The Fourth Arkadi M. Rywlin International Pathology Slide Seminar Symposium in Anatomic Pathology

5-6 June 2010

İSTANBUL

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SESSION I

CASE 1

Janez Lamovec, M.D.

History: In a 58-year-old female patient who was admitted because of mushroom poisoning, an asymptomatic tumor of the liver and retroperitoneum was found by ultrasound examination and subsequently by computerized tomography. Aspiration cytology showed malignant myxoid tumor. The patient was operated upon and two segments of the liver, gall bladder and part of duodenum were resected.

Pathologic findings: Grossly, in the resected liver segments a well delineated lobulated partly grey-yellow myxoid, partly hemorrhagic tumor was found that measured 11 x 8 cm in two dimensions. Tumor was surrounded by a narrow layer of liver parenchyma and partly by connective-adipose tissue of hepatoduodenal ligament. Gall bladder and partially resected duodenum were unremarkable; the latter was adherent to tumor. The total weight of the specimen was 470 grams.
Histologically, the tumor shows uneven cellularity. Tumor cells are for the most part set in an edematous/myxoid stroma that is focally very loose and only in some areas condensed, hyalinized. Tumor cells are of different shapes – spindle, stellate, oval, many of them pleomorphic, bizarre, multinucleated, some lipoblast or rhabdomyoblast-like; some floret-like shaped. Nuclei are hyperchromatic, many misshapen, giant, lobulated, with unevenly distributed chromatin, some are vacuolated, clearer. Multinucleated cells are numerous, some nuclei are wreath-like shaped. Nucleoli are indistinct. Mitoses are innumerable, most of them

atypical. Stromal vasculature is delicate, many vessels dilated, in several areas numerous intra- and extracellular eosinophilic hyaline globules of different sizes are seen. Rare foci of extramedullary hematopoesis are also seen.
Tumor is focally necrotic, in larger areas hemorrhagic, entrapped bile ductules are seen in more peripheral parts, close to liver tissue; some of them are cystic. From the liver tissue tumor tissue is separated by a fibrous capsule, only focally small foci of direct infiltration of liver parenchyma are seen. The latter shows focal fatty metamorphosis and slight portal inflammation and fibrosis. Similar fibrous capsule is also found on tumor-hepatoduodenal ligament interface. Duodenal wall was not infiltrated.
Immunohistochemically, tumor cells are diffusely positive for vimentin and bcl-2 in all or vast majority of tumor cells, and focally for smooth muscle actin, muscle specific actin, cytokeratin MNF116, CK AE1/AE3, CAM 5.2, chymotrypsin and in rare cells also for desmin. MyoD1, myogenin, CD34 Q, CD117, S-100, HMB45, CD68 and Hepat are negative.
Electron microscopy of the tumor showed undifferentiated mesenchymal cells with rough cytoplasmic reticulum with some larger electron dense structures corresponding to hyaline droplets on H&E. Bundles of intermediate filaments were also seen in some cells that lacked specific organization.

Diagnosis: Undifferentiated (embryonal) sarcoma of liver in adult.

Follow up: The patient received 4 cycles of adjuvant chemotherapy (cisplatin, 5-FU, oncovin); no metastases of tumor were clinically and radiologically discovered. Nine months after operation, she developed acute B lymphoblastic leukemia and died one month later. Autopsy was not performed.

Comment:

Undifferentiated (embryonal) sarcoma of the liver is a very rare neoplasm predominantly occurring in children, particularly between 6 and 10 years of age (1, 2). In adults and children over 15 years of age, it is even rarer, with reported 67 cases in the recent publication (3). The chief complaint of patients with this tumor is abdominal mass and/or abdominal pain. Incidentally discovered tumor, such as in our case, is an extremely unusual occurrence (4). Grossly, these tumors are well delineated, often gelatinous, of variegated color, and in more than half of the cases cystic (1). Microscopically, they show a sarcomatous growth, with predominantly spindle to stellate cells, with numerous pleomorphic and giant cell, many multinucleated, some lipoblasts-like, some rhabdomyoblasts-like. Small cells, monocytoid or stellate, with scant cytoplasm and picnotic nuclei may dominate in the overall appearance of the tumor. Matrix is usually myxoid. Intra and extracytoplasmic PAS positive globules are seen in most cases. Extramedullary hematopoesis may be observed in some cases. Tumors are microscopically surrounded by fibrous capsule. Entrapped bile ductules are almost always found (1, 2). The tumor presented here very much corresponds to this general microscopic description. Although it was suggested that tumors in adults microscopically somewhat differ from those in childhood (5), this was not so obvious in our case.
The histogenesis of the tumor is still in debate, anecdotical reports associate some cases with the presence of mesenchymal hamartoma of liver (6, 7). Immunohistochemical findings do not contribute much in this regard (8, 9). Tumors are positive for vimentin, alpha-antitrypsin, variably for CD68, focally for desmin, alpha-smooth muscle actin, cytokeratin 18 and 19, and negative for S-100 protein, MyoD1, myogenin, CD34, CD117, AFP, and a number of other markers.

In differential diagnosis, in children, hepatoblastoma, embryonal rhabdomyosarcoma, infantile hemangioendothelioma, and mesenchymal hamartoma should be considered, while in adults some other sarcomas such as liposarcoma, leiomyosarcoma, and other tumors, e.g. sarcomatoid hepato or cholangiocarcinoma, carcinosarcoma, GIST, and also angiomyolipoma may be confounded with undifferentiated sarcoma (1, 2, 8). Clinical data, careful morphological analysis and immunohistochemical examination are helpful to properly classify an individual tumor.

Undifferentiated embryonal liver sarcoma were originally considered to have a dismal prognosis (1). Recently, however, it has been shown, in childhood cases (10), as well as in adult cases (3), that curability is possible when combined multimodal treatment – conservative surgery and adjuvant chemotherapy is used.

The association of undifferentiated sarcoma of the liver with other malignancy must be exceedingly rare. There is one report of a patient who had surgery and postoperative chemotherapy because of ovarian high-grade serous papillary carcinoma and developed liver tumor 18 months following completion of chemotherapy; the latter was proven to be an undifferentiated liver sarcoma (11). Another case, a 17-year-old boy developed therapy related acute myeloid leukemia 3 years after cessation of chemo-radiotherapy for undifferentiated liver sarcoma (12). There is another report of a pediatric case with concurrent cerebellar pilocytic astrocytoma (13). In our case, acute B lymphoblastic leukemia developed right after the last cycle of chemotherapy and was the cause of patient's death.

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CASE 2

Janez Lamovec, M.D.

