Immunocytochemical Detection of Ovarian Carcinoma Cells in Serous Effusions

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Monoclonal antibodies OC 125, OV632, OV-TL 3, MOv18 and OV-TL 23, directed against distinct ovarian carcinoma-associated antigens, were examined for their value in cytopathologic diagnosis. Their sensitivity and specificity in staining ovarian carcinoma cells in serous effusions was determined using the indirect immunoperoxidase technique. Smears prepared from 140 serous effusions (73 benign, 67 malignant) were immunostained with the five antibodies. OC 125 and MOv18 reacted positively with 96% and 81% of the smears of effusions from ovarian carcinoma patients, respectively, while OV-TL 3, OV632 and OV-TL 23 stained a lower percentage of the samples (73%, 65% and 62%, respectively). In discriminating ovarian carcinoma cells from benign (mesothelial or inflammatory) cells in serous effusions, MOv18 demonstrated the highest specificity (100%) since none of the 73 samples from benign effusions were stained upon incubation with this antibody. OC 125 cannot be used for this purpose due to its reactivity with mesothelial cells in benign samples. Staining cytologic preparations of malignant effusions from cancer patients with carcinomas not originating in the ovary revealed that OV632 and OV-TL 23 may be useful adjuncts to determine the origin of the carcinoma cells found in serous effusions. The reactivity of these antibodies was highly selective for ovarian carcinoma cells, staining only 6% and 0% of the samples from the non-ovarian carcinoma samples, respectively. It is concluded that MOv18 is the most suitable antibody for distinguishing ovarian carcinoma cells from mesothelial cells in serous effusions, while OV632 and OV-TL 23 especially may help to assess whether carcinoma cells found in effusions originate in the ovary.

Cytopathologic analysis of serous effusions can provide important information for the management of patients presenting with effusions. However, it may be difficult to determine whether the effusion is caused by a benign (reactive or inflammatory) process or is a result of a malignancy. Mesothelial cells cannot always be distinguished from neoplastic cells.

Immunocytochemical staining with pancarcinoma antibodies may facilitate the distinction between mesothelial and carcinoma cells. Especially, antibodies against carcinoembryonic antigen7,22 and epilasin
(EMA, Ca-1, HMFG-2) have been shown to react more or less specifically with carcinoma cells. Similarly, B72.3 antibody, reactive with a broad range of adenocarcinoma cells and rarely reactive with mesothelial cells, is a useful tool in cytopathologic practice. Application of combinations of such antibodies has been shown to optimize the diagnostic accuracy of cytopathologic analysis.

Since a peritoneal effusion may be one of the first symptoms of ovarian cancer, cytologic examination may play an important role in the management of these patients. In these cases cytologic examination of effusions may be used to establish malignancy and to indicate the nature of the primary tumor. Assessment of the origin of the malignant cells may have therapeutic consequences because treatment modalities for ovarian cancer differ from those for several other carcinomas. For this purpose pan-carcinoma antibodies (anti-CEA, anti-TAG 72, anti-EMA) cannot be used because of their reactivity with a broad range of adenocarcinoma cells. In addition, following debulking surgery, ovarian cancer patients generally undergo several courses of chemotherapy. Cytologic analysis of peritoneal washings may be used to evaluate the efficacy of these treatment steps.

During the past few years, several monoclonal antibodies mainly (but not exclusively) reactive with ovarian carcinoma cells have been described. It has been shown that these antibodies can be used to obtain additional information in cases of differential diagnostic problems in surgical pathology.

In the present study we evaluated the reactivity patterns of five anti-ovarian cancer monoclonal antibodies (OC 125, OV-TL 3, OV632, MOv18 and OV-TL 23) in cytologic preparations from serous effusions. Their specificity and sensitivity in the positive identification of ovarian cancer in cytologic smears were determined to assess their potential value for cytopathologic practice.

Materials and Methods

Clinical Material

Serous effusions were obtained by aspiration and centrifuged for five minutes at 1,500 rpm; smears were made and stained by routine procedures using both Papanicolaou and May-Grünewald-Giemsa stain. Smears from 140 effusions (113 patients) were used in this study. Based on cytopathologic, histologic and clinical follow-up data, 67 smears were diagnosed as malignant (23 ovary, 21 breast, 2 endometrium, 7 lung, 3 pancreas, 2 kidney, 1 adenocarcinoma, 1 melanoma, 1 sarcoma, 6 lymphoma). The remaining 73 nonmalignant preparations contained atypical mesothelial cells and/or inflammatory cells. Unfixed smears were stored at –80°C until used for the immunocytotochemical staining procedures.

