Epithelial Tumors in the Ovary
The Good, The Bad, and the Ugly

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Case 1
High Grade Serous Carcinoma

**Clinical History:** The patient was a 68-year-old woman with bilateral adnexal masses and an elevated CA125 (793). She had had a hysterectomy in 2008 for postmenopausal bleeding. In 2011, a CT scan confirmed the presence of bilateral adnexal masses and revealed multiple small omental nodules. At operation in 2011 she had 6-8 L of ascites. She underwent bilateral salpingo-oophorectomy and tumor debulking including a transverse colon resection. Tumor was noted to extensively involve the diaphragm, the anterior peritoneum, and the transverse colon, the mesentery of the small bowel, the sigmoid colon and the bladder peritoneum.

**Gross Pathology:** The ovaries were small. The right ovary was a pale-tan firm multinodular solid mass 4 cm in maximum dimension with tumor involving the surface. The left ovary was 5 cm in maximum dimension and was cystic and solid, with papillary excrescences in the linings of some cysts. Solid areas were yellow-white. No abnormalities were noted in the fallopian tubes. A 21 cm segment of transverse colon and omentum was riddled with small nodules measuring 0.2 to 1 cm, with a dominant mass 4.5 cm in greatest dimension in the mesentery. The bowel mucosa was unremarkable.

**Diagnosis:** High-grade serous carcinoma of the left fallopian tube and left and right ovaries with extensive extraovarian tumor spread.

**High Grade Serous Carcinoma**

Serous carcinoma is the most common type of ovarian cancer, accounting for 68% of ovarian cancers and 88% of stage III and IV cancers in a large population based study. (1) Mutations of the P53 gene are present in most cases and are thought to be critical events in the pathogenesis of this type of carcinoma. (2) Intraabdominal metastases are usually present at the time of diagnosis, as in this patient, involving the omentum, the peritoneum or the abdominal lymph nodes. (1) Occasional metastases are found in unusual distant sites, such as the brain, lung, distant lymph nodes or the breast. (3)

**General Clinical Features of Epithelial Tumors, Especially High Grade Serous Carcinoma**

Ovarian carcinoma occurs mainly in peri- and postmenopausal women. About 10% of patients with ovarian cancer have an inherited predisposition to develop the cancer (BRCA mutation or Lynch Syndrome). (4) More than 70 percent of women with ovarian cancer have extensive extraovarian tumor spread at the time of diagnosis. One reason for this is that the symptoms caused by epithelial tumors are vague and non-specific. Common symptoms include pelvic discomfort or pain, a sensation of abdominal fullness or pressure, gastrointestinal disturbances, urinary frequency, and occasionally, menstrual abnormalities. Women with advanced ovarian cancer often have ascites, which interferes with gastrointestinal function, leading to nausea and vomiting. Ovarian enlargement in a woman over 45 raises the question of ovarian cancer and requires further evaluation. The identification of a solid or complex mass by sonography or some other imaging technique is worrisome.
The CA-125 monoclonal antibody blood test detects an antigenic site on MUC16, a high molecular weight glycoprotein of uncertain function. (5, 6) The test is most useful in women with serous carcinoma although women with other types of ovarian cancer sometimes have elevations of CA-125 as well. The CA-125 is usually elevated in women with advanced borderline and malignant epithelial tumors and in some women with localized disease. (7, 8) The response to therapy correlates with the serum CA-125 level, with the CA-125 dropping into the normal range in patients in clinical remission and rising again if the tumor recurs. (9) Increased CA-125 levels also occur in patients with other types of cancers, and in some with benign conditions including benign ovarian tumors and cysts, pregnancy, endometriosis, pelvic inflammatory disease, leiomyomas, liver disease, and some collagen-vascular disorders. (10)

The treatment of ovarian tumors is primarily surgical. Invasive carcinoma of the ovary directly invades into adjacent organs or spreads via the peritoneal fluid to the omentum, the peritoneum, the serosal surfaces of the abdominal viscera and the diaphragm. Lymph node metastases are common and distant metastases are occasionally detected in the lungs and other sites. The stage, which is based on the surgical and pathologic findings, is the most important prognostic factor.

<table>
<thead>
<tr>
<th>FIGO Staging of Ovarian Cancer</th>
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<tbody>
<tr>
<td>IA – One ovary, intact capsule, no tumor on surface, cytology negative</td>
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<tr>
<td>IB - Both ovaries, intact capsule, no tumor on surface, cytology negative</td>
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<tr>
<td>IC – One or both ovaries, with ruptured capsule, tumor on surface or positive cytology</td>
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<tr>
<td>IIA – Spread to uterus and/or fallopian tubes, cytology negative</td>
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<tr>
<td>IIB – Spread to other pelvic tissues, cytology negative</td>
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<tr>
<td>IIC – Pelvic spread, any site, positive cytology</td>
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<tr>
<td>IIIA – Microscopic peritoneal metastases outside the pelvis</td>
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<td>IIIB - Macroscopic peritoneal metastases outside the pelvis ≤ 2 cm</td>
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<tr>
<td>IIIC – Macroscopic peritoneal metastases outside the pelvis &gt; 2 cm and/or lymph node metastases</td>
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<td>IV – Distant metastases (excludes peritoneal metastases)</td>
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The treatment of ovarian cancer usually includes surgery and chemotherapy. (11-13) The standard surgical treatment is hysterectomy, bilateral salpingo-oophorectomy, omentectomy, pelvic and para-aortic lymph node dissection, and staging biopsies and appendectomy if indicated. Gynecologic oncologists generally try to remove as much extraovarian tumor as possible (“cytoreductive surgery”). The prognosis is most favorable in early (stage I–II) stage disease. In this group the patient’s age, the stage, the tumor grade and the results of peritoneal cytology studies influence the prognosis. (14) A tumor type of high-grade serous carcinoma and a positive cytology are the most important unfavorable prognostic findings in early stage disease. (15) Young women with some types of stage IA or IC adenocarcinomas can be treated by unilateral salpingo-oophorectomy, omentectomy, and thorough staging if they are unwilling to have a hysterectomy. (16-18) Some women with advanced ovarian cancer (stage IIIC-IV) receive chemotherapy prior to surgery. This is known as neoadjuvant chemotherapy and it can reduce tumor volume and make resection easier. Neoadjuvant chemotherapy followed by cytoreductive surgery resulted in approximately the same survival rate as primary cytoreductive surgery followed by chemotherapy in a recent European trial, but there was less morbidity. (19) The survival results were less favorable than those currently reported for patients treated with primary cytoreductive surgery followed by chemotherapy in the US, and American gynecologic
oncologists appear to be taking a cautious approach to adopting neoadjuvant chemotherapy as the standard treatment of all patients. (20) Neoadjuvant chemotherapy sometimes causes changes in the histologic appearance of a tumor such that it is difficult to classify, (21, 22) although immunohistochemical staining patterns are less altered and may be of some use in classifying treated tumors. (23)

Combination chemotherapy with carboplatin and paclitaxel is the standard first line chemotherapy for women with high-grade stage IA carcinomas and for those with extraovarian spread or positive peritoneal cytology. (24) Optimally debulked patients with stage III carcinomas may be candidates for treatment with IV paclitaxel followed by intraperitoneal chemotherapy with cisplatin and paclitaxel. (25, 26) This regimen is reported to result in increased survival compared to standard IV chemotherapy, but it is more toxic. Chemotherapy results in partial or complete clinical remission in about 85 percent of women with advanced cancer, but most patients relapse within 2–3 years and the long-term survival rate is less than 20–30 percent. (27)

**Gross Pathology**

Serous carcinoma tends to be large and is often bilateral. There is often a mixture of cystic, papillary, and solid growth. The solid areas are tan or white and there are foci of hemorrhage and necrosis. Carcinoma frequently invades through the ovarian capsule and grows on the surface of the ovary. Serous surface papillary carcinoma grows predominantly on the surface of the ovary, with minimal parenchymal invasion and no intracystic growth. When there is extensive extraovarian serous carcinoma with only focal (<0.5cm) surface growth on the ovaries, the process can be viewed as primary peritoneal serous carcinoma with involvement of the ovaries. While most serous carcinomas are large tumors, small high-grade serous carcinomas occur and can be associated with widespread metastasis throughout the abdomen.

**Microscopic Pathology**

The tumor cells exhibit marked nuclear atypia and frequent (often 30-50 or more mf/10 hpf) mitotic figures. The nuclei are hyperchromatic and variably pleomorphic, and often contain one or more prominent nucleoli. The nucleus to cytoplasm ratio is high, and the nucleus typically appears to occupy most of the cell. Papillary growth is usually present at least focally and is frequently the predominant growth pattern (Fig. 1-1). Some papillae have fibrovascular cores that are lined by stratified tumor cells. The tumor cells often pile up to form tufts that project above the surfaces of the papillae. A micropapillary pattern in which long thin tufts of tumor cells grow into cystic spaces is also common. Tumor cells line glands and cystic spaces, diffusely infiltrate fibrotic stroma, or form solid nests and sheets (Fig. 1-2). Elongated slit-like glands within foci of solid growth are a characteristic finding in high-grade serous carcinoma.

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<th>Growth Patterns in High Grade Serous Carcinoma</th>
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<tr>
<td>Papillary</td>
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<tr>
<td>Micropapillary</td>
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<tr>
<td>Glandular</td>
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<tr>
<td>Slit like glands</td>
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<tr>
<td>Cribriform</td>
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<td>Cystic</td>
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<tr>
<td>Microcystic</td>
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<tr>
<td>Solid</td>
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<td>Transitional cell carcinoma like</td>
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Foci of microcystic growth, sometimes with admixed signet ring type cells, are noted occasionally. (28) Geographic zones of necrosis may be present. Rare examples of high grade serous carcinoma apparently evolve from a borderline serous tumor or a low grade serous carcinoma, (29, 30) and in these, areas of high grade carcinoma are
admixed with areas of borderline tumor or low grade carcinoma.

The histologic classification of high-grade ovarian carcinomas is of controversial. Some authorities think that a diagnosis of high-grade serous carcinoma is only appropriate for predominantly papillary tumors with minor glandular or solid areas. (31) Some who favor this view think that neither the immunophenotype nor the presence or absence of p53 mutations separates high grade serous carcinoma from high grade endometrioid carcinoma and that the distinction must be made based on the histology, such that tumors with papillae and slit like glands are high grade serous carcinomas and those where there is sheet like growth of tumor cells or glands are high grade endometrioid carcinomas. (32) Others have adopted a more inclusive classification scheme, and view most high grade carcinomas with some features of serous differentiation as types of high grade serous carcinoma, including tumors with extensive solid areas, tumors with high grade glandular areas without squamous differentiation, and some tumors with clear cell or transitional cell areas. (33-37) High grade serous carcinomas with clear cell areas are variants of high grade serous carcinoma and not mixed serous-clear cell carcinomas, and they should be classified accordingly. (38) Immunohistochemical and molecular studies support the concept that these are variants of high-grade serous carcinoma and diagnosing them as such results in a more reproducible and useful classification system. (33) Tubal intraepithelial carcinoma is associated with high-grade serous carcinoma.

