Fig. 3, Table 2). Other immunohistochemical results were as follows: positivity for cytokeratin 7 (CK7), CK AE1/AE3 and CEA; and negativity for placental alkaline phosphatase (Pl-ALP), MIC-2 gene product (CD99), TTF-1 and vimentin (Fig. 3, Table 2). The large bizarre cells were positive for CK7, CK AE1/AE3 and CEA, but negative for other markers including neuroendocrine markers, suggesting that these large cells were undifferentiated malignant epithelial cells. Thus, we diagnosed this case as OSCCPT admixed with undifferentiated carcinoma. The left ovarian tumor had infiltrated into the muscularis propria of the rectum, and the right ovarian tumor had infiltrated into the muscular layer of the uterus. Multiple peritoneal masses were regarded as dissemination of OSCCPT. Para-aortic lymphatic metastasis was seen. A microscopic 2 mm-sized distant metastasis was seen in the apex of the left lung.

The cause of death was considered to be circulatory insufficiency from abundant ascites due to peritoneal dissemination of bilateral ovarian tumor.

3. Discussion

OSCC is classified into OSCCHT and OSCCPT by the World Health Organization (WHO) Classification of ovarian tumors. OSCCHT typically occurs in young women and is associated with hypercalcemia. These tumors are unilateral in most occasions. Histologically, they form macrofollicle-like space in approximately 80% of cases, and have a component of large cells with abundant eosinophilic cytoplasm in about half of the cases [1,2]. On the other hand, OSCCPT typically occurs in postmenopausal women without hypercalcemia. These tumors are bilateral in about half of the cases. Histologically, they resemble PSCC of pulmonary type.
neuroendocrine type [1,3]. In the present case, histologically, the small atypical cells proliferated diffusely with alveolar pattern and, in some parts, they formed a cord-like structure. The nuclear-cytoplasmic ratio was high, and the nuclei showed increased chromatin. These cells showed neuroendocrine differentiation, including positivity for chromogranin A. These features are typical of small cell carcinoma. Furthermore, the tumors occupied the bilateral ovaries, and macrofollicle-like space was histologically absent. Clinically, this patient was an elderly woman without hypercalcemia. Thus, a diagnosis of OSCCPT was made.

Immunohistochemically, it has been reported that tumor cells of OSCCHT are rarely positive for chromogranin A but are positive for vimentin in about half of the cases [2,17]. On the other hand, tumor cells of OSCCPT are positive for chromogranin A in 12 out of 23 cases and are negative for vimentin in all 17 examined cases from review of the literature (Table 1) [3–16]. In addition to the description ‘chromogranin positivity may be very focal with punctuate cytoplasmic immunoreactivity’ as indicated by WHO classification of ovarian tumours [1], interpretation of recently reported cases (Table 1)

**Fig. 2** Histopathology of the tumor: tumor cells grow diffusely (A: H&E, ×40) with some cord-like structure (B: H&E, ×100). Small atypical cells (C: H&E, ×400) are admixed with large bizarre cells (D: H&E, ×400).

**Fig. 3** Immunohistochemical study of the tumor: tumor cells are positive for CK7 and negative for vimentin and TTF-1 (×200). Chromogranin A immunolabels small atypical cells, but not large ones.
suggested that positivity for chromogranin A and negativity for vimentin in OSCC could be key features of OSCCPT as also shown in the present case and as proposed by other authors [18].

In the diagnosis of OSCCPT, it is very important to exclude metastatic PSCC by searching for the primary focus. Immunohistochemically, it has been reported that about 90% of PSCC are positive for TTF-1, and almost all cases of OSCC are negative for TTF-1 [19,20]. In this case, only a 2 mm-sized mass was found in the lung, and the tumor cells were negative for TTF-1, which ultimately excluded metastasis of PSCC to the ovaries.

From the ante-mortem cytology of ascites in the present case, we diagnosed presence of carcinoma, suspicious of adenocarcinoma, in consideration that the majority of carcinomas in the ascites were adenocarcinomas. Upon review of the cytology, however, we noticed the occurrence of some small atypical cells which were admixed with reactive mesothelial cells and histiocytes in the center of the preparation, in contrast to the large atypical cells that were abundantly found on the side of the preparation where the smear examination started. Thus we modified the initial diagnosis to small cell carcinoma. Immunocytochemical study of cell block sections revealed neuroendocrine differentiation, compatible with small cell carcinoma (Fig. 1). However, in the present case, chest imaging suggested the exclusion of PSCC.

Although OSCCPT is currently thought to be aggressive regardless of the stage and little is known about the effectiveness of chemotherapy so far [1,3,18], review of the literature does suggest that its prognosis can be improved by surgery and post-operative chemotherapy; 8 out of 17 cases with operation and post-operative chemotherapy were in complete remission regardless of the stage (Table 1). Indeed, no accepted and consistent postoperative chemotherapy regimens have been established yet, but postoperative chemotherapy was shown to be effective in some cases despite the natural aggressiveness of this disease as in this case which progressed naturally without operation or chemotherapy. Thus, studies on early introduction of chemotherapy may shed light on the effectiveness of this treatment for this intractable tumor.

Furthermore, because of its natural aggressiveness, OSCCPT have been reported to spread beyond the ovary as assessed by laparotomy in more than half of the patients. Cytology of ascites could be assessed in sixteen out of 25 past OSCCPT cases in the literature because they had stage Ic, IIc, III or IV tumors, which were exposed to the abdominal lumen, thus, tumor cytology of ascites could be obtained (Table 1). One of the objects of this report was to recognize the diagnostic significance of cytology of the ascites for OSCCPT. We propose that to diagnose OSCCPT by cytology of ascites, it is important to consider that OSCCPT often exists with other histological components, thus, all cells on the preparation should be carefully screened, lest the small atypical cells of OSCCPT behind reactive mesothelial cells or histiocytes are overlooked.

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**Table 2 Immunohistochemical study in the present case.**

<table>
<thead>
<tr>
<th>Markers of antibody</th>
<th>Source</th>
<th>Dilution</th>
<th>Immunolabeling</th>
<th>Small atypical cells</th>
<th>Large atypical cells</th>
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<tr>
<td>CK7</td>
<td>Dako</td>
<td>1:200</td>
<td>(+)</td>
<td>(+)</td>
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<td>Epithelial membrane antigen</td>
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<td>(+)</td>
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<td>Vimentin</td>
<td>Leica</td>
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<td>(−)</td>
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<tr>
<td>CEA</td>
<td>Dako</td>
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<td>(+)</td>
<td>(+)</td>
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<td>CA125</td>
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<td>(−)</td>
<td>(−)</td>
</tr>
<tr>
<td>WT1</td>
<td>Dako</td>
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<td>CD56</td>
<td>Dako</td>
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<td>Chromogranin A</td>
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<td>Synaptophysin e-kit</td>
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<td>(+)</td>
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<td>Human chorionic gonadotropin</td>
<td>Nichirei</td>
<td>Prediluted</td>
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<td>PI-ALP</td>
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<td>(−)</td>
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<td>TTF-1</td>
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<td>(−)</td>
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<td>p53</td>
<td>Dako</td>
<td>1:200</td>
<td>(+)</td>
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<td>(−)</td>
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<td>Estrogen receptor</td>
<td>Thermo Scientific</td>
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<td>(−)</td>
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<td>Progesteron receptor</td>
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<td>Ki-67</td>
<td>Dako</td>
<td>1:400</td>
<td>About 37% cells were positive</td>
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References