History: On ultrasound examination for bile stones in a 68-year-old woman. a solid tumor in the lower abdomen was found. In addition, a small round tumor in the kidney was also discovered, ultrasonographically considered to be angiomyolipoma. Aspiration cytology was performed and mesenchymal tumor, not otherwise specified, was diagnosed. At laparotomy, an oval tumor in the mesentery of the small intestine was found adherent to ileum. Tumor and a segment of intestine were resected.

Pathologic findings: Grossly, the specimen was represented by a 10 cm long segment of small intestine with a segment of mesentery in which a 5.5 x 4.8 cm oval, clearly circumscribed tumor was found. Tumor was relatively firm, yellow-brown on cut surface. It didn't infiltrate the intestinal wall.
Histologically, the tumor shows non-homogenous structure – irregular admixture of solid areas of dense eosinophilic spindle and epithelioid cell and clear areas of lipocytes and lipoblast-like cells. Epithelioid/spindle cells show some fascicular arrangement and also vague nests are formed. Prominent dilated, mostly thin-walled vascular structures are present throughout the tumor, some thick-walled vessels are also thin. Perivascular perpendicular orientation of cells is focally seen. Epithelioid and spindle tumor cells are variably pleomorphic, with abundant eosinophilic cytoplasm; some cells are strapped shaped, rhabdomyoblasts-like. Cytoplasm of many eosinophilic epithelioid tumor cells appears slightly granulated and vacuolated. Neoplastic nuclei are large, oval, round, elongated, spindle, for the most part clear, with prominent nucleoli. Mitoses are extremely rare. In clear cell areas that are relatively intimately admixed with solid areas, lipocytes and lipoblast-like cells of different sizes are seen, many of them multivacuolated, some of brown adipocyte-like appearance. Tumor tissue is diffusely sprinkled with mastocytes, some predominantly lymphocytic infiltrates are seen focally as well as many foci of extravasated red cells. Necroses are not present, tumor is not incapsulated but is clearly separated from surrounding adipose tissue of mesentery. Intestine was not infiltrated.
Immunohistochemically, many cells were positive for vimentin and CD68. Only very rare epithelioid cells, mostly individually, showed HMB- 45 and Melan A positive reaction, and positive reaction for desmin was found in only one small focus. Smooth muscle actin was practically negative except for some splaying cells from rare vessels. CD34, caldesmon, CD117, S-100 protein, ER and PR and keratins were negative. Mast cells were even more clearly shown by positive toluidin blue reaction and Giemsa staining. MIB-1 staining decorated less than 1 to 2 % of tumor cells.

Diagnosis: Extrarenal (mesenterial) angiomyolipoma

Follow-up: Half a year after surgery the patients shows no evidence of disease. No evidence of tuberous sclerosis has been proven.

Comment: Angiomyolipoma (AML) is an unusual tumor, most often found in kidney and liver although many individual case reports and small series show that this is an ubiquitous lesion encountered in many different tissues and organs, such as heart, lung, G-I tract, adrenal, mediastinum, retroperitoneum, skin, genital tract, bone, parotid gland. Its classical morphological appearance is that of a mixture of mature adipose tissue with intermingled

disorganized bundles of spindly and epitheloid smooth muscle cells, some of bizarre forms, and network of thick walled vessels. There is a great variation of the proportion of the three component in an individual tumor (1). Besides, monomorphic variants exist, such as epithelioid types of angiomyolipoma that may mimic epithelial neoplasms such as renal oncocytoma or renal carcinoma (2 - 4). Lipoma-like and leiomyoma like variants are also known.

It was originally thought that AML is a type of hamartoma; however, it has been later demonstrated that it is a clonal disorder, i.e. neoplasm (5 - 6); not only one but different clones may be involved, for each different component arising independently (7).

It has been shown that AML belongs to a family of lesions that are characterized by a distinctive cell type of origin, so-called perivascular epithelioid cell (PEC). The latter typically shows immune reaction with melanogenesis marker HMB-45. The lesions of the family included, AML, pulmonary and extrapulmonary clear cell ‘’sugar’’ tumor, lymphangiomyoma, lymphangioleiomyomatosis, renal capsuloma and myomelanocytic tumor of hepatic ligaments (8-9). Many of PEC lesions are related to genetic syndrome of tuberous sclerosis, with disease determining genes on chromosome 9 and 16, their loci called TSC1 and TSC2 (8).

Morphology of extrarenal AML is essentially similar to that of tumors of renal origin, with monotypic variants also on record (10-11). In differential diagnosis, other variants of PEComas should be differentiated from AML, an exercise not too difficult when classical three tumor component are present (9). When some of the three component is predominant, such as smooth muscle tissue, the tumor may be confounded with leiomyoma, leiomyosarcoma, GIST and for liposarcoma when adipose component is most prominent (1-2, 12).

Immunohistochemical examination is of critical importance in diagnosing AML. Tumor cells are, as a rule positive for HMB-45, although rare negative cases with typical morphology are on record (1). Besides, another melanocytic marker Mart-1/Melan-A, microphthalmia transcription factor and some others are also positive. Smooth muscle cells are variably positive for smooth muscle actin, calponin, and less consistently for desmin. It should be stressed that reactivity for any of the mentioned markers could be quite focal, and sometimes difficult to detect (1). In one study estrogen and progesterone receptors were found in 25% of 46 renal cases (1). CD117 (KIT) may also be expressed in AML (13).

AML is essentially a benign tumor, while monotypic variants may behave in a malignant way; they were separated from the rest of AML in the latest WHO classification (14).

The treatment of AML is surgical, in malignant cases with metastases adjuvant chemotherapy is used (15).

Our case is not quite typical, particularly in regard to the lack of tortuous hyalinized vessels, although this feature may be encountered in renal AML cases (1). In addition, it is focally similar to hibernoma, also known from the literature (16). Furthermore, the expression of HMB-45 and smooth muscle actin was seen only in very small rare foci in sections from many paraffin blocks. An additional feature, rarely specifically mentioned in previous reports (13), was the presence of very large number of mastocytes, that were sprinkled throughout the tumor. The significance of this finding is unclear to us.

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CASE 3

Janez Lamovec, M.D.

History: A 44-year-old extremely obese woman was admitted with a palpable tumor in upper outer quadrant of the left breast of unknown duration. FNAB showed carcinoma. A quadrantectomy and axillary dissection was performed.