Monoclonal Antibodies

Several murine monoclonal antibodies were used in this study. First, OC 125 was raised against a serous ovarian carcinoma cell line. Biochemical studies have shown that the OC 125–defined antigenic determinant is expressed on a high-molecular-weight glycoprotein. Second, OV-TL 3 was obtained after immunization with a cell suspension prepared from an endometrioid ovarian carcinoma specimen; it was recently shown to recognize a 30-kd membrane protein. Third, OV632 was obtained after immunization with cyst fluid from a serous cystadenocarcinoma. The antibody, MOv18 was raised against a crude membrane preparation of a poorly differentiated ovarian carcinoma. Recently the antigen that is recognized by MOv18 was characterized as a folate-binding protein. Last, OV-TL 23 was isolated after immunization with cyst fluid from a serous ovarian carcinoma.

OC 125 (CIS Laboratories, Gif-sur-Ivette, France) and OV632 (Sanbio, Uden, the Netherlands) were obtained commercially and used at optimal dilutions, 1:100 and 1:40, respectively. MOv18-induced ascitic fluid was the gift of Dr. M. I. Colnaghi (Milan, Italy) and was used at a 1:2,000 dilution. The hybridoma culture supernatant of OV-TL 3 was diluted 1:10 in normal goat serum, while the supernatant of OV-TL 23 was used undiluted.

Immunocytotochemical Studies

Smears were dried overnight and fixed briefly in acetone by dipping at room temperature; endogenous peroxidase activity was quenched with 3% H2O2 in phosphate-buffered saline (PBS)/methanol (1:1) for 15 minutes. Subsequently the preparations were incubated with the appropriate primary antibody for 45 minutes at room temperature. The smears were then washed (3×10 minutes in PBS) and treated with peroxidase-conjugated rabbit antimouse immunoglobulins (Dako, Glostrup, Denmark) diluted 1:50 in PBS containing 5% human serum. After another series of washing steps, peroxidase activity was revealed with 1 mol/L 3-amino-9-ethylcarbazole and 0.01% H2O2 in NaAc buffer, pH 4.85. The preparations were counterstained with hematoxylin and mounted in glycerin/gelatin. In the
negative controls the first antibody was replaced by PBS.

Staining patterns were scored arbitrarily by two independent observers, unaware of any clinical data or histologic or cytologic findings, as negative or weakly, moderately or strongly positive according to the intensity of the staining. Smears with only limited numbers of cells left after completing the staining procedure were excluded from the evaluation. Sections of a serous ovarian carcinoma sample reactive with all antibodies used in this study served as a positive control in each staining session. The sensitivity of the antibodies for detecting ovarian cancer in smears was calculated as follows:

\[
\text{Sensitivity} = \frac{\text{number of smears with ovarian carcinoma cells stained positively}}{\text{total number of smears with ovarian carcinoma cells examined}} \times 100\%.
\]

The specificity of the antibodies in the differential diagnosis between ovarian carcinoma cells and benign (mesothelial or inflammatory) cells was calculated as follows:

\[
\text{Specificity (ovarian carcinoma vs. benign) = } \frac{\text{number of smears from benign effusions stained negatively}}{\text{total number of smears from benign effusions examined}} \times 100\%.
\]

The specificity of the antibodies in distinguishing ovarian carcinoma cells from carcinoma cells not originating in the ovary was calculated as:

\[
\text{Specificity (ovarian carcinoma vs. non-ovarian carcinoma) = } \frac{\text{number of smears from non-ovarian carcinoma}}{\text{total number of smears from non-ovarian carcinoma patients examined}} \times 100\%.
\]

Results

Sensitivity

The five monoclonal antibodies were used to stain smears from 140 serous effusions. The results are listed in Table I. The highest sensitivity for staining ovarian carcinoma cells in smears was found for OC 125 (96%), while MOv18 and OV-TL 3 scored 81% and 73%, respectively. OV632 and OV-TL 23 exhibited lower sensitivity for ovarian carcinoma cells—i.e., 65% and 62%, respectively.