Grading of Serous Carcinoma

Grading of ovarian carcinomas has not been standardized. Silverberg and colleagues developed a “universal” grading system that they thought could be used for all types of ovarian carcinomas. (39) In their system, the grade is determined by the degree of nuclear atypia, the mitotic index, and the extent to which the tumor cells form papillae or glands. More recently, a binary grading system, in which low-grade serous carcinoma almost always falls into grade 1 of the “universal” grading system, has gained greater acceptance than the “universal” grading system because it better reflects the current concept that high and low grade serous carcinomas represent two different tumor types rather than two different grades of the same tumor. In the binary system, low-grade serous carcinoma exhibits mild to moderate nuclear atypia and 12 or fewer mitotic figures per 10 high power fields. (40) In

<table>
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<th>High Grade vs Low Grade Serous Carcinoma</th>
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<tr>
<td>Atypia</td>
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<tr>
<td>LG Serous</td>
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<td>HG Serous</td>
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BL = associated with borderline tumor elements
high-grade serous carcinoma there is marked nuclear atypia, considerable pleomorphism and often prominent macronucleoli. Mitotic activity is high; while a floor of 12 mf/10 hpf has been proposed as a lower limit of mitotic activity in high grade serous carcinoma, there was a median of 38 mf/10 hpf in one study. (40) Apart from their differences in nuclear atypia and mitotic activity, low and high-grade serous carcinoma grow in different architectural patterns, and the correct diagnosis can generally be determined at low magnification without any need for mitotic counting. Also, most low-grade serous carcinomas are associated with a borderline serous tumor, so in a primary ovarian tumor the finding of patterns of a borderline tumor favors low-grade serous carcinoma. Tumors with intermediate nuclear grades (“grade 2 serous carcinoma”) are similar in their growth patterns and molecular features to high-grade serous carcinoma and exhibit the same aggressive behavior, so they are viewed as high-grade tumors. (41)

**P53 and High Grade Serous Carcinoma**

Mutations of the TP53 gene have long been associated with high-grade serous carcinoma. TP53 mutation has been thought to be an early event in serous carcinogenesis, a point that has been re-emphasized by recent studies of a p53 positive putative cancer precursor in the fallopian tube known as the p53 signature lesion. (42) p53 signatures show positive immunostaining for p53 and they have TP53 mutations. The p53 signature is currently viewed as a non-obligate premalignant condition, illustrating just how early in the carcinogenic process TP53 mutations may occur. Recent studies of high-grade serous carcinoma reveal a high frequency of TP53 mutations in such tumors. Yemelyanova et al studied 43 high-grade serous carcinomas; only 6 lacked TP53 mutations. (43) Ahmed and colleagues found TP53 mutations in 96.7% (119/123) of patients with advanced stage high-grade serous carcinomas. (2) The Cancer Genome Atlas Research Network analyzed whole exome DNA sequences in 316 high-grade high stage serous carcinomas and found that 96% had TP53 mutations. (44) Thus, almost all high-grade serous carcinomas have TP53 mutations. The high and rather consistent frequency of TP53 mutations is somewhat surprising, since I would have expected that variations in the way high-grade serous carcinoma is diagnosed and classified to impact the percentage of positive cases.

High-grade serous carcinoma frequently shows immunohistochemical evidence of the presence of a p53 mutation. This can be manifest as diffuse strong staining of nearly all tumor cell nuclei. (45) In some cases, the p53 mutation results in the complete absence of p53 protein, in which case there is no staining in any tumor cell nuclei. Both of these results are considered positive test results. Tumors that do not have a p53 mutation generally show weak to moderate

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<th>Interpretation of p53 Immunostaining</th>
<th>Test Result</th>
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<tr>
<td>Diffuse strong positive nuclear staining (Positive staining in &gt; 50% of tumor cell nuclei and usually in &gt; 80%)</td>
<td>Positive</td>
</tr>
<tr>
<td>Patchy weak staining in &lt; 50% of tumor cell nuclei</td>
<td>Negative</td>
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<tr>
<td>Absolutely no staining in any tumor cell nuclei</td>
<td>Positive</td>
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staining in some tumor cell nuclei. In one study, there was complete absence of p53 expression in 30.3% of cases, focal expression in 12.0% and overexpression in 57.7%, (46) and similar results were observed in another study. (43) By recognizing the patterns of positive staining pathologists can identify which ovarian cancers are likely to have TP53 mutations and hence which are best classified as high-grade serous carcinomas. Among patients with probable TP53 mutations, those with complete absence of expression had an increased risk of recurrence compared with those with overexpression. (46) Other markers that are generally positive in high-grade serous carcinoma, and that complement p53 as immunohistochemical markers of high-grade serous carcinoma, are p16 and HGMA2. A positive stain for p16 in this context is diffuse strong staining of the cytoplasm and nuclei of all or nearly all tumor cells. (47-49) The range of staining that should be expected for HGMA2 is less well worked out; in one study about two thirds of high grade serous carcinomas showed moderate to strong nuclear staining in 40-100% of tumor cells. (50) Low grade serous carcinoma, in contrast, rarely shows the type of strong staining for p53 or p16 seen in high grade serous carcinoma, (45, 48, 51, 52) and these markers are generally negative in borderline and benign tumors as well.

BRCA Mutations and High Grade Serous Carcinoma

High-grade serous carcinoma is the type of ovarian cancer most associated with mutations in the BRCA genes. Large, clinically detected tumors in BRCA mutation carriers are mostly high-grade serous carcinomas, although other types of ovarian cancer occasionally occur in these patients. (53) In the range of 10-15% of ovarian carcinomas occur in women with germline mutations of BRCA1 or BRCA2. In a recent analysis of 1342 unselected Canadian women 176 or 13.3% carried a mutation (107 BRCA1, 67 BRCA2, and 2 with mutations in both genes). (54) The hereditary breast and ovarian cancer syndrome, caused by mutations in these genes, is inherited in an autosomal dominant fashion. The lifetime risk of a woman with a BRCA mutation developing breast cancer is 50-80% and the lifetime risk of developing ovarian cancer is 30-50%. The risks reported in various studies depend partly on how the cases were ascertained, with higher risks reported in studies of cancer families and lower risks in studies where carriers were identified independent of their family histories. Women with BRCA mutations or who have a family history of ovarian cancer are treated by risk-reducing bilateral salpingo-oophorectomy (RRSO), which has been shown to markedly lower the risk of developing carcinoma. (55-58) Cancer risk is not eliminated as these patients are still at risk for peritoneal serous carcinoma and for breast cancer, although the risk for peritoneal cancer is very low.

There are two BRCA genes, BRCA1 and BRCA2. They are classified as tumor suppressor genes. BRCA1 is located on chromosome 17 at 17q21-22 and BRCA2 is located on chromosome 13 at 13q12-13. The BRCA2 gene is larger, having about twice as many amino acids as the BRCA1 gene (3148 vs 1863). These genes are both involved in many intercellular processes but their main function is to protect the genome from double-stranded DNA damage during DNA replication. BRCA1 is involved in checkpoint activation and DNA repair and both BRCA1 and BRCA2 are involved in homologous recombination as a means to repair double stranded DNA breaks. (59) When BRCA is deficient, double stranded breaks cannot be repaired by the error free homologous recombination process, and they are instead repaired by alternate methods, such as end joining and single strand annealing, that lead to genomic instability resulting in the cancer predisposition that is associated with BRCA loss.
BRCA associated ovarian cancers theoretically might be more responsive to chemotherapy with drugs like cisplatin that damage DNA, since their tumor cells are less able to repair the DNA breaks that such drugs cause. In addition, BRCA associated cancers may be amenable to therapy with PARP (poly (ADP-ribose) polymerase) inhibitors, the activity of which are dependent on loss of BRCA function. In recent analyses, conflicting results have been published. Analysis of the Cancer Genome Atlas Project data indicated that patients with BRCA1 associated tumors were likely to be slightly younger than those with BRCA2 or wild type cancers. Patients whose tumors had somatic mutations had the same clinical characteristics as patients with wild type tumors. Among those with germline mutations, only patients with BRCA2 mutations had improved survival and better response to platinum based chemotherapy. (60) On the other hand, patients with BRCA1 inactivation due to germline mutations or promoter hypermethylation had the same outcome as those with wild type BRCA. The authors of another study, which pooled data from 26 studies, concluded that women with BRCA1 and BRCA2 germline mutations both had improved survival relative to those with wild type BRCA, although those with BRCA2 mutations appeared to have the best survival. {Bolton, 2012 #36408}

Loss of BRCA function is often due to a germline mutation in either BRCA1 or BRCA2, but it can also be due to somatic BRCA mutations and in the case of BRCA1, to promoter hypermethylation. (61) Regardless of the cause of BRCA dysfunction, recent pathologic studies suggest that a combination of histologic findings may predict that a given ovarian tumor is BRCA related. One set of histologic criteria was published by Soslow et al, {Soslow, 2012 #36680} and another was published in abstract form only by Fujiwara et al. Histologic features that may be BRCA related include serous/undifferentiated histologic type; solid, pseudoendometrioid or transitional growth patterns; marked atypia and giant bizarre nuclei; numerous tumor infiltrating lymphocytes; and a very high mitotic rate. According to these authors, combinations of these features, when present, may be indicative of BRCA dysfunction, but it is not clear that the histologic criteria differentiate tumors with BRCA1 mutations from those with BRCA2 mutations, nor do the criteria differentiate tumors with germline mutations from those with BRCA dysfunction due to somatic mutations or promoter hypermethylation.

| Causes of BRCA Loss of Function |  |
|---------------------------------|  |
| Germline BRCA mutation          | 10-15% |
| Somatic BRCA mutation           | 5-10%  |
| Promoter Hypermethylation       | 30%    |
| Other (microRNA, etc)           | ?      |

Fallopian Tube Surprise

While small tumors are occasionally identified in the ovaries, most of the intraepithelial and early invasive serous carcinomas detected in RRSO specimens have been found in the fallopian tubes.(62-69) Tubal intraepithelial carcinoma is less common in salpingectomy specimens from patients without a BRCA mutation, but it is occasionally detected; in one study tubal intraepithelial carcinoma was found in 8% of patients with a BRCA mutation and in 3% of patients with no mutation. (69) Interestingly, complete sectioning of the fallopian tubes in patients with a clinical diagnosis of a primary ovarian or primary peritoneal serous carcinoma, regardless of whether it occurs in a woman with a germline BRCA mutation, frequently reveals foci of serous tubal intraepithelial carcinoma or small apparently primary foci of invasive serous carcinoma of the fallopian tube. (66, 70-72) This has led to the hypothesis that many, if not all, serous carcinomas of the ovary and peritoneum are
actually metastases from tubal tumors, not primary neoplasms of the ovary or peritoneum. (70, 73, 74) Rare high-grade serous carcinomas appear to arise from a low grade serous carcinoma or a borderline serous tumor. (30) The histogenesis of serous carcinoma of the ovary and its relationship to tumors of the fallopian tube is an area of active research.