Pathologic findings: In the quadrantectomy specimen, a 21 x 19 mm relatively well circumscribed tumor was found. On cut surface, it was grey-white, firm. Resection margins were well away from the tumor. In none of the 18 isolated lymph nodes from the axillary specimen gross mestastases were found.
Microscopically, tumor is focally well delineated from the surrounding, in many parts tongues and irregular trabeculae of tumor tissue infiltrate lipomatous breast tissue. Small nodules of tumor tissue, centrally, and also peripherally are present, separated by sclerosed septae. On high power view, neoplastic tissue is composed of small nests and loose clusters of tumor cells, irregular anastomosing trabeculae of cells, and also dissociated tumor cells. No ductal or glandular structures are seen; in rare places some intracytoplasmic lumina in tumor cells may be observed. In a few foci, more centrally, ducts with intraductal cancer (DCIS) are embedded in the tumor infiltrate. There are no necrotic foci although individual apoptotic cells are common. The dominant feature of the tumor is a dense, almost diffuse lymphoid infiltrate composed of mature lymphocytes, plasma cells, histiocytes, and rare eosinophils. Some lymphatic follicles are also seen. The infiltrate surrounds, permeates and dissociates neoplastic cell nests, and in many foci almost obscures carcinoma cells. The latter are relatively large, with moderate amount of amphophilic cytoplasm, and with generally large, oval vesicular nuclei with distinct nucleoli. Some nuclei are quite large. Rare multinucleated cells are also seen. Mitoses are numerous, up to 12 per HPF. Many are atypical. According to Nottingham grading, this is a grade III tumor.
In the lipomatous parenchyma surrounding the tumor, some small irregular infiltrates of neoplastic tissue, identical to that of the dominant tumor are seen, and in addition, several foci of DCIS with lobular cancerization as well (not present in the submitted slides). In the immediate vicinity of the tumor, foci of mild to moderate lymphocytic lobulitis are seen.
In none of the 18 lymph nodes microscopic metastases were found.
Tumor cells were positive for ER and PR and negative for HER2 (immunohistochemically and by FISH method).
Immunohistochemically, tumor cells were positive for CK8, CKAE1/AE3, CK18, EMA, weakly and focally for CK19; they were mostly positive for E-cadherin and were negative for CK5, CK14, BRST-2, p53 and S-100 protein; bcl-2 was positive. Lymphoid cells were in great majority CD3 positive cells, CD8 positive cells predominating; there were no cytotoxic Granzyme B and TIA-1 positive cells present in the inflammatory infiltrate. CD68 positive cells (PGM1) were numerous; CD138 positive cells were relatively few. MIB1 decorated around 20 % of tumor cells.
In situ hybridization failed to reveal any positivity for EBV genome.

Diagnosis: Lymphoepithelioma-like carcinoma of the breast.

Follow-up: The patient was not suited for adjuvant chemotherapy so she was given hormonal treatment; five years after operation she shows no evidence of disease.

Comment: Lymphoepithelioma-like carcinoma of the breast (LEC) is a relatively recently recognized type of breast carcinoma (1); the original report was followed by other publications describing single cases or small series of cases (2-9). Such tumors were previously described in several other organs as well and were morphologically similar to a prototypic lymphoepithelioma of the nasopharynx, a tumor known from many decades ago. The latter was described in two histological patterns – Schminke and Regaud variants (10). The same two patterns may be observed in breast lymphoepithelial-like carcinoma, both patterns may appear together.

The main problem in this type of carcinoma is how to differentiate it from better known medullary and atypical medullary carcinoma (infiltrating duct carcinoma with medullary features). Lymphoepithelial carcinoma is not so well delineated, stromal lymphoid infiltrate contains less plasma cells than medullary carcinoma, it may be ER and PR positive, show less commonly syncytial growth if ever, the nuclear grade is rarely high (2).

Although, the strict morphologic criteria proposed for medullary carcinoma by Ridolfi et al appear to be well established they are nonetheless relatively poorly reproducible (11). The same holds true for infiltrating duct carcinoma with medullary features. However, it appears that many of the medullary and atypical medullary carcinomas are part of a basal-like carcinoma spectrum as demonstrated by immunoreactivity for basal keratins (CK5/6, CK14), triple negativity and genomic investigations (12-13). In addition, some of them are BRCA-1 associated cancer (14).

The reproducibility of histologic criteria for lymphoepithelioma-like carcinoma has never been really established, particularly because of their rarity. However, it seems that criteria, apart from the non-circumscribed margins and dense lymphoid infiltration, vary somewhat between different publications (2-9). Even syncytial growth pattern in > 75 % of the tumor, characteristic feature of medullary carcinoma was described in one case of LEC (3). Immunohistochemically, tumors are positive for low molecular weight keratins, EMA, and E-cadherin in one study of a small series (3); these results vary in some other publications (1-2, 4-9). ER and PR may be positive. Most tumors are ductal in nature, some also lobular (2, 5-6). Both, Schminke and Regaud pattern of growth were observed in breast LEC (4). In one case, glandular differentiation of the carcinoma was confirmed by EM (8). Some cases may be associated with sclerosing lobulitis (4).

In our case, immunohistochemical results to some extent confirm those of others; specifically, tumor were negative for basal type keratins but positive for luminal ones (CK8, 18, 19); it was also positive for E-cadherin, thus proving its ductal luminal phenotype. Our case is so far the first in which in situ ductal component was evident, outside and also inside the dominant tumor mass; in the latter location it was clearly demonstrated by CK5 positivity of myoepithelial cells and its negativity of intraductal tumor cells.

As in all other studies, our case was also EBV negative, demonstrated by in situ hybridization. The recent study demonstrated a human papilloma virus positivity in one case (9).

In regard to prognosis, it seems that LEC of breast has a good prognosis although lymph node metastases were found in two described cases (4-5); in another case the patient developed distant metastases (8). It should, however, be mentioned that in the recent study it was shown that all types of breast carcinoma of high histologic grade with prominent inflammation, whichever their designation – medullary, atypical medulary or grade 3 ductal carcinoma did not have significantly different prognosis but all of them had better prognosis than grade 3 ductal carcinoma without inflammation (15). Although not included in the latter study, LEC of the breast may well share such a prognostic feature.

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CASE 4

Elvio G. Silva M.D.

45-year-old female patient who consulted because of abdominal enlargement. After different tests, the patient underwent an exploratory laparotomy. The section is from a 5 x 4 x 4 cm. right ovarian mass.

The microscopic slides on this biopsy show that the patient had a papillary carcinoma and the papillae were composed of only epithelial cells without stroma, which are the characteristics of papillae of serous carcinoma. In addition, there was numerous psammoma bodies. Mitoses were rare, and there were no significant

atypia. Based on these findings, the diagnosis of low-grade serous carcinoma was made.