In addition to sensitivity, the intensity of the staining of carcinoma cells may be an additional criterion for selecting antibodies for use in cytopathology. Figure 1 summarizes the staining intensity for ovarian cancer cells in the effusions examined (n = 23) for each antibody. OC 125 strongly stained the ovarian cancer cells in most (91%) samples. With the other antibodies strong staining was found in approximately 27-48% of the samples, while an additional 25% of the samples stained moderately or weakly positive. OC 125, OV-TL 3, MOv18 and OV-TL 23 each predominantly stained the membrane of the carcinoma cells, while OV632 was directed against an antigen that is localized in the cytoplasm. The immunocytochemical reactivity of each of the five antibodies with ovarian carcinoma cells from a peritoneal effusion is shown in Figure 2A-E.

Specificity in the Distinction Between Ovarian Carcinoma and Benign Cells (Figure 3, Table I)

In samples of benign effusions the reactivity of OC 125 was most striking. In 50% of the benign effusions (35 of 70), OC 125 stained the atypical mesothelial cells. Generally, staining of mesothelial cells was less intense than staining of carcinoma cells with OC 125 (Figure 2F). OV632, OV-TL 3 and OV-TL 23 stained only a few of the benign samples. In most of these cases atypical mesothelial cells reacted positively. The specificity of OV-TL 3, OV632 and OV-TL 23 was 95%, 90% and 97%, respectively. MOv18 was consistently negative in all the cells in these samples, thus revealing 100% specificity in distinguishing ovarian carcinoma from nonmalignant cells.

Specificity in the Distinction Between Ovarian Carcinoma and Non-Ovarian Carcinoma Cells

The reactivity patterns of the five monoclonal antibodies with samples prepared from the serous effusions from patients with malignancies not originating in the ovaries are summarized in Table I. Again, OC 125 stained positively in most (28 of 35) of these non-ovarian carcinoma samples, resulting in a specificity of 20% (Figure 3). The reactivity of the other antibodies was much more restricted to ovarian carcinoma cells. OV-TL 3 and MOv18 showed some reactivity with samples from breast cancer patients (8 of 21, respectively) and scored a sensitivity of 78% and 86%, respectively. OV632 stained positively in only two smears from non-ovarian carcinoma patients (specificity, 94%). In this group of samples OV-TL 23 demonstrated its highly ovarian carcinoma-specific reactivity, staining none of the 34 non-ovarian carcinoma samples examined (specificity, 100%).
Table 1  Reactivity of the Five Anti-Ovarian Carcinoma Monoclonal Antibodies in Smears of 140 Serum Effusions

<table>
<thead>
<tr>
<th>Disease</th>
<th>OC 125</th>
<th>OV-TL 3</th>
<th>OV632</th>
<th>MOv18</th>
<th>OV-TL 23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian carcinoma</td>
<td>22/23a</td>
<td>16/22</td>
<td>11/17</td>
<td>17/21</td>
<td>13/21</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>2/2</td>
<td>1/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>16/20</td>
<td>5/21</td>
<td>1/16</td>
<td>3/21</td>
<td>0/21</td>
</tr>
<tr>
<td>Lung carcinoma</td>
<td>5/7</td>
<td>0/7</td>
<td>1/7</td>
<td>2/7</td>
<td>0/7</td>
</tr>
<tr>
<td>Pancreatic carcinoma</td>
<td>3/3</td>
<td>1/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>1/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>1/1</td>
<td>1/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Melanoma</td>
<td>1/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Sarcoma</td>
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<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>3/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>Benign effusion</td>
<td>35/70</td>
<td>4/73</td>
<td>7/72</td>
<td>0/72</td>
<td>2/73</td>
</tr>
</tbody>
</table>

*aNumber of positive cases vs. number of cases examined is indicated.

*bTwo small cell carcinomas, four adenocarcinomas and one squamous cell carcinoma.

*cAdenocarcinoma of the salivary gland.

Discussion

To investigate the usefulness for application in cytopathologic differential diagnosis, we studied the reactivities of five anti-ovarian carcinoma monoclonal antibodies in smears from malignant and nonmalignant effusions.

OC 125 intensely stained most samples of effusions from ovarian cancer patients. For this antibody, comparably high sensitivity for staining ovarian carcinoma tissue sections was found in three independent immunohistochemical studies.2,15,21 These studies also showed that a wide range of nonovarian carcinomas stain positively with OC 125. This relatively broad reactivity was also found in carcinoma cells in serous effusions. In the present study 28 of 35 samples from effusions from nonovarian carcinomas stained positively. Despite this high sensitivity for staining carcinoma cells in body fluids, OC 125 cannot be used to positively identify carcinoma cells in effusions due to its reactivity with mesothelial cells, which has been described in previous papers.14,16,27 Kabawat et al14 have shown that the OC 125–defined antigenic determinant is expressed in colon- or stomach-derived tissues—i.e., peritoneum, pleura and pericardium.