**Immunohistochemistry of Serous Tumors**

All serous tumors have immunohistochemical features in common. They usually show positive staining for cytokeratin 7 and they do not stain for cytokeratin 20 or CDX2. (75) They show variable staining for estrogen and progesterone receptors, with less staining being present in high-grade serous carcinoma than in lower grade serous tumors. Borderline serous tumors and serous carcinomas generally show membrane staining for OC-125. (76) One of the most helpful features of serous tumors is that they show nuclear staining for WT-1. (77-80) This helps differentiate them from other types of ovarian tumors, such as endometrioid and clear cell carcinomas. Staining for WT-1 is also helpful in differentiating ovarian serous carcinoma from endometrial serous carcinoma, which has a similar microscopic appearance but is less likely to stain for WT-1. (77, 81, 82) Borderline serous tumors and low grade serous carcinomas are more likely to show nuclear staining for PAX2 than are high grade serous carcinomas; variable strong staining is generally seen in the former categories of serous tumors while staining is typically absent in high grade carcinomas. (83) PAX8 appears to be a reliable marker of female genital tract tumors, and it is almost always positive in serous tumors, although staining can vary in extent and intensity. (84-86) HMGA2 is another marker that is positive in a majority of high-grade serous carcinomas but uncommon in other types of ovarian cancer. (50) Patients with high-grade serous carcinoma are frequently treated with chemotherapy, either after or before surgery. The postchemotherapy immunophenotype is similar to that of untreated serous carcinoma. (23)

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<thead>
<tr>
<th>Immunohistochemical Staining in High Grade Serous Carcinoma</th>
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<tbody>
<tr>
<td>CK7</td>
<td>+</td>
</tr>
<tr>
<td>CK20</td>
<td>-</td>
</tr>
<tr>
<td>PAX8</td>
<td>+</td>
</tr>
<tr>
<td>CA125</td>
<td>+</td>
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<tr>
<td>WT-1</td>
<td>+</td>
</tr>
<tr>
<td>p53</td>
<td>Diffuse strong positive or completely negative</td>
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<tr>
<td>p16</td>
<td>Diffuse strong positive</td>
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<tr>
<td>HMGA2</td>
<td>+</td>
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<tr>
<td>PAX2</td>
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**References**


Case 2

Borderline Serous Tumor, Micropapillary Type

Clinical History: None provided.

Gross Pathology: The left ovary was 29 cm in maximum diameter. The surface was smooth to finely granular but there were areas of surface papillary growth measuring up to 5 cm in maximum dimension. Most of the cyst lining was smooth, but areas of soft tan papillary growth up to 2 cm were present in the lining. The right ovary was 10 cm in diameter. An area of papillary growth on the surface measured 8 cm in maximum dimension. The cut surface showed tan cysts up to 0.3 cm.

By report, the tumor spread beyond the ovaries to involve the omentum, the uterine serosa and the cul de sac.

Diagnosis: Borderline Serous Tumor, Micropapillary Variant.

Borderline Serous Tumor

Tumor is confined to the ovaries in most patients (60-85%). (1, 2) A minority of patients has pelvic or peritoneal implants and pelvic or abdominal lymph nodes contain tumor in up to 40% of patients. (3) Spread to parenchymal organs or outside the abdominal cavity is uncommon, although it does rarely occur. (4) Long-term survival is more than 90% for patients with all stages of disease, (1, 2, 5, 6) but the longer patients with extraovarian disease are followed the greater the percentage who will have recurrences, since many recurrences are detected more than 5 years after initial diagnosis. (7) Survival approaches 100 percent for patients with tumor confined to the ovaries (stage I). Tumor-related deaths fall into three main categories: 1) the patient develops low grade serous carcinoma and dies of carcinoma; 2) the patient develops a fatal complication of a borderline tumor, such as fibrous adhesions leading to bowel obstruction; or 3) the patient dies of a complication of treatment.

Conservative treatment is generally indicated for patients with borderline tumors, except for the small proportion whose tumors exhibit features consistently associated with aggressive behavior, such as invasive peritoneal implants or recurrence as low-grade serous carcinoma. Oncologists treat patients with invasive implants or low-grade serous carcinoma with chemotherapy although such patients tend to have long survivals regardless of therapy and tumor response to chemotherapy is less than satisfactory (i.e., available chemotherapy is less than optimal for such patients).

The standard surgical treatment for a borderline tumor is total abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy, and complete staging with resection of extraovarian tumor implants if any are present. Many women with borderline tumors are young and want to retain their childbearing capability. Unilateral salpingo-oophorectomy, or even cystectomy, can be considered as a treatment option in some circumstances, although patients treated in this way have about a 25 percent risk of recurrence in the contralateral ovary. (1, 8) Laparoscopic management has been utilized, even in women with small peritoneal implants. (9, 10) Conservative surgery to preserve fertility appears possible in some circumstances, even in
patients with extraovarian tumor spread. Patients with micropapillary borderline serous tumors are more likely than those with typical borderline serous tumors to have tumor spread beyond the ovary. When corrected for stage and implant type, however, no difference in survival has been demonstrated. (11) Whether women who have been inadequately staged during their initial operation should be restaged is controversial. The tumor stage is changed in about 15% of women who have restaging surgery, but the risk of recurrence appears to be the same in women who are restaged and those who are not. Restaging is most valuable in women with micropapillary borderline serous tumors, since they have a greater risk of having invasive peritoneal implants. Recurrences are generally detected many years after primary therapy, and recurrent disease can be slowly progressive. Recurrent tumor can be as borderline serous tumor, low grade serous carcinoma, or, rarely, high grade serous carcinoma. (12, 13) Some authors have reported an increased rate of disease progression when recurrence is as low-grade serous carcinoma. (1) Surgical resection of contralateral or extraovarian tumor deposits is the most effective treatment for women with progressive or recurrent tumors. Oncologists are not in complete agreement, but most give chemotherapy or radiotherapy only when there is progressive disease that cannot be resected. The survival rate is high, even for women with advanced stage tumors. (14)

**Gross Pathology**

Borderline serous tumors are large, usually multilocular cystic neoplasms. They are bilateral in 35–40% of cases. Coarse papillary excrescences arise from the cyst linings and, in 40–50% of cases, from the surface of the ovary. Papillary growth is a focal finding in some tumors and an extensive confluent one in others. Areas of solid growth are unusual except in the form of adenofibromatous and hemorrhage or necrosis are seldom present.

**Microscopic Pathology**

Borderline serous tumors have a characteristic pattern of papillary growth with a complex “hierarchical” branching pattern (Fig. 2-1). The papillae grow from the cyst linings into the lumina, or from the surface of the ovary. Complex papillary and glandular patterns and secondary cyst formation are typical. The papillae have fibrovascular cores that are conspicuous even in smaller branches. The papillae are lined by proliferating columnar cells that pile up into several layers. Ciliated cells are not uncommon. The cells form tufts from which clusters of cells and single cells are detached into the cyst lumens. There is low grade nuclear atypia and scattered mitotic figures are present. Cells with abundant eosinophilic cytoplasm, the “indifferent” or “metaplastic” cells, are scattered singly or in small clusters among the columnar tumor cells; they tend to be most conspicuous at the tips of the papillae. Plaques or nodules of loose fibrous tissue containing glands and papillae are occasionally noted in borderline serous tumors, either inside the tumor or more commonly on the surface of the ovary. They resemble desmoplastic peritoneal implants (see below) and have been termed “autoimplants”.

![Branching papillary growth](image_url)
Autoimplants tend to be detected in high stage tumors, but do not appear to have prognostic significance. (1, 15) The wall of a borderline serous tumor is generally thicker than that of a cystadenoma. Some tumors have a fibromatous stromal component that is prominent enough that they can be classified as borderline serous adenofibromas or cystadenofibromas.

The main microscopic finding that differentiates a serous borderline tumor from a serous carcinoma is the absence of diffuse stromal invasion in the borderline tumor. In a borderline tumor, papillae and glands that appear to be within the stroma are usually an artifact resulting from tangential cutting of complicated infoldings of the cyst lining. Such glands are not infiltrative and there is no stromal fibroblastic or inflammatory reaction around them.

**Stromal Microinvasion**

Limited foci of stromal invasion are occasionally identified in a borderline serous tumor, called microinvasion by analogy with foci of minimal invasion seen in other sites, such as the cervix. Various arbitrary size limits have been proposed for microinvasion, ranging from 3 mm to 5 mm, but in practice foci of microinvasion are almost always smaller than 3 mm. (16) A largest dimension of invasive growth of 3 mm is accordingly a reasonable maximum size limit for microinvasion. (17) Multiple foci of microinvasion are typically present, usually 2 or 3 but ranging up to 10 or even more. A number of patterns of microinvasive growth have been described. The most common is one in which small clusters and cords of cells with eosinophilic cytoplasm, round vesicular nuclei, and prominent nucleoli are haphazardly distributed in the fibrous stroma of the cyst wall or a papilla. A stromal reaction usually is not seen around the microinvasive cells. They are occasionally found within lymphatic spaces, but the clinical significance of this finding is unclear. (18) A systematic study of borderline serous tumors using sections stained with the lymphovascular endothelial marker D2-40 revealed intratumoral lymphovascular space invasion in 60% of borderline tumors with microinvasion but in none of the tumors without it. (19) In a second pattern the stroma is invaded by papillae, small glands, cribriform glands, cords of cells, or even confluent nests of tumor cells. (18) Less common patterns include complex branching micropapillae in the stroma, usually within clear clefts, and a macropapillary pattern of invasion in large papillae with thick fibrous cores, also generally surrounded by clear spaces, are lined by one or two layers of tumor cells. Intrastromal tumor cells can lie in unremarkable stroma or they can be surrounded by inflamed or myxoid fibrous stroma. When microinvasion is detected the pathologist should conduct a thorough evaluation to exclude larger areas of invasion that would be indicative of low-grade serous carcinoma. It is difficult to identify stromal microinvasion at low magnification; immunostains for cytokeratin or epithelial membrane antigen can be used to accentuate the microinvasive epithelial cells in the stroma in questionable cases.

The clinical significance of microinvasion is unclear. Most patients have been reported to have an uneventful course and an unfavorable outcome observed in a few patients has been attributed to other factors, such as invasive implants or incomplete staging. (18, 20-23) The authors of recent studies of borderline serous tumors have noted progressive disease in a few patients with microinvasion, (24, 25) and one group found microinvasion to be a significant adverse finding. (1)

The epithelium in borderline serous tumors includes eosinophilic “metaplastic” or “indifferent” cells. These cells tend to be more numerous in tumors in which microinvasion is identified, and they are often prominent in tumors removed from pregnant patients. For unknown reasons, microinvasion seems to be detected more frequently in tumors from pregnant women.
(26) Mucin secretion and stromal decidual reactions have also been noted in tumors removed from pregnant women.

**Micropapillary Variant of Borderline Serous Tumor**

The term “micropapillary serous carcinoma” was proposed for a group of proliferative serous tumors with excessive epithelial proliferation, including some non-invasive tumors in the borderline category as well as some low grade invasive serous carcinomas. (27, 28) While no one doubts that invasive micropapillary tumors are forms of low grade serous carcinoma, the nature of the non-invasive tumors is still controversial, with most gynecologic pathologists considering them to be variants of borderline serous tumors. Gene expression analysis indicates that micropapillary borderline serous tumors are more closely related to low grade serous carcinoma than to typical borderline serous tumors. (29) Clinical follow-up of patients with micropapillary serous tumors indicates that most patients with stage I tumors are cured by surgery. (30, 31) However, patients with micropapillary borderline serous tumors are more likely to have bilateral tumors, surface papillary growth, extraovarian disease and invasive implants. Survival rates appear to be similar to those for conventional borderline serous tumors, once adjusted for stage and implant type. (11, 24, 31-34) Although it remains a somewhat controversial entity, I view non-invasive micropapillary serous tumors as “high grade” or “excessively proliferative” variants of borderline serous tumors.

Grossly, micropapillary borderline serous tumors are cystic and solid tumors that average 8–9 cm in diameter. They tend to be bilateral, and to exhibit both intracystic and surface papillary tumor growth.