Case 4-**Diagnosis: Low grade serous carcinoma**

The patient received seven different treatments in ten years. It was necessary to change the chemotherapy almost every year because of the lack of response to the treatment. Even if we did not see the microscopic slides this typical multiple treatments without response is characteristic of low-grade serous carcinoma, because the patients are still alive after several years. We divide the serous neoplasm in three groups: benign, borderline, and carcinoma. What separates benign from borderline is the presence or absence of cell detachments, which are an expression of epithelial proliferation. Borderline from carcinomas are separated based on the presence of invasion, and the carcinomas are graded as low and high based on significant atypia and 12 mitoses per 10 high power fields. Regarding mitoses in most cases of low-grade serous carcinoma is difficult to find more than 3 or 4 mitosis in 10 high power fields. Two other important features are that in low-grade serous adenocarcinoma, calcifications are always seen and in high-grade serous carcinoma are present in only 50% of the cases. Low-grade serous carcinoma is a very homogeneous tumor, while high grade is a heterogeneous tumor. Low-grade serous carcinoma is not a benign lesion and most patients will die of the disease; however, the difference between low grade and high grade is the time of survival. The 10-year survival for high-grade serous carcinoma is approximately around 5% while for low grade serous carcinoma is 40-50%. We designated these tumors as low grade and high-grade serous carcinomas; however, most probably they represent different tumors and not different grades of the same tumor. This is based on two important findings: 1) there is no transformation of the low grade into the high grade and, 2) both tumors have different mutations, P53 typical of the high grade, and KRAS typical of the low grade.

Everybody agrees that the invasion separates borderline from low-grade serous carcinoma. The invasive component needs to be larger than 3 mm in any given direction; however, there are some other new findings that separate these lesions, too.

Micropapillary pattern:
This pattern has been described by the group of Dr. Kurman several years ago, and the proposal was to use an area larger than 5 mm.

In 2008, we thought that a large borderline tumor with very extensive micropapillary pattern would be different than a large tumor with only a small 3-mm area of micropapillary pattern. Therefore, we reviewed our cases and found out that if the micropapillary pattern represents more than 10% of the entire tumor the recurrence rate is around 92%. Based on these findings I diagnosed borderline tumors with a micropapillary pattern larger than 10% as noninvasive low-grade serous carcinoma. We believe that it is important to separate the noninvasive from the invasive low-grade serous carcinoma, because the time to recurrence and the survival time are different. In the noninvasive recurrences occur at 50 months and the time to death is180 months, while in the invasive tumors recurrences occur at 30 months and death at 120 months.

Other interesting findings of low-grade serous carcinoma are the different types of stromal invasion. One of the types of invasion in the low-grade serous carcinoma is the NELS (nonepithelial lined spaces). In this pattern, we see invasion of the stroma by a small group of cells, which are within the spaces that do not have epithelial cells around. Within these spaces, we can see small groups of cells, micropapillae, macropapillae, or single cells. Using this definition of invasion, we do not need to consider where the cells are, in the septum of the omentum, or invading the stroma underneath the mesothelium. These foci of invasion can be recognized because there are groups of cells in spaces that look like lymphatic spaces, but electron microscopy shows that they are just artifactual spaces probably created by fixation. The other more unusual pattern is the invasion by glands characterized by a group of glands in a haphazard distribution. They do not have the NELS; however, the haphazard distribution and the significant fibrosis present around the glands are typical of invasion.

Other special features in low-grade serous carcinoma are the orphan papillae, calcifications, and mucin. We designate orphan papillae to the papillary formation that are not attached to either other papillae or to the border of the cystic space. They are totally disorganized and since they are not attached to a mother papillae we designate them as orphan. These orphan papillae are seen only in low-grade serous carcinoma. In the regular borderline case, including those with micropapillary pattern, the papillae follow an organized architecture having the typical hierarchical structure.

Calcifications:
Calcifications are always seen in low-grade serous carcinoma, sometimes there are few calcifications, and sometimes there are so many that it is difficult to find the tumor cells. In this latter situation, the diagnosis of psammocarcinoma can be made. We believe that this type of carcinoma is a low-grade serous carcinoma and the good prognosis of this type of carcinoma is due to the fact that the amount of epithelial cells is minimal. Most of the tumor represents calcification.

Mucin:
In approximately 70% of low-grade serous carcinomas, mucin is found within the cytoplasm or the epithelial cells.

In summary, noninvasive low-grade serous carcinoma is a borderline tumor with more than 10% of micropapillae, and invasive low-grade serous carcinoma has tumor cells within NELS in areas larger than 3 mm or with desmoplasia. Another rare type of invasion is invasion by glands. Special features important to remember are the orphan papillae, calcifications always present, and mucin seen in 70% of the cases. Regarding the implants of serous borderline tumor, we do not use the term invasive implants for lesions that have tumor cells in NELS because we call these lesions low-grade serous carcinoma. If you prefer to designate the lesion as invasive implants, it is important to mention in the report that 65% of these cases recur as low-grade serous carcinoma.

High-grade serous carcinoma
To make a diagnosis of high-grade serous carcinoma it is important to have papillary

projections without stroma, composed of only epithelial cells, the papillae have multiple layers of tumor cells and it is a papillary tumor. If you do not see papillae in a high-grade carcinoma, and it is a solid neoplasm, it would be an undifferentiated carcinoma. If the papillae do not have multiple layers or they have a very smooth border, I would not designate the tumor as a papillary serous carcinoma. Papillae with smooth borders and thick areas are most probably transitional cell carcinoma. There is another variety of high-grade ovarian carcinoma that we designate as microcystic, which is a tumor composed of multiple microcyst and also within the groups of epithelial cells there are signet ring cells containing mucin. The signet ring cells; however, are never seen as individual cells in the stroma. We believe that since we are getting into the personalized treatment of ovarian carcinoma, it is very important to separate the different types of high-grade carcinoma including papillary serous clear cell, transitional cell, undifferentiated and microcystic carcinoma. Personalized treatment of ovarian cancer requires first proper classification of the tumor.

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CASE 5

Elvio G. Silva M.D.

57-year-old female patient who had vaginal bleeding for 2 years and consulted now because it became very profuse. The patient underwent an endometrial curettage followed by hysterectomy. The section is from a tumor found in the endometrium and myometrium.

The histologic sections of the tumor show that there was a carcinoma with endometrioid type of glands, but in several areas there were large groups of cells without forming glands. I believe this is a very important finding in the endometrioid type of carcinoma. A high-grade endometrioid carcinoma should have glands. If glands are not seen in the solid component it should be diagnosed as undifferentiated carcinoma. Therefore, if we see areas of differentiated and undifferentiated, we will call the tumor a dedifferentiated endometrioid

adenocarcinoma.

Case 5 diagnosis: **Dedifferentiated endometrioid adenocarcinoma**.