OV-TL 3 stained positively in 73% of the smears from ovarian cancer patients. OV-TL 3 generally did not stain mesothelial or inflammatory cells and thus can be used in cytopathology to discriminate between malignant and nonmalignant effusions. The occasional reactivity of OV-TL 3 with carcinoma cells not originating in the ovary (8 of 36), especially mam-

![Figure 1](attachment:image.png)

Figure 1
Percentage of ovarian carcinoma samples stained strongly positive (■), moderately positive (□) and weakly positive (○) for each of the five antibodies used in this study.
mary carcinoma cells, has been observed in tissue sections as well.²

The sensitivity of OV632 in this study was relatively low (65%). In a similar study, OV632 stained 34 of 35 samples of effusions from epithelial ovarian carcinoma patients,¹⁶ while a specificity of 100% was found. In the present study some reactivity of OV632 with mesothelial cells was found, be it only occasionally and weakly. Still, the specificity of OV632 was high (90%), allowing discrimination between malignant and nonmalignant effusions. In addition, the antibody may be used to help determine the origin of
carcinoma cells in serous effusions since it almost exclusively stained ovarian carcinoma cells in this study.

MOv18 appears to be a very useful antibody in cytopathology, especially because it did not stain any of the samples from nonmalignant effusions. The paper describing the production and initial characterization of this monoclonal antibody indicates that MOv18 stained cytospin preparations from ovarian cancer effusions with high sensitivity (eight of eight) and high specificity (zero of five benign effusions). In this study the high sensitivity and specificity of MOv18 for staining ovarian carcinoma cells in serous effusions was confirmed on a much larger series of samples. In addition, the antibody appears to be useful for differential diagnosis of carcinoma cells in body fluids. However, in those cases the occasional reactivity of MOv18 with mammary carcinoma cells must be kept in mind.

OV-TL 23 showed the lowest sensitivity, staining only 62% of the samples from ovarian cancer patients. However, the specificity of OV-TL 23 for discrimination between ovarian carcinoma and benign cells was very high (97%). In addition, the reactivity of OV-TL 23 with carcinoma cells was restricted completely to ovarian carcinoma cells. Therefore, a positive reaction with OV-TL 23 strongly suggests that these cells are ovarian carcinoma cells, indicating that this antibody may be a useful adjunct in assessing the origin of carcinoma cells in body fluids. This highly specific reactivity of OV-TL 23 was also observed in our previous immunohistochemical study.

We conclude that antibodies OV-TL 3, OV632, MOv18 and OV-TL 23 can be used for the detection of ovarian carcinoma cells in smears from body fluids. Immunocytochemical staining of smears with these antibodies can help to make a precise diagnosis in samples suspected of containing malignant ovarian tumor cells. Especially, the highly specific MOv18 appears most useful for discriminating ovarian carcinoma cells from benign (mesothelial and inflammatory) cells in cytopathology. Furthermore, OV-TL 23 (and to a lesser extent OV632) may be used in panels with pan-carcinoma antibodies (anti-TAG 72, anti-

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Figure 2

Immunocytochemical reactivity of (A) OC 125, (B) OV-TL 3, (C) OV632, (D) MOv18 and (E) OV-TL 23 with carcinoma cells in a smear of the peritoneal effusion from a serous ovarian carcinoma patient. Note the psammoma bodies in the carcinoma cells. In (F) note the OC 125 reactivity in a cluster of atypical mesothelial cells from a benign pleural effusion. The five ovarian carcinoma-associated antigens were stained using an indirect immunoperoxidase technique with 1 mol/L 3-amino-9-ethylcarbazole and 0.01% H2O2 in the substrate solution. Cells were counterstained with hematoxylin (×500).
epigsialin) in cases in which the primary tumor is unknown. Various studies have demonstrated that B72.3 and anti-epigsialin antibodies (e.g., EMA, HMFG-2, Ca 1) permit specific recognition of carcinoma cells in effusions,9,17,20,25,27 Additional staining with OV-TL 23 and/or OV632 could positively identify these cells as ovarian carcinoma cells.

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References