Microscopically, micropapillary borderline serous tumors are characterized by foci of micropapillary growth in the background of a typical borderline serous tumor. The micropapillary growth must be > 0.5 cm (an arbitrary size) for a borderline serous tumor to be classified as the micropapillary type. (30) If there is micropapillary growth < 0.5 cm the tumor is simply classified as a borderline serous tumor.

This pattern is characterized by the presence of long micropapillary tufts of epithelial cells with little or no fibrovascular stroma sprouting from bulbous fibrovascular stromal papillae, from the cyst wall. When proliferative micropapillae are tangentially sectioned, they are packed together with larger papillae resulting in a very busy histologic appearance. However, the pattern is one of proliferation into cystic spaces rather than growth into the stroma, so the proliferation is not an invasive one. In another arbitrary definition, the papillae supposedly should be five or more times longer than they are wide. Tumors with foci of cribriform growth along the surfaces of papillae are also classified as micropapillary borderline serous tumors. The tumor cells are often somewhat different from those in the background borderline serous tumor. They tend to be cuboidal, hobnail-shaped, or low columnar, rather than the taller columnar cells seen in typical borderline serous tumors. The tumor cell nuclei are uniform and there is only mild or moderate

![Fig. 2-2. Micropapillary growth.](image)
atypia. Mitotic figures are infrequent. Ciliated cells are seen less often in micropapillary borderline serous tumors than in typical borderline serous tumors.

**Peritoneal and Omental Implants**

Microscopic or macroscopic peritoneal or omental tumor implants are found in 15–30 percent of women with serous borderline tumors. These are most often small superficial excrescences measuring only a few millimeters, although larger solid or cystic implants are occasionally present. There is controversy as to whether these represent metastases from the ovarian tumor or sites of synchronous peritoneal neoplasia, although an increasing number of studies show similar genetic profiles in the ovarian and extraovarian tumors, favoring the view that they represent spread from the ovarian tumor. (35-39) Several types of implants occur.

In non-invasive epithelial implants, papillary serous borderline tumor grows on the surface of the peritoneum or in cystic spaces just beneath it. The implants are circumscribed and do not invade the underlying stroma. Often, immunostains for calretinin delineate mesothelial cells in the lining of the subsurface cysts, although the tumor cells are negative.

Non-invasive desmoplastic implants are plaques of vascular fibrous stroma that contain a few epithelial cells, small clusters of cells, or scattered small glands lined by bland epithelial cells. The implants appear plastered on to the peritoneal surface and there is no invasion into the underlying stroma.

Mixtures of these two types of non-invasive implants are common; sometimes non-invasive papillary growth overlies a desmoplastic implant or the patterns may lie side by side. Patients with non-invasive implants tend to have a favorable prognosis, although a small percentage develop progressive disease and die of tumor. (1, 7, 33) Non-invasive implants can also cause adhesions leading to bowel obstruction.

Invasive implants are rare (5–10 percent) but, together with advanced tumor stage, are the most significant adverse prognostic findings in patients with borderline serous tumors. (6, 11, 24, 34, 40) Some studies are difficult to evaluate because the authors did not use standard definitions of invasive implants. No invasive implants were found in one hospital-based study of 57 patients that did not include any consultation cases, indicating that practicing pathologists will not see invasive implants very often. (41) The distinguishing features of invasive implants are 1) an infiltrative pattern of growth into the surrounding tissues and 2) abundant epithelium, often in the form of micropapillae surrounded by clear clefts. Invasive implants essentially have the microscopic appearance of low grade serous carcinoma. In practice, I like to use the term “invasive implant” for microscopic foci of invasive growth most typically seen in the omentum of patients with borderline serous tumors in the ovary, and “low grade serous carcinoma” for macroscopic amounts of invasive growth as seen in the ovaries or the peritoneum/omentum.

**Classification of Peritoneal/Omental Implants of Borderline Serous Tumors**

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
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<tbody>
<tr>
<td>Non-invasive epithelial implant</td>
<td></td>
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<tr>
<td>Non-invasive desmoplastic implant</td>
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<tr>
<td>Indeterminate implant</td>
<td></td>
</tr>
<tr>
<td>Invasive implant (generally viewed as a form of low grade serous carcinoma)</td>
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</table>
classify implants. When clear cut invasion is not present the term “indeterminate” implant is often used. Pathologists need to realize that a diagnosis of “invasive implant” generally leads to chemotherapy. Since chemotherapy in this setting is not particularly effective, I prefer to be conservative with the diagnosis of invasive implants; I like to see tissue invasion as well as increased cellularity before making such a diagnosis.

**Pathologic Findings in Lymph Nodes**

Tumor is present in pelvic or para-aortic lymph nodes in up to a third of patients with advanced borderline tumors. (3, 43) There are two main patterns of lymph node involvement. In one, the tumor grows in a branching papillary pattern similar to that observed in a primary ovarian borderline serous tumor. In the other, tumor cells are present singly or in small nests in the subcapsular sinuses. Lymph node involvement by hyperplastic mesothelial cells is a rare mimic of the latter pattern. (44) This is a very rare finding that I have never encountered. The presence of peritoneal mesothelial hyperplasia, the appearance of the cells, and positive immunostaining for mesothelial markers such as calretinin differentiate hyperplastic mesothelial cells from tumor cells. A rare but prognostically unfavorable pattern of lymph node involvement by borderline serous tumor is one in which the lymph node parenchyma is replaced by a nodular proliferation of tumor cells growing in reactive fibroblastic stroma. This pattern represents low grade serous carcinoma in the lymph node and is similar in appearance to an invasive peritoneal implant. Borderline serous tumors mainly involve intra-abdominal lymph nodes, but tumor is rarely found in extra-abdominal lymph nodes, either at the time the ovarian tumors are removed or at some later time. (4, 45)

Epithelial inclusions lined by low columnar cells, many of which are ciliated, are found in the pelvic or para-aortic lymph nodes in 5–25% of patients who are operated on for uterine cancers. These inclusions, which are called benign epithelial inclusions or endosalpingiosis, are much more common in patients with borderline serous tumors than in the general population. It has been proposed that they represent a precursor lesion or a type of lymph node involvement by a borderline serous tumor. (3, 46, 47) Some pathologists have proposed that lymph node involvement by borderline serous tumors represents synchronous neoplasia arising in epithelial inclusions rather than metastases from the ovarian tumor. For staging purposes the inclusions are viewed as benign and not as lymph node involvement. Regardless of its origin, lymph node involvement by a borderline serous tumor is most often found in women who have peritoneal or omental implants and it does not appear to be an independent adverse prognostic finding. (3, 48, 49) Foci of low grade serous carcinoma in a lymph node on the other hand are an unfavorable finding. (3)

**Low Grade Serous Carcinoma**

Low-grade serous carcinoma is relatively uncommon, accounting for no more than 10% of serous carcinomas. (50) In a large population based study only 3.4% of ovarian cancers were low grade serous carcinomas. (51) Although clearly of serous type, this tumor is now recognized as a distinct type of ovarian carcinoma and it is no longer viewed simply as the low grade end of a continuum with high grade serous carcinoma at the most malignant end of the spectrum. Low-grade serous carcinoma has a different molecular basis than high-grade serous carcinoma. It is characterized by BRAF or KRAS mutations, with the latter the most common, rather than TP53 mutations. (52) The DNA ploidy pattern of low grade serous carcinoma is more similar to that of borderline serous tumor than high grade serous carcinoma. (53) Low grade serous carcinoma has
been proposed to arise via stepwise progression of a serous tumor from benign to borderline to low-grade carcinoma. (54) Low-grade serous carcinoma is typically intermixed with areas of borderline serous tumor, supporting its origin from the borderline tumor. Low-grade serous carcinoma does not appear to be linked to BRCA mutations. (55) Low-grade serous carcinoma occurs in younger patients than high-grade serous carcinoma (average age in the mid-40’s) and the clinical course is usually more indolent. The median survival is considerably greater than for patients with high-grade serous carcinoma; (56) in the largest series published to date the median overall survival of patients with stage II-IV low-grade serous carcinoma was more than 80 months. (57) Although women with low-grade serous carcinoma tend to have slowly progressive disease, it is difficult to treat. Primary and recurrent low grade serous carcinoma tends to be resistant to standard ovarian cancer chemotherapy regimens. (58, 59) The clinical behavior of low-grade serous carcinoma that develops as a recurrence of a borderline serous tumor appears to be similar to that of de novo low-grade serous carcinoma. (60)

Low-grade serous carcinoma cells are cuboidal to low columnar and they have relatively uniform round to oval nuclei with evenly distributed chromatin and small nucleoli. There is mild to moderate nuclear atypia, and the mitotic rate is low (<12/10 hpf). Crowded glands, simple or complex micropapillae, and solid or cribriform nests of tumor cells infiltrate fibrous stroma (Fig. 2-3). (54) Less common patterns of invasion include a macropapillary pattern in which large papillae with broad stromal cores lined by tumor cells invade the stroma, (61) a solid pattern of growth and a single cell pattern of invasion. (62) Variable amounts of mucin are present, in tumor cell cytoplasm, in gland lumens, or in the stroma. Clusters of tumor cells are often surrounded by clefts or clear spaces. Zones of invasive carcinoma may be mixed with zones of serous borderline tumor of the typical or more often the micropapillary type. (56) Distant metastases from an ovarian low grade serous carcinoma, such as those to lymph nodes, breast or to the mediastinum, can be difficult to diagnose because they can mimic borderline serous tumors or other types of carcinomas. (63, 64)

Psammoma bodies are small laminated calcifications that form around products of cellular degeneration. They are often seen in serous tumors, particularly serous carcinomas, and are occasionally numerous. Rarely, psammoma bodies are so numerous in a low-grade serous carcinoma that they obscure the epithelial elements of the tumor. Carcinomas with innumerable psammoma bodies are called serous psammocarcinomas. They are extremely rare, but they seem have a favorable prognosis when they can be completely removed. (65) Similar tumors can arise as primary peritoneal neoplasms. The prognosis for a high-grade serous carcinoma with numerous psammoma bodies is unfavorable and such tumors should not be diagnosed as psammocarcinomas. Psammoma bodies are suggestive of a serous tumor, but they are also found in other types of tumors, especially those with papillary growth, and in association with non-neoplastic conditions such as epithelial inclusion cysts and endosalpingiosis.
References

Case 3
Clear Cell Carcinoma

Clinical History: The patient was a 72 year old woman admitted to the hospital for evaluation of a large pelvic mass. A pelvic examination performed 8 months prior to admission had not revealed a mass. She was treated by TAHBSO followed by instillation of radioactive chromic phosphate into the abdominal cavity.

Gross Pathology: The left ovary was enlarged and cystic, measuring 12 cm in diameter. The capsule was intact, but there were adhesions to the uterus. The cut surfaces revealed many cysts as well as solid areas. The cysts were up to 10 cm and solid areas were up to 3 cm. Small dark pink velvety tumor masses lined the inner surfaces of the cysts.

Diagnosis: Clear Cell Carcinoma

Clear Cell Tumors

Clear cell tumors were for many years thought to be of mesonephric origin, but were shown by Scully and Barlow to arise from endometriosis or from the surface epithelium or epithelial inclusions. (1) Most clear cell tumors are carcinomas, but benign and borderline clear cell tumors also occur. (2-6) Women with borderline clear cell tumors generally have a favorable prognosis. Rare patients with borderline clear cell tumors have developed metastases or died of their tumors. (6, 7) While borderline clear cell tumors, even those with intraepithelial carcinoma, are thought to have a favorable prognosis, it is important to keep in mind that few cases have been studied, and even fewer have had long follow-up, so the behavior of such tumors should be viewed as incompletely determined.