The FIGO grading system in the 70s determined that endometrioid adenocarcinoma should be graded as Grade 1, Grade 2, and Grade 3 based on the solid component which included <5%, 6% to 50%, and more than 50%, including undifferentiated carcinomas; however, in 1978, the revision of the FIGO grading dropped the undifferentiated carcinoma from the Grade 3, but they gave no comment on the significance of the diagnosis of undifferentiated carcinoma. The survival rate of endometrial carcinoma is excellent for Grades 1 and 2, for Grade 3, a five year survival rate is 50 to 75%. However, the survival of an undifferentiated carcinoma is different because it is a very aggressive lesion. Therefore, it is important to separate Grade 3 adenocarcinoma from undifferentiated. If we see cords or glands, we call the tumor high-grade endometrioid, and if we see that there is no pattern, we call it undifferentiated carcinoma. The five year survival rate for undifferentiated carcinoma is 25%. The situation could be extremely important when we make a diagnosis of Grade 2 endometrioid adenocarcinoma because of the presence of a Grade 1 plus solid areas. In this situation, it is extremely important to make certain that the solid areas have glands. If we do not see glands, we should make the diagnosis of dedifferentiated carcinoma and because of the undifferentiated component the survival rate for five years is going to be around 25%. We found out about these tumors, reviewing cases of grade 2 endometrioid adenocarcinoma that have rapid progression. The dedifferentiation can occur in the primary or in metastatic tumors. Another important point is that sometimes because of this solid component, we question the possibility of neuroendocrine differentiation and we request stains for synaptophysin, chromogranin, and CD56. We found out that it is not important to separate undifferentiated carcinomas of the endometrium from tumors
with neuroendocrine differentiation, because tumors with neuroendocrine differentiation behave similarly to those without neuroendocrine differentiation. This is important in areas like cervix where tumors with neuroendocrine differentiation respond poorly to radiotherapy, but in the endometrial lesions the undifferentiated carcinoma is extremely aggressive and therefore it does not make any difference whether there is neuroendocrine differentiation or not.

It is also important to remember that in serous carcinoma, clear cell carcinoma, and undifferentiated carcinoma, most of these tumors are associated with areas of differentiated endometrioid adenocarcinoma. Most pathologists will recognize areas of serous or clear cell; however, we need to avoid using the undifferentiated areas just to grade the tumor. The undifferentiated component could be completely solid, or with areas of a rhabdoid type of tumor. I believe that some of the reports on rhabdoid tumors of the endometrium included cases of undifferentiated carcinomas.

An important consideration is that even when these are carcinomas, frequently they are negative for keratin. EMA has been shown to have better results in recognizing epithelial cells in this type of tumor.

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CASE 6

Elvio G. Silva M.D.

54-year-old female patient who presented with vaginal bleeding. An MRI showed the presence of a mass involving the cervix and the endometrium. A biopsy of the cervix was performed followed by hysterectomy. The slide is from the hysterectomy specimen.

The biopsy on this patient shows an epithelial type of tumor completely solid without glands or keratin. In tumors of the GYN tract with this kind of appearance we perform a keratin stain and if the keratin stain is focally positive, most probably the tumor represents a poorly differentiated carcinoma, which could be adenosquamous ; however, if all the tumor cells are positive for keratin, then we think about a possibility of the tumor of intermediate trophoblasts because all intermediate trophoblastic cells are diffusely positive for keratin. To prove that the tumor is a lesion of intermediate trophoblast we use stains, which are HLA, CD10, and hCG. We do not depend on HPL because it's positive in only 60% of intermediate trophoblast tumors. Since carcinomas of the cervix need to be differentiated from intermediate trophoblast tumors, we also perform stains to recognize the carcinomas of the cervix including p16, because of the correlation with HPV
and keratin 5/6 because many of the poorly differentiated carcinomas are squamous.

Case 6: Diagnosis: **Intermediate trophoblast tumor**.

Is it necessary to separate ETT from PSTT? How about calling them Intermediate Trophoblast Tumors? Both tumors have similar behavior, but they have different morphology, immunophenotype, and differential diagnosis. ETT can be present in the cervix and extrauterine sites, while PSTT is present only in the uterus. Immunohistochemistry separates both lesions because hPL is typical for PSTT, while P63 is an excellent stain for ETT. In my opinion, it is not necessary to separate these lesions, because the behavior is the same and the treatment is also similar. Most PSTT tumors that infiltrate the myometrium and form solid areas. They do not have areas of fibrinoid material. ETT is a tumor from multiple nodules of tumor cells, which are round areas of extensive fibrinoid changes.

What is the significance of intermediate trophoblast in complete mole? We are certain that there is a population of intermediate trophoblasts in complete mole and in choriocarcinoma. We are not sure of the significance of the amount of intermediate trophoblast. Probably it would be important to study this issue because choriocarcinoma and complete mole respond very well to chemotherapy, while tumors of the intermediate trophoblast do not. Therefore, the significance of the intermediate trophoblast in hydatidiform mole and choriocarcinoma is currently uncertain.

Between placental cell islands and plaques, and tumors of the intermediate trophoblast, are there hyperplastic lesions of the intermediate trophoblast? It is possible, placental site nodules, plaque, and exaggerated implantation site are more heterogeneous than intermediate trophoblastic tumors. I do not use KI67 to separate placenta site nodules, plaques, and tumors of intermediate trophoblasts because trying to determine the presence of more or less than 5% of cells positive for KI67, is an extremely difficult issue. I depend mainly on the mixture of trophoblast and decidual cells. If the decidual cells are mixed with intermediate trophoblastic cells then I believe it represents an implantation site, and if there are only intermediate trophoblast cells, I believe it represents a tumor. The only exception that I am aware of is in the placenta previa, because in these areas, there are no decidual cells mixed with intermediate trophoblast.

The question of identifying intermediate trophoblasts in hydatidiform mole and choriocarcinoma became extremely important; therefore, we use immunostains to separate intermediate trophoblastic cells from cytotrophoblast, positive Beta-catenin, negative Mel-CAM, negative HLA, and negative MUC-4 for cytotrophoblast, and the opposite is typical of intermediate trophoblast. Another important issue is that intermediate trophoblastic cells are also important to be recognized in abortions because in the absence of chorionic villi, we can still make the diagnosis of products of conception. Usually, this is a very simple task; however, it could be difficult when intermediate trophoblast cells are completely necrotic. In this situation, it would be important to request a keratin stain, because keratin works even in areas of necrotic tumors. Having positive single cells in areas of fibrinoid and necrotic changes is diagnostic of implantation site. Therefore, the
presence of these cells warrants the diagnosis of products of conception. This might become an important issue because there have been patients in whom pregnancy changes were not recognized; however, they developed a trophoblastic tumor.