Clear Cell Tumors of the Ovary

<table>
<thead>
<tr>
<th>Clear Cell Tumors of the Ovary</th>
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<tbody>
<tr>
<td>Clear Cell Adenofibroma</td>
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<tr>
<td>Borderline Clear Cell Adenofibroma</td>
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<tr>
<td>Borderline Clear Cell Adenofibroma with Intraepithelial Carcinoma</td>
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<tr>
<td>Clear Cell Carcinoma</td>
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</table>

More than 90 percent of clear cell tumors are carcinomas. Clear cell carcinoma comprises from 5 to 12 percent of ovarian cancers in North America and other western countries, with the highest percentages having been reported most recently. (8) Clear cell carcinoma is often detected at an early stage, so studies that deal only with advanced stage tumors contain a lower percentage of clear cell carcinomas. Clear cell carcinoma constitutes a higher percentage of epithelial ovarian cancers (20-25 percent) in Japan than in western countries. (9, 10) Survival rates that are favorable, or at least equivalent to those observed with other types of ovarian carcinoma have been found in many studies of clear cell carcinoma, particularly for patients with early stage tumors. (11-14) The trend recently, however, has been to view clear cell carcinoma as an unfavorable histologic type because of a worse prognosis in advanced stages compared to other common epithelial tumors, and a poor response to platinum-based chemotherapy. (9, 15-18) Nevertheless, current NCCN guidelines call for treatment of patients with clear cell carcinoma, even in stage IA, with 3-6 cycles of IV taxane/carboplatin therapy. Recurrent clear
Clear cell carcinoma appears to be particularly resistant to chemotherapy and it is accordingly difficult to treat. (19)

Several paraneoplastic syndromes occur in women with clear cell carcinoma. (20) Clear cell carcinoma is more likely than other types of epithelial carcinoma to be associated with hypercalcemia, (21) and women with clear cell carcinoma also are more likely to have thromboembolic events such as deep venous thrombosis and pulmonary emboli. (22)

There is a strong association between clear cell carcinoma and endometriosis, with clear cell carcinoma patients often having endometriosis in the ovaries or elsewhere in the pelvis. (23-25) Atypical endometriosis has been proposed as a possible precursor of clear cell carcinoma, (24, 26). Recently, mutations in the ARID1A gene have been detected in ovarian cancers associated with endometriosis, and in endometriosis itself, including 46-57 per cent of patients with clear cell carcinoma. (27, 28). An origin in or an association with endometriosis has correlated with a more favorable outcome in some studies. (24, 29) Clear cell carcinoma appears overrepresented among women with the Lynch syndrome. (30)

**Benign Clear Cell Tumors**

Benign clear cell tumors are unilateral solid tumors. They range from small tumors 3 or 4 cm in diameter to large ones measuring up to 15 cm in diameter. Small cysts are usually visible on close inspection of the white or tan cut surfaces. Microscopically, all benign clear cell tumors are adenofibromas. (4, 6). The fibromatous stroma contains spindle-shaped fibroblasts and collagen bundles. Scattered within the stroma are small tubules or cysts lined by cuboidal or hobnail cells with clear or granular eosinophilic cytoplasm. There is no cytologic atypia or mitotic activity.

**Borderline Clear Cell Tumors**

Borderline clear cell tumors are unilateral and predominantly solid. They typically measure 10–15 cm in diameter. The cut surface is white, gray, or tan and contains small to medium sized cysts. Borderline clear cell tumors, like the benign ones, are all adenofibromas. (4, 6) Two tumors reported as purely cystic borderline clear cell tumors might also be interpreted as cysts lined by intraepithelial clear cell carcinoma. (31) Tubules and cysts are irregularly distributed in a fibrous stroma. The tubules and cysts are lined by cuboidal or hobnail cells that have clear or eosinophilic cytoplasm. Occasionally, the epithelium is stratified or tufted, or grows as small solid circumscribed nests. Some tumor cells exhibit mild to moderate nuclear atypia and there may be scattered mitotic figures (usually <1 per 10 high power fields). Rare clear cell tumors grow in a parvilocular pattern in which cysts lined by clear cells are surrounded by stroma. Most of such tumors exhibit sufficient atypia or mitotic activity to be classified as borderline tumors. The absence of stromal invasion or confluent growth differentiates clear cell adenofibroma from clear cell carcinoma. Non-invasive clear cell adenofibromas with significant nuclear atypia or mitotic activity can be classified as borderline tumors with intraepithelial carcinoma. As mentioned above, very few of these have been reported and their clinical behavior is accordingly not well defined. The diagnosis of a benign or borderline clear cell tumor should be made only after thorough histologic study, since bland-appearing areas are often present in clear cell carcinoma.
Clear Cell Carcinoma

Gross Pathology

Clear cell carcinomas are usually large tumors, ranging from 10 to 30 cm in diameter. They can be solid or cystic with solid gray-tan nodular areas in their walls. The cut surfaces are soft and tan or gray-white. Some clear cell carcinomas may arise from clear cell adenofibromas like Case 3, while others appear to arise from endometriosis. In one study, cystic tumors were more likely to be diagnosed at an early stage, to be associated with endometriosis, to exhibit a papillary growth pattern and to have a more favorable outcome than solid adenofibromatous tumors, while in another study adenofibromatous tumors were more likely to be of low stage at diagnosis and to have a more favorable prognosis. The significance, if any, of the various growth patterns is thus unclear and needs further study. Tumors that are confined to the ovaries (stage I) are usually unilateral, but when tumors of all stages are considered, about 30 percent are bilateral.

Microscopic Pathology

Clear cell carcinoma grows in a variety of patterns and contains various cell types, including, usually, cells with clear cytoplasm. The diagnosis is not based simply on the presence of cells with clear cytoplasm, as many types of ovarian tumors contain clear cells, but on the combination of the presence of characteristic cell types growing in the typical patterns of clear cell carcinoma.

Clear cell carcinoma contains clear cells, cells with granular eosinophilic cytoplasm, and hobnail cells; usually, a mixture of the various cell types is present. Clear cells are flat, cuboidal, low columnar, or polygonal and have abundant clear cytoplasm, central often fairly uniform vesicular nuclei and, usually, conspicuous nucleoli. PAS stains and electron microscopic studies reveal that they contain abundant cytoplasmic glycogen, which accounts for the cytoplasmic clearing. Cells with eosinophilic cytoplasm are similar in size, shape, and nuclear features to the clear cells, but they have granular eosinophilic cytoplasm. Hobnail cells are columnar and have either granular eosinophilic or clear cytoplasm. Their most dramatic feature is their hyperchromatic apical nuclei that bulge into the lumina of cysts or tubules. Mitotic activity tends to be lower in clear cell carcinoma than in other types of ovarian carcinoma (average, about 5 mf/10 hpf); the low proliferation rate has been proposed as a possible reason for the poor response to chemotherapy.

<table>
<thead>
<tr>
<th>Cytologic Features of Clear Cell Carcinoma</th>
<th>Cell Types</th>
<th>Cytoplasm Types</th>
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<tbody>
<tr>
<td></td>
<td>Flat</td>
<td>Clear</td>
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<tr>
<td></td>
<td>Cuboidal</td>
<td>Eosinophilic</td>
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<tr>
<td></td>
<td>Low Columnar</td>
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<tr>
<td></td>
<td>Polygonal</td>
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<tr>
<td></td>
<td>Hobnail</td>
<td>Oxyphilic</td>
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The rare oxyphilic clear cell carcinoma is composed predominantly of large polygonal cells with abundant eosinophilic cytoplasm. Thorough sampling is necessary to reveal areas that are more typical of clear cell carcinoma, thereby establishing the diagnosis.

There is typically a mixture of growth patterns in clear cell carcinoma. The tumor cells line short stubby papillae with fibrous or sometimes hyaline cores in the papillary pattern (Fig. 3-1) and line tubules or cysts in the tubulocystic pattern (Fig. 3-2). Occasionally, long branching papillae are present, but marked stratification or tufting of tumor cells on the surfaces of papillae is not a feature of clear cell carcinoma. Ring-like tubules lined by cuboidal cells with clear cytoplasm and filled with eosinophilic secretions are particularly characteristic. There can be areas of solid growth composed of polygonal cells with clear or eosinophilic cytoplasm. Adenofibromatous clear cell carcinomas contain crowded angulated and infiltrative tubules growing through fibrous stroma. Compared to what is seen in a borderline clear cell adenofibroma, the tubules are more crowded and infiltrative in carcinoma, and there are usually typical areas of confluent tubulocystic or papillary growth, or sheets of tumor cells.

<table>
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<tr>
<th>Histologic Patterns of Clear Cell Carcinoma</th>
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<tbody>
<tr>
<td>Tubulocystic</td>
</tr>
<tr>
<td>Not glands lined by columnar cells</td>
</tr>
<tr>
<td>Papillary</td>
</tr>
<tr>
<td>Not papillae with surface stratification or tufted growth</td>
</tr>
<tr>
<td>Solid sheets of polygonal cells</td>
</tr>
<tr>
<td>Adenofibromatous</td>
</tr>
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</table>

A few ancillary findings can help suggest the possibility of clear cell carcinoma. Eosinophilic hyaline globules are often scattered among the tumor cells. Amorphous eosinophilic hyaline material is a frequent finding in the stroma and the cores of the papillae. The hyaline material is PAS positive and appears to be basement membrane-like material, based on its ultrastructural appearance and immunohistochemical staining for type IV collagen and laminin.
Clear cell carcinoma can be graded using the universal grading system proposed by Silverberg and his colleagues, but histologic grading has not proven to be of prognostic value in most studies. (38, 39) Clear cell carcinoma is accordingly not graded.

Clear cell carcinoma is strongly associated with endometriosis, either within the involved ovary or elsewhere in the pelvis, and occasional examples of clear cell carcinoma arise directly from endometriosis. The percentage of cases associated with endometriosis, including atypical endometriosis, approaches or exceeds 50 percent in some series. (24, 29, 40, 41)

### Immunohistochemistry and Molecular Pathology of Clear Cell Tumors

Clear cell tumors share many immunohistochemical features with other primary epithelial tumors of the ovary. Clear cell carcinoma is typically positive for cytokeratin, cytokeratin 7 and epithelial membrane antigen. (42, 43) It stains for CD15 and is usually cytokeratin 20 negative. (42) Staining for estrogen and progesterone receptors is minimal or totally absent. (43, 44) A new antibody, hepatocyte nuclear factor-1beta (HNF-1β) has emerged as a sensitive marker for clear cell tumors, particularly clear cell carcinoma. (44-46) HNF-1β is a nuclear antigen, and diffuse strong nuclear staining is observed in more than 80% of clear cell carcinomas. A panel that includes HNF-1β, WT-1, and ER may help differentiate between clear cell carcinoma and serous carcinoma. (44) HNF-1β is likely to be positive in clear cell carcinoma, while WT-1 and ER are likely to be positive in serous carcinoma. HNF-1β has been reported as being highly specific for clear cell tumors and unlikely to stain serous carcinoma (95% specific). (44) However, the most commonly used antibody is a polyclonal antibody and we have recently begun to observe positive staining in cases of serous and endometrioid carcinoma. Therefore, diffuse strong nuclear staining for HNF-1β supports a diagnosis of a clear cell tumor, but I do completely rely on positive staining to exclude a serous or endometrioid tumor. Diffuse positive nuclear staining for p53 has been reported by some, (42) but not by others. (47, 48) We find that staining for p53, when present, is usually irregular in distribution and less intense than is observed in serous carcinoma, in which nuclear staining tends to be strong and diffuse when present. Clear cell carcinoma frequently contains waxy eosinophilic hyaline material in its stroma. This material appears to be basement membrane material based on its electron microscopic appearance and positive staining for laminin and type IV collagen. (49, 50) In summary, the typical clear cell tumor immunophenotype is cytokeratin 7, EMA, and hepatocyte

<table>
<thead>
<tr>
<th>Stain</th>
<th>Result</th>
<th>Pattern</th>
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<tbody>
<tr>
<td>CK7</td>
<td>Positive</td>
<td>Cytoplasm/membranes</td>
</tr>
<tr>
<td>EMA</td>
<td>Positive</td>
<td>Cytoplasm/membranes</td>
</tr>
<tr>
<td>HNF-1β</td>
<td>Positive</td>
<td>Nucleus</td>
</tr>
<tr>
<td>ER</td>
<td>Usually negative</td>
<td>Nucleus</td>
</tr>
<tr>
<td>PR</td>
<td>Usually negative</td>
<td>Nucleus</td>
</tr>
<tr>
<td>P53</td>
<td>Negative; weak moderate staining in some tumor cells</td>
<td>Nucleus</td>
</tr>
<tr>
<td>P16</td>
<td>Negative; patchy positive staining</td>
<td>Cytoplasm and nucleus</td>
</tr>
<tr>
<td>WT1</td>
<td>Negative</td>
<td>Nucleus</td>
</tr>
<tr>
<td>PAX8</td>
<td>Positive</td>
<td>Nucleus</td>
</tr>
<tr>
<td>SALL4</td>
<td>Negative or focal and weak</td>
<td>Nucleus</td>
</tr>
<tr>
<td>Glypican-3</td>
<td>Negative or focal and weak</td>
<td>Cytoplasm</td>
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</tbody>
</table>
nuclear factor-1beta positive and estrogen receptor, progesterone receptor, WT-1 and p53 negative. (34)

Recent developments in molecular pathology have provided interesting information about clear cells carcinoma, some of use to surgical pathologists. The observation that HNF-1β is one of the genes that is upregulated in clear cell carcinoma led to the introduction of HNF-1β immunostaining as a diagnostic aid. (51) More recently, it has been observed that mutations of the tumor suppressor gene ARID1A occur in clear cell carcinoma and result in loss of ARID1A immunostaining. (27, 28) ARID1A mutations also occur in endometrioid carcinoma, but it is possible that immunostaining for ARID1A may prove helpful in the differential diagnosis with serous carcinoma and other types of ovarian tumors with clear cells. In tumors that show loss of ARID1A, the protein is also generally lost in adjacent precursors, such as atypical endometriosis, endometriosis, and clear cell adenofibromas, suggesting that ARID1A mutation, along with mutations of PIK3CA are early events in tumorigenesis. (52) Mutations are known to be present in the following genes in clear cell carcinoma: PIK3CA, KRAS, ARID1A and PPP2R1A. (28)

**Differential Diagnosis**

A wide variety of tumors that can involve the ovary contain clear cells and can be considered in the differential diagnosis of clear cell carcinoma.

<table>
<thead>
<tr>
<th>Clear Cell Carcinoma</th>
<th>Differential Diagnosis</th>
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<tbody>
<tr>
<td>Serous Carcinoma</td>
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<tr>
<td>Borderline Serous Tumor</td>
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<td>Endometrioid Carcinoma</td>
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<tr>
<td>Yolk Sac Tumor</td>
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<td>Dysgerminoma</td>
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<tr>
<td>Steroid Cell Tumor</td>
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<tr>
<td>Metastatic Clear Cell Carcinoma</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td></td>
</tr>
</tbody>
</table>

Some clear cell carcinomas contain minor admixtures of endometrioid carcinoma or other types of surface epithelial carcinoma, but this is a diagnosis that should be made with caution. Other types of epithelial ovarian tumors, especially serous carcinoma and endometrioid carcinoma, occasionally contain clear cells and pathologists are frequently tempted to diagnose them as having a component of clear cell carcinoma. (53) The diagnosis of clear cell carcinoma should only be made if there is a combination of the characteristic cytologic features, architectural growth patterns, and immunophenotype, rather than simply the presence of tumor cells with cytoplasmic clearing. Carcinomas with overlapping features of clear cell carcinoma and serous carcinoma are occasionally encountered; these are best regarded as variants of serous carcinoma. (54) Papillary clear cell carcinomas are occasionally mistaken for borderline serous tumors, a significant diagnostic error. (55) Features that suggest the correct diagnosis include a unilateral tumor, non-branching papillae, a monotonous tumor cell population, the presence of more characteristic patterns of clear cell carcinoma, and an association with endometriosis.

Yolk sac tumor and clear cell carcinoma were thought for many years to be variants of the same tumor type, the mesonephroma, so it is not surprising that yolk sac tumor is sometimes
considered in the differential diagnosis of clear cell carcinoma. Knowledge of the clinical details of the case, especially the patient’s age and the serum alpha-fetoprotein level is helpful in arriving at the correct diagnosis. Clear cell carcinoma is generally alpha-fetoprotein negative while yolk sac tumor is positive although staining for alpha-fetoprotein can be weak or focal. (56) Other recently introduced stains for yolk sac tumor such as glypican-3 and SALL4 tend to be negative in clear cell carcinoma or to stain only focally and weakly. (57, 58) Keep in mind that both clear cell carcinoma and yolk sac tumor show positive nuclear staining for HNF-1β. Clear cell carcinoma stains strongly for epithelial markers such as CD15, (59) EMA and cytokeratin 7, (56), which are generally negative in yolk sac tumor, although exceptions occasionally occur. (60)

Metastatic clear cell renal cell carcinoma is generally not a diagnostic issue as renal cell carcinoma rarely spreads to the ovaries. (61, 62) Ovarian clear cell carcinoma shows positive staining for CK7 and CA125 while metastatic clear cell renal cell carcinoma is rarely CK7 positive, (43, 63, 64) although it shows positive staining for CD10. (65) Renal cell carcinoma antigen (RCC), and PAX2 are generally positive in metastatic renal cell carcinoma, (43, 66) but ovarian clear cell tumors also occasionally stain for these markers. (67) Both renal and ovarian clear cell carcinomas show positive nuclear staining for PAX8 and hepatocyte nuclear factor-1beta. (68)

References


Case 4
Metastatic Adenocarcinoma

Clinical History: The patient was a 43-year-old Mexican woman. She had bilateral ovarian tumors and peritoneal metastases. Review of the history revealed that the patient had had a gastric resection 7-8 years previously in Mexico, but the pathologic findings were unknown.

Gross Pathology: The ovaries were solid and moderately enlarged, the left measuring 7 cm and the right 8 cm in maximum diameter. The cut surfaces were rubbery and lobulated, with gray-white glistening trabeculated cut surfaces.

Diagnosis: Metastatic adenocarcinoma, signet ring cell type, compatible with a gastric primary (Krukenberg Tumor).

Metastatic Tumors in the Ovary
Metastatic tumors involve the ovaries more often than any other part of the female genital tract. It is often said that 5–10% of all malignant ovarian tumors are metastatic, so the possibility that an ovarian tumor might be metastatic always has to be considered, particularly if the gross or microscopic pattern is unusual for a primary tumor. Possible metastatic pathways to the ovary include retrograde lymphatic spread, transperitoneal spread, and hematogenous metastasis. (2) Based on the appearance of the involved ovaries and on clinical and operative findings, lymphatic and transperitoneal spread account for most ovarian metastases, particularly from primary sites within the abdomen. (3) Adenocarcinomas of the breast, large intestine, endometrium, and stomach are the most common primary sites, but a wide variety of malignant tumors can metastasize to the ovaries. (1) These include cancers of the cervix, (4-6) appendix, (7, 8) pancreas, (9, 10) bile duct and gallbladder, (11, 12) liver, (13, 14) kidney, (15-18) urinary tract, (19) and lung, (20) as well as melanoma, (21, 22) malignant lymphoma and various types of soft tissue and gastrointestinal sarcomas. (21, 23-26) It is often difficult to differentiate metastatic endometrial adenocarcinoma in the ovary from synchronous primary endometrioid adenocarcinomas of the endometrium and ovary.

Clinically significant ovarian metastases are most often detected in women who are already known to have an extraovarian cancer, most often one of the colon or rectum. (27) The average time between diagnosis of the primary tumor and detection of the ovarian metastases is about 2 years, but the length of the interval varies and ranges from a few months to many years.

<table>
<thead>
<tr>
<th>Nongenital Primary Sites of Ovarian Metastases (1)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Site</td>
<td></td>
</tr>
<tr>
<td>Colorectal</td>
<td>32.3</td>
</tr>
<tr>
<td>Appendix</td>
<td>20.3</td>
</tr>
<tr>
<td>Breast</td>
<td>8.5</td>
</tr>
<tr>
<td>Stomach</td>
<td>6.8</td>
</tr>
<tr>
<td>Small intestine</td>
<td>6.8</td>
</tr>
<tr>
<td>Pancreas</td>
<td>5.1</td>
</tr>
<tr>
<td>Other or unknown</td>
<td>20.3</td>
</tr>
</tbody>
</table>

(28) Some patients have no history of a primary extraovarian tumor. They present with
symptoms of a pelvic tumor, such as pelvic or abdominal pain, gastrointestinal or urinary disturbances, abdominal distention, or abnormal uterine bleeding, and are at least initially thought to have primary ovarian cancer. In most such patients a locally advanced gastrointestinal primary cancer is discovered at the same time as the ovarian metastases but occasionally the primary site is only discovered months or years after the ovarian tumors are removed. Not all ovarian tumors that are detected in women with a history of cancer are metastases. For example, when a woman with a history of colorectal cancer develops a new pelvic mass the most likely diagnosis is metastatic adenocarcinoma, but primary benign or malignant ovarian tumors are discovered in a significant percentage of cases (26 percent benign and 17 percent primary ovarian cancers in one study). (28) The possibility of treating women with cancers that have a significant risk of metastasizing to the ovaries with prophylactic oophorectomy is sometimes considered, but it has not been shown to improve survival and its efficacy is at present unclear. (29, 30) The prognosis is poor for women with ovarian metastases, (27) but resection appears to lengthen survival and relieve symptoms. Some have found that ovarian metastases are more likely to develop in premenopausal women, but this has not been confirmed by others.

**Gross Pathology**

Metastatic tumors in the ovary vary in appearance depending on the primary site. Metastatic colorectal cancer is bilateral in 50–70 percent of cases. The ovarian metastases average 10–11 cm in diameter, have a smooth surface, and tend to be cystic or solid and cystic. (31-33) The cysts are unilocular or multilocular and are filled with mucin. In one study, 90% of mucinous carcinomas were correctly classified as primary or metastatic by assuming that all bilateral mucinous tumors or mucinous tumors smaller than 10 cm were metastatic, and all unilateral mucinous tumors larger than 10 cm were primary. (34) The criteria were subsequently modified by increasing the size cutoff to 13 cm, resulting in improved performance of the algorithm. (35) Metastatic colorectal and cervical adenocarcinomas proved to be the most difficult to correctly identify.