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CASE 7

Masaharu Fukunaga, M.D.

History: A 32-year-old, gravida 0, para 0, Japanese woman presented with lower abdominal and left leg pain that had been present for 11 months. MRI revealed a 5-cm subserosal mass in the uterine fundus. Intraoperatively, a rubbery solid uterine mass attached to the left broad ligament was found and locally excised. The patient did not have the tuberous sclerosis complex. Fortunately, she gave a birth at 24 moths postoperatively. The patient had no evidence of tumor at 31 months.

Immunohistochemical studies: vimentin, h-caldesmon, HMB45, alpha-smooth muscle actin: (+).
Desmin, HHF35, CAM5.2, EMA, S-100 protein, CD34, CD10, Melan A, synaptophysin, chromogranin A, GFAP, CD117: (-).

Diagnosis: **Perivascular epithelioid cell tumor (PEComa) of the uterus.**

Histology and Comments: Its preoperative diagnosis was a subserosal leiomyoma. A clinician asked me for an intraoperative consultation. He said that the lesion looked like 'a carcinoma' not a leiomyoma and it was soft and hemorrhagic. My frozen section diagnosis was 'epithelioid smooth muscle tumor, borderline, most likely'
Grossly, the mass was fragmented and measured 5.0cm in aggregates. Its sectioned surface was tan-pin, unencapsulated, rubbery, and solid with foci of hemorrhage and necrosis. Microscopically, the tumor was composed of round to polygonal cells with a round nuclei and abundant clear to slightly eosinophilic cytoplasm and the cells were arranged in short fascicles and focally in a perivascular location. No melanosomes or premelanosomes were found in EM studies. Because of the presence of coagulative necrosis, infiltrative growth and the size of the mass 5cm, it is considered that it is a PEComa with an uncertain malignancy potential (1, 2).

The PEComa family of tumors includes angiomyolipoma, lymphangioleiomyomatosis, clear cell sugar tumor of the lung, and clear cell myomelanocytic tumor of the falciform ligament/ligamentum teres. There have been only 26 reported cases of uterine PEComa (1-7). The histogenesis of this family of tumor has been controversial. It has been proposed to be of melanocytic, smooth muscle, pericytic or perivascular epithelioid cell origin. Basic characteristic features of perivascular epithelioid cells are immunoprofiles of HMB45 (+)/ S-100 protein (-) or (+) (rarely) and abundant clear to eosinophilic granular cytoplasm. Other features include expression of the other melanoma-associated antigen Melan A, coexpression of muscle markers without cytokeratin expression, and the presence of melanosomes or premelanosomes.

There is also controversy about relationship between PEComa and epithelioid leiomyosarcoma with clear cells or HMB45 positivity (8). HMB45 immunoreactivity has been noted in uterine tumors, including leiomyomas, epithelioid leiomyosarcomas, stromal tumors, and even in normal myometrium (7). However, generally its expression was focal and weak. Silva et al. (8) asserted that some epithelioid cells in uterine smooth muscle tumors most likely undergo clear cell changes and become positive for HMB45 and argued that the concept of PEComa still needs some refinement. I consider the possible origin of primitive mesenchymal cells that have ability of differentiating to both smooth muscle and HMB45-positive perivascular epithelioid cells. Its degree of differentiation can vary from case to case. Comprehensive studies of uterine PEComas are needed to ascertain whether they represent a distinct entity or an unusual variant of epithelioid smooth muscle tumors exhibiting HMB45 positivity.

Folpe et al. (1) have classified PEComa into "benign", "uncertain malignant potential" and "malignant" categories based on tumor size (> 5cm); infiltrative margins; high grade nuclear atypia and cellularity; mitotic index (>1 MF/50 HPF); necrosis: and vascular invasion. PEComas with nuclear pleomorphism and /or multinucleated giant cells only or size> 5 cm are "of uncertain malignant potential", while PECOmas with two or more worrisome features are considered to be "malignant" or at "high risk or aggressive behavior".

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CASE 8

Masaharu Fukunaga, M.D.

Case a 37-year-old female (gravida 2, para 2**) with a right ovarian tumor**

Clinical history the patients presented with lower abdominal pain. Physical examination, CT and EMR indicated a right ovarian tumor. Abdominal hysterectomy, bilateral salpingo-oophorectomy, omentectomy and pelvic lymph node dissection were performed. Serum calcium levels were within normal range. The patient died of the spread disease 4 months after the surgery.

Macroscopic findings The right ovary revealed a 15x9x8cm, yellowish white, soft solid tumor with prominent hemorrhage and necrosis. The left ovary showed 1cm solid tumor on the surface and there were numerous white nodules in the omentum.

Immunostaining Some tumor cells arranged in follicles or nests showed CAM5.2, CK7 and EMA immunostaining. The tumor was uniformly negative for inhibin- alpha, calretinin, CD99, and LCA.

Pathologic diagnosis small cell carcinoma of the ovary, hypercalcemic type.

Discussion: The tumor was composed of a proliferation of small to medium-sized round cell in a solid sheet or follicular arrangement. Follicles contained PAS-positive proteinacious material. The rumor cells had hyperchromatic round or oval nuclei and moderate amount of cytoplasm. Atypia was moderate. The stroma was fibrous but inconspicuous. Tumor necrosis and hemorrhage was prominent. The mitotic activity was 20/10 HPF. The small nodules in the left ovary and omentum revealed the same histology of the right ovarian tumor. The immunohistochemical studies confirmed an epithelial nature. Electron microscopical examination failed to reveal specific features to identify the cell type of the tumor. The tumor cells had desmosomes, moderate amounts of mitochondria and dilated rough endoplasmic reticulum. No neurosecretory granules were identified.

The patients with small cell carcinoma, hypercalcemic tumor have ranged from 14 months to 43 (mean, 24) years of age (1-6). Most patients present with signs and symptoms related to an abdominal or pelvic mass, but rarely the clinical presentation is related to the hypercalcemia. Approximately 66% of patients presented with hypercalcemia (2). Some studies have documented serologically the presence of parathyroid hormone-related protein (PTHrp). This type of ovarian carcinoma has a dismal prognosis. About 5% of the tumors have spread beyond the ovary at the time of laporatomy. The overall survival rate is approximately 16%.

The tumors are almost always unilateral, usually large, solid, soft and white. An important feature that is seen in about 80% of the tumors is follicles that vary from small to large. There is a variant of "large cell type" in which large cells have eccentric nuclei and dense globular cytoplasm (5.6).

This tumor is often confused with a granulosa cell tumor, adult type and the juvenile type (7, 8). Adult granulosa cell tumor is rare in the young. Small cell carcinoma has spread beyond the ovary at presentation, which would be unusual for either variant of granulosa cell tumor. In granulosa cell tumors, tumors are usually positive for inhibin-alpha and calretinin, but negative for EMA. These profiles are opposed to those of small cell carcinoma.

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CASE 9

Masaharu Fukunaga, M.D.

History: A 79-year-old, gravida 0, para 0, Japanese female presented with lower abdominal distention. CT revealed a left adnexal mass measuring 5.0 cm. In laboratory examinations before surgery, serum levels of CA125 and alpha-fetoprotein were elevated. Serum enzymes of hepatic function were all within normal limits. Intraoperatively, a solid mass in the left tube and small, masses in the pelvic cavity were found. No abnormalities were observed in the liver, gall bladder and stomach. No lymphoadenopathy was noted. A total abdominal hysterectomy, bilateral salpingooophorectomy, omentectomy and tumorectomy were performed. An extensive systemic examination revealed no other primary site. The patient is alive with no frank evidence of disease at 2 months after the surgery.

Macroscopic findings: the left tube contained an unencapsulated yellowish-white soft tumor measured 5.0 X 5.0 X 4.5 cm with extensive necrosis and hemorrhage.

Immunohistochemical studies: alpha-fetoprotein, CAM5.2, polyclonal CEA, EMA, alpha-1-antitrypsin, alpha-1-antichemothrysin, hepatocyte paraffin 1, albumin, WTI. (+). CK7, chromogranin, synaptophysin, CA125, vimentin: (-).

Diagnosis: Hepatoid carcinoma (with serous component) of the fallopian tube.

Discussion: The tubal tumor resembled histologically and immunohistochemically hepatocellular carcinoma There was a secondary minor tumor component, serous adenocarcinoma, which was not observed in the distributed sections. A panel of antibodies including polyclonal CEA, alpha-fetoprotein, hepatocyte paraffin 1 is needed to identify the hepatocytic differentiation. There has been only one reported case of tubal hepatoid carcinoma in the English literature (1). Its histogenesis and biologic behavior are controversial.

The differential diagnosis includes clinically and pathologically hepatoid yolk sac tumor (HYST) and metastatic hepatocellular carcinoma. HYST occurs in the ovary of younger patients and usually in the reproductive age group (2, 3). Hepatoid carcinoma occurs in generally older patients. The present tumor, which was of tubal origin with a focal serous carcinoma component, was not associated with germ cell neoplastic components or gonadal dysgenesis. Large polygonal cells histologically characterize HYST with abundant eosinophilic cytoplasm growing in compact masses separated by fibrous bands. In most cases, classic yolk sac tumor elements such as reticular pattern or Schillar Duval bodies are found al least focally in HYST (3). In this particular case the presence of serous carcinoma is a helpful finding in differentiation from metastatic hepatocellular carcinoma. Metastasis is only rarely an initial mode of presentation for hepatocellular carcinoma. The sharp drop in alpha-fetoprotein postoperatively offered convincing support for the diagnosis of a tubal hepatoid carcinoma.

Hepatoid carcinomas of the ovary sometimes accompany serous or endometrioid carcinoma components (4-6). These findings and the current tubal case indicate mullerian epithelial origin. In addition hepatocytic differentiation has also been described in ovarian sex cord-stromal tumors (7-9) and in germ cell tumors (10-12). The origin of the hepatoid components is yet to be determined in these neoplasms. It is interesting that most of the reported cases of hepatoid carcinoma are from Japan. There may be an ethnic or epidemiological factor.

Most of hepatoid carcinoma in the ovary, lung, urinary bladder and stomach had a poor prognosis (4-6, 13, 14). The levels of alpha-fetoprotein may be a useful tool for following up. The prognosis of tubal hepatoid carcinoma remains unknown because of the paucity of case (1). The follow-up term is limited in this case. The patient with tubal hepatoid carcinoma previously reported was alive 2 years after the operation. Comprehensive studies of tubal hepatoid carcinoma are needed to evaluate the prognosis.

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CASE 10

Thomas Krausz M.D.

Clinical History: 100-year-old male with a 6 x 2.8 x 2.5 cm retroareolar mass in the right breast. Simple mastectomy performed.

Pathology: This is a multinodular, highly cellular, compact but richly vascular variant of breast carcinoma with structural and cytological features suggestive of an endocrine tumor. The tumor is composed of both in situ and invasive components, although in places it is difficult to determine invasion without immunohistochemical study. The following description is based on examination of the whole tumor; thus some of the features may not be well represented on the submitted slide.
The striking feature of this neoplasm is its organoid appearance with palisaded cells outlining some of the neoplastic units. This essentially solid, compact tumor has a framework of vascular connective tissue permeating it, so that, in two dimensions, ramifying islands of connective tissue are enclosed by the tumor cells. Frequently, the amount of collagenous, focally hyalinized, fibrous tissue is small, so the blood vessels in these areas are the dominant stromal component.
The structural features include solid islands, pseudorosettes, palisades, ribbons, tubules and polypoid formations in distended ducts. There are also foci of adjacent papilloma.
Focally the tumor exhibits palisades and pseudorosettes. Palisades form a seam around some of the tumor lobules, while pseudorosettes are oriented around a central blood vessel, with or without accompanying connective tissue. The tumor cells forming the palisades and pseudorosettes exhibit a columnar shape, with elongated nuclei arranged towards the stroma. Anastomosing trabeculae and ribbons are also seen focally. The cells and their nuclei are arranged transverse to the axes of trabeculae. The trabeculae are two to three cells wide and show interlocking of the constituent cells. In these areas the nuclei are more elongated than the nuclei in the solid areas. The cytoplasmic granularity is also less obvious.
In places, there are glandular structures lined by columnar cells with basally located nuclei. The luminal margins of the tumor cells are focally truncated while elsewhere they show apical snouts. There are also polypoid projections into distended ducts in the form of solid tumor enclosing ramifying islands of vascularized connective tissue.
The solid structures lack the streaming pattern of epithelial hyperplasia, with which this tumor can be confused. The tumor cells are slightly larger than those of epithelial hyperplasia. They are mostly rounded or polygonal, sometimes slightly ovoid. Cell borders vary between indistinct and moderately sharp. The cytoplasm is pale eosinophilic to amphophilic and slightly granular. The nuclei are rather uniform, ovoid and either central or eccentric. The chromatin is finely dispersed with small, inconspicuous nucleoli. There are 4 mitoses/10 HPF.
Immunohistochemical study: >50% of the tumor cells were immunoreactive for neuroendocrine marker synaptophysin, while chromogranin was positive only in the minority of the cells. The tumor was positive for ER, PR and AR but negative for HER2/neu.