Metastatic stomach cancer usually has the appearance of a Krukenberg tumor. The ovaries tend to retain their shape but are symmetrically or asymmetrically enlarged. They are firm and have areas of nodularity on the surface. The cut surface is gray, tan, or white and edematous and honeycombed with small mucinous cysts. Most other types of metastatic tumors tend to be solid and grow as multiple nodules in the cortex and medulla, often with implants on the serosa.

**Microscopic Pathology**

General microscopic features that raise the possibility that an ovarian tumor might be metastatic include bilaterality, a multinodular growth pattern, implants on the surface of the ovary or just beneath it, numerous emboli of metastatic carcinoma in lymphatic spaces, especially in the hilum and mesovarium, a desmoplastic or unusually fibrous or myxoid stromal reaction, an unusual microscopic pattern for a primary ovarian tumor, such as a signet ring cell appearance, a colloid carcinoma, a linear so-called “Indian file” pattern of invasion, and a higher nuclear grade than would be expected for the architectural pattern (for example, high grade nuclei and frequent mitotic figures in a purely glandular tumor with low grade architecture). (36) Luteinization of the stroma occurs around some metastatic tumors, but it also occurs around primary ovarian tumors and does not necessarily imply that a tumor is metastatic. The luteinized
cells occasionally secrete sufficient amounts of estrogen or androgen to cause clinical symptoms. (37)

**Krukenberg Tumor and Metastatic Stomach Cancer**

A Krukenberg tumor is a form of metastatic adenocarcinoma, sometimes found in a young woman, in which malignant signet ring cells comprise more than 10 percent of the tumor and often infiltrate a hypercellular stroma. (38, 39) The last case of Krukenberg tumor that we saw was an unexpected finding in a 25 year old woman in who a gastric primary was subsequently detected at endoscopy. Gastric cancer is not common in young patients, but when it occurs, it occurs in women more often than me and more than 50% of young women with signet ring cell carcinoma of the stomach develop Krukenberg tumors. While the primary carcinoma is usually in the stomach, signet-ring cell carcinomas of the breast, colon, gallbladder, and other sites can also give rise to metastases of this type. Krukenberg tumors are relatively rare in the USA and Europe, but are common in populations in which there is a high incidence of gastric carcinoma, such as in Japan and in women of Japanese extraction.

Microscopically, the signet-ring cells grow as single cells, in variably sized nests or cords, or in widely scattered tubules. The malignant cells contain cytoplasmic mucin globules that compress and flatten the hyperchromatic nucleus against the cell wall (Fig. 4-1). Small polygonal or cuboidal cells with eosinophilic cytoplasm and eccentric nuclei are present in some tumors. Some Krukenberg tumors contain prominent tubular glands in addition to signet ring cells. These have been designated tubular Krukenberg tumors. (40) The hilar lymphatics often contain tumor cells, which may be cohesive or form glands. The stroma of a Krukenberg tumor is abundant, hypercellular, and focally edematous, and it may contain pools of mucin. The malignant cells can be obscured by the stroma, but they are easily be identified in sections stained for neutral mucins with PAS or mucicarmine, or with immunohistochemical stains for cytokeratin or epithelial membrane antigen. The tumor cells tend to be cytokeratin 7 positive, but staining for cytokeratin 20 and CDX2 is variable. Diffuse types of gastric carcinoma can lack membrane staining.

<table>
<thead>
<tr>
<th>General Features Suggesting that an Ovarian Tumor Could Be Metastatic</th>
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<tbody>
<tr>
<td>History of an extraovarian primary carcinoma</td>
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<tr>
<td>Clinical evidence of an extraovarian primary carcinoma</td>
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<tr>
<td>Bilateral tumors</td>
</tr>
<tr>
<td>Small size (for mucinous tumors &lt; 13 cm)</td>
</tr>
<tr>
<td>Tumor or mucin grossly present on the surface of the ovary</td>
</tr>
<tr>
<td>Microscopic implants on or just beneath the surface of the ovary</td>
</tr>
<tr>
<td>Multinodular pattern of invasive growth</td>
</tr>
<tr>
<td>Desmoplastic or myxoid stromal reaction</td>
</tr>
<tr>
<td>High nuclear grade or frequent mitotic figures but low architectural grade</td>
</tr>
<tr>
<td>Unusual histologic pattern – signet ring cells, colloid carcinoma pattern, etc.</td>
</tr>
<tr>
<td>Lymphovascular space invasion, especially in the hilum of the ovary</td>
</tr>
</tbody>
</table>

![Fig. 4-1 Metastatic signet ring cell carcinoma](image)
for e-cadherin, similar to what is seen in lobular carcinoma of the breast. It is unclear whether staining for e-cadherin, along with ER and other breast markers, might help to pinpoint the primary site if it is unknown. The e-cadherin stain was negative in this case. The reactive stromal cells may show strong staining for inhibin. Rare Krukenberg tumors have been designated as “primary Krukenberg tumors” because an extraovarian primary could not be identified. (41) Rare ovarian mucinous tumors have a component of signet ring cells and have overlapping features with a Krukenberg tumor, although they are generally not classified as such. (42) Gastrointestinal primary cancers, particularly those of the stomach, can remain undetected even after careful investigation, so it is best to consider all Krukenberg tumors to be metastatic until proven otherwise by clinical follow-up or autopsy. Nevertheless, a rare patient survives long-term after resection of a Krukenberg tumor or no primary site is identified at autopsy, raising the possibility of the existence of a very rare primary type of signet ring Krukenberg tumor. (43)

<table>
<thead>
<tr>
<th>Krukenberg Tumor Primary Sites</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>76</td>
</tr>
<tr>
<td>Colon and rectum</td>
<td>11</td>
</tr>
<tr>
<td>Breast</td>
<td>4</td>
</tr>
<tr>
<td>Gallbladder and biliary</td>
<td>3</td>
</tr>
<tr>
<td>Others</td>
<td>6</td>
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</table>

“Other” primary sites includes small intestine, appendix, pancreas, uterus, bladder, renal pelvis

Metastatic Colorectal Adenocarcinoma

Colorectal carcinoma is the cancer that most often metastasizes to the ovaries. Metastatic colorectal adenocarcinoma often simulates a primary adenocarcinoma of the ovary. (31-33, 44, 45) The typical colorectal adenocarcinoma, in which absorptive cells predominate and goblet cells are inconspicuous, mimics endometrioid adenocarcinoma. Metastatic colorectal adenocarcinoma forms large complex glands and cysts that contain necrotic debris. The debris is coarsely granular and contains nuclear fragments and inflammatory cells, resulting in a distinctive appearance, termed “dirty” necrosis (Fig. 4-2). While extensive necrosis is characteristic of metastatic colorectal adenocarcinoma, it can also be seen in a primary ovarian adenocarcinoma, especially an endometrioid adenocarcinoma. The malignant cells lining the glands and cysts stratify or grow in cartwheel, garland, or cribriform patterns with foci of segmental epithelial necrosis. The degree of nuclear atypia and mitotic activity tends to be greater than is seen in an endometrioid adenocarcinoma of similar architectural grade. Findings that are typical of primary endometrioid carcinoma, such as squamous metaplasia, a focal adenofibromatous pattern of growth and adjacent endometriosis are generally absent.

Metastatic colorectal adenocarcinoma mimics primary mucinous carcinoma of the ovary when goblet cells are numerous and there is abundant mucin production. (34) Metastatic adenocarcinoma from the pancreas or biliary tract typically has a mucinous phenotype and thus also enters the differential diagnosis, (9-12, 45) as can metastases from urachal adenocarcinomas.
of the bladder. (46, 47) Metastatic mucinous adenocarcinomas exhibit the general gross and microscopic features of metastases, including surface implants and at least focally infiltrative growth, (36) but it may be necessary to compare the microscopic appearance of the metastasis with that of the primary or to perform immunohistochemical studies in order to arrive at the correct diagnosis.

Immunohistochemical stains are often essential in the differential diagnosis between a primary adenocarcinoma of the ovary and metastatic colorectal adenocarcinoma. (2, 48, 49) Immunostains for cytokeratin subtypes and CDX2 are the most useful ones. Primary adenocarcinoma of the ovary is almost always strongly positive for cytokeratin 7, (50) while colorectal adenocarcinoma is usually cytokeratin 7-negative. (33, 51) Immunostains for cytokeratin 20 and CDX2 are negative in endometrioid carcinoma. Stains for cytokeratin 20 and CDX2 are generally positive in ovarian mucinous adenocarcinoma but staining is often only weak to moderate and focal (50, 52) in contrast to the diffuse strong positive staining for cytokeratin 20 and CDX2 in most cases of metastatic colorectal adenocarcinoma. (33, 50, 53) Thus, a cytokeratin 7 positive/cytokeratin 20 negative or weak/CDX2 negative or weak immunophenotype strongly suggests a primary ovarian carcinoma, while a cytokeratin 7 negative/cytokeratin 20 positive/CDX2 positive immunophenotype strongly favors metastatic adenocarcinoma. (50, 51, 53, 54) While colonic adenocarcinoma is almost invariably cytokeratin 7 positive, some rectal adenocarcinomas and adenocarcinomas from more proximal portions of the gastrointestinal tract, including the appendix, small intestine, and stomach, can be cytokeratin 7 positive. (54-56) Other immunohistochemical stains that may help differentiate between primary and metastatic neoplasms include beta-catenin, (57, 58) which shows positive nuclear staining in some colorectal and endometrioid carcinomas, but which is usually negative in primary mucinous carcinoma; OC 125 (CA 125), which shows positive membrane staining in endometrioid carcinoma, but is usually negative in primary mucinous carcinoma and metastatic colorectal carcinoma; (59, 60) PAX8, which shows positive nuclear staining in endometrioid adenocarcinomas and often to at least a limited degree in mucinous carcinoma, but which is negative in metastatic colorectal adenocarcinoma; (61) alpha-methylacyl-CoA racemase (also known as P504S), (58) which shows granular cytoplasmic staining in some metastatic colorectal adenocarcinomas and primary mucinous adenocarcinomas, but which tends to be negative in endometrioid adenocarcinomas; and carcinoembryonic antigen (CEA), where negative or weak staining favors an ovarian primary over metastatic colorectal adenocarcinoma. (32)

<table>
<thead>
<tr>
<th>Immunohistochemistry of Metastatic Colorectal Adenocarcinoma</th>
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<td>------------------</td>
</tr>
<tr>
<td>CK7</td>
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<tr>
<td>CK20</td>
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<td>CDX2</td>
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<td>PAX8</td>
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<tr>
<td>CA125</td>
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<td>ER</td>
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Metastatic Tumors from the Appendix and Pseudomyxoma Peritonei

Metastatic tumors from the appendix are rare and account for only 1 percent of ovarian metastases. (62) Most appendiceal tumors that metastasize to the ovary are signet ring cell
adenocarcinomas that arise from the base of the mucosa and infiltrate the wall of the appendix. They contain signet ring cells, tubules, and goblet cells in varying proportions. (7, 8) Carcinomas with a prominent tubular component lined by goblet cells have been classified as goblet cell or mucinous carcinoids, or as mixed carcinoid-adenocarcinomas in the past. (63) Such tumors are biologically aggressive and do not always exhibit the typical staining pattern of a carcinoid tumor (argentaffin positive and immunoreactive for chromogranin and/or synaptophysin). Such metastases often fulfill criteria for classification as a classical or tubular Krukenberg tumor (see above). Other metastatic appendiceal adenocarcinomas are glandular adenocarcinomas of colorectal or mucinous intestinal types. (7) These metastases are usually immunoreactive for cytokeratin 20, but are also cytokeratin 7 positive in about 50 percent of the cases, so they can potentially be mistaken for primary ovarian carcinomas. (7)

Pseudomyxoma peritonei is an uncommon condition in which mucinous ascites causes progressive abdominal distention and gastrointestinal dysfunction. The mucus is viscous, loculated, yellow-brown or red, and contains foci of fibrous organization that form adhesions to adjacent structures. Pseudomyxoma peritonei in women is often associated with ovarian tumors that resemble borderline mucinous tumors or, less often, a mucinous cystadenoma, and in the past it was thought to be the cause of death of nearly all women who die of borderline mucinous tumors. It also occurs in patients with tumors of the gastrointestinal tract, particularly those of the appendix, and rarely with tumors of other sites.

Three different microscopic patterns have been described in pseudomyxoma peritonei. (64-67) In the first, the mucin is superficial. It lies on the surface of the ovaries, on the peritoneal surfaces or on the omentum and may contain inflammatory cells, macrophages, fibroblasts and an ingrowth of capillaries. In some cases no epithelium is present, while in others there is a small amount of low-grade mucinous epithelium. This has been termed the “acellular” or “superficial organizing” pattern of pseudomyxoma peritonei, depending on whether or not epithelium is present. Second, and most common, is a pattern in which the mucus dissects through the involved tissue. Bands of fibrous tissue surround the mucin and there is organization, with ingrowth of fibroblasts and capillaries. Occasional strips of low-grade mucinous epithelium are present in the mucin or in or adjacent to the fibrous bands. (68) This is the pattern that has been designated as disseminated peritoneal adenomucinosis (DPAM) by some authors. (65) It is found in women with benign or borderline-appearing mucinous tumors of the ovary. Finally, in a third pattern, the mucus contains more epithelial cells and the cells exhibit high-grade nuclear atypia or there are clear-cut features of mucinous adenocarcinoma, such as proliferative malignant glands, solid growth, or signet ring cells. This pattern can be designated as metastatic mucinous carcinoma or peritoneal mucinous carcinomatosis. The prognosis is related to the type of peritoneal mucinous lesion present. Patients with acellular or superficial organizing mucin have the best prognosis. A diagnosis of “acellular mucin” requires careful study, since epithelial cells are usually found if sufficient slides are studied. Patients with so-called DPAM have an intermediate prognosis with a high rate of recurrence, a protracted clinical course and, in many cases, eventual death due to complications of pseudomyxoma peritonei. Patients with markedly atypical or malignant cells in the mucus have an unfavorable prognosis, and usually die of carcinomatosis within a few years of diagnosis. Radical forms of therapy for pseudomyxoma peritonei involving peritonectomy and intra-abdominal chemotherapy have been developed in recent years and appear to have some therapeutic benefit. (69-71)

The nature of the ovarian tumors that occur in women with pseudomyxoma peritonei has been a source of controversy. Recent clinicopathologic studies have shown that nearly all women
with pseudomyxoma peritonei have a tumor in the appendix or, in a few cases, elsewhere in the gastrointestinal tract. These studies have led to the conclusion that the ovarian tumors are secondary, with the primary site usually being in the appendix. (64, 72, 73) Findings that favor this hypothesis include: the tumors in the ovary and appendix are usually synchronous; the tumors in the ovary and appendix are histologically similar; the ovarian tumors are frequently bilateral, a finding that is more in keeping with a secondary neoplasm than with a primary borderline mucinous tumor; when unilateral, the ovarian tumors are most often right sided, near the appendix; implants are present on the surfaces of the ovaries, a finding characteristic of metastases; the gross and microscopic appearance is not exactly typical of a primary mucinous tumor; the ovarian involvement may be superficial; pseudomyxoma ovarii is almost always present, and frequently contains epithelial cells with an appearance identical to those in the abdominal mucin; immunostains for cytokeratin 7 are negative in some ovarian tumors; (74) the ovarian and appendiceal tumors show immunohistochemical staining and molecular marking for MUC2, a marker of intestinal neoplasms, while primary ovarian mucinous tumors express MUC5AC but not MUC2; (75, 76) and there are molecular similarities between the ovarian and appendiceal tumors in some cases. (77-79) The current consensus is that the ovarian tumors in women with pseudomyxoma peritonei are secondary to a gastrointestinal neoplasm, usually one located in the appendix, in almost all cases. (68, 80, 81) The pathologist should keep in mind that the appendix tends to be abnormal in patients with pseudomyxoma peritonei, even when it is grossly unremarkable and ovarian tumors are present. If possible, the appendix should be removed and processed in its entirety for histologic study. The type of tumor present in the appendix, if any, and the histologic appearance of the tumor cells in the peritoneal mucin are important prognostic factors in pseudomyxoma peritonei, and should be described in the pathology report. In most patients, the appendiceal tumor is a low-grade appendiceal mucinous neoplasm, sometimes associated with a diverticulum, (82) although in a minority of patients it is an adenocarcinoma. (83-85) In a small minority of patients, no extraovarian primary tumor can be identified. In some of these patients, the explanation for the pseudomyxoma peritonei appears to be that an intestinal mucinous type tumor arising in a benign cystic teratoma has spread to the peritoneum and given rise to pseudomyxoma peritonei. (86-88) Such tumors can have the immunophenotype of intestinal (cytokeratin 7 negative, cytokeratin 20 positive, CDX-2 positive), not ovarian (cytokeratin 7 positive, cytokeratin 20 and CDX-2 variable) mucinous tumors. In a few patients, the derivation of the pseudomyxoma peritonei is difficult to reconcile with current concepts of the disease and, in such cases, the pseudomyxoma peritonei may arise from an ovarian mucinous tumor or from an occult primary mucinous tumor in some other organ.

**Metastatic Breast Cancer**

Breast cancer is a common metastatic tumor of the ovary, but it is rarely clinically significant. Historically, most examples of metastatic breast cancer have been small, sometimes even microscopic, foci of tumor cells found in ovaries removed for hormonal therapy of breast cancer. (89, 90) Adnexal masses in women with a history of breast cancer are more likely to be primary ovarian
neoplasms than metastases. (91) It is unusual to find metastatic breast cancer in risk reducing salpingo-oophorectomy specimens, even though many patients who undergo this procedure have been diagnosed with breast cancer. (92) Metastatic breast cancers that present clinically as primary ovarian tumors, prior to discovery of the breast primary, often pose diagnostic problems. (27, 93) Metastases from the breast are sometimes an incidental microscopic finding, but in about one-third of cases the ovaries are enlarged by solid diffuse or nodular metastases. Most metastases resemble infiltrating ductal (Fig. 4-3) or lobular carcinoma, and are easy to recognize as metastatic from the breast. Uncommon microscopic patterns that can be difficult to differentiate from primary tumors or metastases from other organs include a single cell pattern, a diffuse, solid growth pattern, and a cribriform growth pattern. (89)

Metastatic breast cancer needs to be differentiated from ovarian cancer not only in the ovary but also in various intra-abdominal metastatic sites. Considerable study has been devoted to the use of immunohistochemistry to resolve this differential diagnosis, and antibodies are now available that make it possible to correctly diagnose most cases. Breast cancer is typically cytokeratin 7 positive, cytokeratin 20 negative, and it may show nuclear staining for estrogen and progesterone receptors, an immunophenotype that overlaps with that of many primary ovarian carcinomas. Positive immunohistochemical staining for gross cystic disease fluid protein (GCDFP)-15 and mammaglobin provide support for a diagnosis of metastatic breast cancer, (94) although it should be noted that mammaglobin is occasionally positive in primary tumors of the female genital tract. (95) Metastatic breast cancer is generally negative for WT-1, OC125 and PAX8, (96, 97) so staining for GCDFP-15, mammaglobin, WT-1, OC125 and PAX8 can generally clarify whether a tumor is a primary ovarian neoplasm or metastatic breast cancer. Metastases from a signet ring cell type of infiltrating lobular carcinoma are a rare cause of Krukenberg tumors in the ovary; the primary site can generally be correctly identified due to positive staining for ER, mammaglobin, and, sometimes GCDFP-15. (98)

<table>
<thead>
<tr>
<th>Metastatic Carcinoma - Breast vs Ovarian</th>
<th>Breast</th>
<th>Ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK7</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>ER</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GCDFP-15</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mammaglobin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PAX8</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>WT-1</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CA125</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Female Genital Tract

Carcinomas of the endocervix and endometrium not infrequently metastasize to the ovaries where they can be difficult to differentiate from primary neoplasms. Synchronous endometrioid carcinomas in the endometrium and ovary are not uncommon; there is endometrioid carcinoma in the endometrium in 10–20% of women who have ovarian endometrioid carcinomas. These are thought most often to be separate synchronous primary tumors. The endometrial cancer is usually superficial and well-differentiated, and would not be expected to spread to the ovary. The survival rate is high when the carcinomas are confined to the uterus and ovary. (99, 100) This favors the interpretation that the ovarian and endometrial carcinomas are separate, synchronous primary tumors since advanced stage endometrial or ovarian carcinoma would have such a favorable prognosis. In some patients with simultaneous tumors, however, the ovarian tumor is a metastasis from the endometrial carcinoma. Features that suggest that the ovarian carcinoma might be metastatic include: the endometrial carcinoma
is high grade, the endometrial cancer is deeply invasive into the myometrium, there is
myometrial or ovarian hilar lymphovascular invasion; aggregates of malignant cells are present
in the fallopian tube lumen, the ovarian tumor is small; the ovarian tumor is multinodular and
solid, there is a desmoplastic stromal reaction, the ovarian tumor is bilateral, surface implants are
present on the ovary, and extravarian metastases are present in a distribution characteristic of
endometrial adenocarcinoma (i.e., lymph node metastases more likely than peritoneal
metastases). Immunostains do not help determine whether the ovarian tumor is primary or
metastatic. In the future molecular diagnostic techniques may provide more definitive diagnostic
information but at this time they are not in routine clinical use.

Endocervical adenocarcinoma metastasizes to the ovaries, but less often than endometrial
carcinoma. When it involves the ovaries cervical adenocarcinoma can mimic either endometrioid
adenocarcinoma or mucinous adenocarcinoma. The ovarian tumors average about 13 cm in
diameter, are usually multicystic, and are unilateral in two thirds of cases. These features would
certainly prompt consideration of a primary ovarian tumor in many instances, as would the
microscopic resemblance to a primary borderline tumor or low-grade adenocarcinoma. Clues to
the correct diagnosis include knowledge that the patient has or had a cervical adenocarcinoma
and recognition in the ovarian tumor of certain morphologic features of endocervical
adenocarcinoma, including an endometrioid appearance at low magnification but apical
mucinous cytoplasm at high magnification, numerous mitotic figures and elongated
hyperchromatic nuclei that are more abnormal than expected for a tumor with well formed
glands, “floating” mitoses (mitotic figures in the apical cytoplasm above the nuclei) and
apoptotic bodies along the base of the epithelium. Somewhat surprisingly, the cervical
adenocarcinoma is not always deeply invasive, (4) and even cases of apparently in situ cervical
adenocarcinoma have been associated with ovarian metastases. (101) Immunohistochemical
staining for p16 and in situ hybridization for HPV can help confirm that an ovarian
adenocarcinoma is metastatic from the cervix.

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