Diagnosis: (Neuro)endocrine ductal carcinoma in situ accompanied by an invasive component

Comments: Neuroendocrine tumors are well recognized in the gastrointestinal tract, pancreas and the lung. Morphologically similar tumors in the breast are rare and the acceptance of endocrine tumor as a distinct entity has taken several decades following its original description by Cubilla and Woodruff in 1977. For years endocrine breast carcinoma as an entity has been questioned by many because of the findings of argyrophilia and immunoreactivity by neuroendocrine markers in a range of breast carcinomas. The simple rule of endocrine surgical pathology that the correct diagnosis of a (neuro)endocrine tumor

requires the recognition of the typical structural and cytological characteristics of the neoplasm was ignored and the presence or absence of argyrophilia and/or expression of neuroendocrine immuno-markers was in focus. A distinctive group of breast carcinomas, frequently in an *in situ* form, that histologically resemble neuroendocrine tumors occurring in other anatomic location do exist (Cross et al, 1985; Tsang and Chan, 1996; Kawasaki et al, 2008). These neoplasms show evidence of endocrine differentiation on histochemical/immunohistochemical and ultrastructural grounds, although a grey zone between pure endocrine and non-endocrine tumors in the form of mixed exocrine-endocrine neoplasms in various organs, including the breast, has been pointed out (Volante et al, 2006). The 2003 edition of the WHO classification of breast and gynecologic tumors recognizes neuroendocrine breast carcinoma as a specific type of breast cancer on the basis of morphological features which are similar to those of neuroendocrine tumors of both gastrointestinal tract and lung. However, the second criterion for diagnosis, namely that "they express neuroendocrine markers in more than 50% of he cell population" is rather restrictive. Knowing that neuroendocrine breast carcinomas have a broad morphologic spectrum, and some, which have the typical endocrine morphology but show immunoreactivity in <50% of tumor cells by neuroendocrine markers are excluded from this group.

Recognizing this problem, the AFIP atlas (series 4) of tumor pathology on the mammary gland (Tavassoli and Eusebi, 2009), adopted the generic "old" WHO (Solcia et al, 2000) definition of endocrine neoplasms in the belief that endocrine tumors of the breast are similar to those recognized in other organs. In the AFIP atlas, endocrine tumors of the breast are discussed in the chapter of "carcinomas of low-grade malignancy" and subdivided into three major groups: (1) well differentiated endocrine carcinoma, (2) poorly differentiated endocrine carcinoma-small cell carcinoma, (3) mixed exocrine-endocrine carcinoma.

The WHO classification lists three subtypes: solid/carcinoid-like neuroendocrine carcinoma, small cell/oat cell carcinoma and large cell neuroendocrine carcinoma. Righi and colleagues (2010) proposed the classification of neuroendocrine breast carcinomas into five groups: (a) solid cohesive carcinomas with features reminiscent of carcinoid tumors, (b) alveolar carcinomas where alveolar-like structures separated by dense stroma; (c) small cell carcinoma, formed by diffuse growth of poorly-differentiated small-sized cells or slightly larger cells similar to those seen in Merkel cell carcinomas; (d) solid papillary carcinomas growing in expansile solid sheets supported by a rich fibrovascular stroma and producing variable amount of extracellular mucin; (e) cellular mucinous carcinomas in which the cellular component arranged in a cribriform fashion or solid islands within mucous lakes. They also point out several unresolved problems regarding the definition, the presence or absence of precursor lesion and the classification of breast carcinomas with neuroendocrine morphology but lacking the neuroendocrine immunophenotype.

The classification of some endocrine tumors under the title of "solid papillary carcinoma of the breast" (Maluf and Koerner, 1995) adds to the controversy and, in my opinion, may distract the surgical pathologist from appreciating the most typical variant of in situ endocrine carcinoma of the breast. I have the same reservations about the term "solid papillary carcinoma of the breast" as Tsang and Chan (1996): " First, this designation does not clearly indicate that it is an in situ process and may lead to misunderstanding or overtreatment. Second, here is little evidence of a relationship of this form of DCIS to the usual papillary form of DCIS. Third, "papillary" is not an accurate description since the primary growth pattern of this form of DCIS is solid rather than papillary; the papillary pattern observed in some cases is only due to cancerization of preexisting papillomas or polypoid protrusion into the larger ducts. In fact, a papillary pattern can be totally lacking."

Another interesting point about this case is that this occurred in a 100-year old male who happens to be still practicing academic medicine. Occurrence of such a tumor in an elderly male has been reported (Papotti 1993).
The submitted case illustrates the structural and cytologic features of both the in situ and invasive components of an endocrine breast carcinoma even though the immunoreactivity of >50% of the tumor cells was seen only by synaptophysin.

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CASE 11

Thomas Krausz M.D.

Clinical History: 54-year-old female with history of right adnexal mass who undergoes total abdominal hysterectomy and bilateral salpingo-oophorectomy. On gross examination both ovaries are enlarged and have a firm consistency (right ovary 15 x 9 x 8.5 cm, left ovary 8.5 x 5 x 2.5 cm). The external surfaces of both ovaries are smooth. The cut surfaces of both ovaries are similar and show replacement of the parenchyma by whorled yellow-tan to white tissue with rare microcysts (up to 2mm in diameter). Intraoperative consultation (frozen section) was requested by the clinician. The fallopian tubes and the uterus were grossly unremarkable. The submitted histologic slides show representative areas of the ovarian

tumors (in order to cut 200 slides three different tumor blocks were used, two from the right ovary and one from the left ovary).

Pathology: On microscopic examination, the right and left ovarian tumors are composed of tubulocystic and glandular structures which are lined by clear cells and imbedded in a dense, fibromatous ovarian stroma. At low power, the right ovarian tumor shows regions of varying cellularity, whereas the left ovarian tumor has a comparatively low cellularity throughout. Different regions of the neoplastic proliferation are separated by areas of morphologically unremarkable ovarian stroma, imparting a vaguely lobular appearance at low power.
The entire left ovarian tumor and a major component of the right ovarian tumor are comprised of a fairly uniform, low-grade adenofibromatous proliferation. The simple tubulocystic structures are evenly dispersed with only occasional glandular crowding. The cysts are round to oval with slight variablilty in size, and are lined by a single epithelial layer. The epithelium is composed of a mixture of flattened to cuboidal to low columnar cells. The small to moderately-sized nuclei are round to angulated, with occasional prominent nucleoli but no significant nuclear atypia. The cytoplasm is clear to pale eosinophilic and granular, and cell borders are distinct. The cyst lumens contain variable amounts of dense inspissated secretions which are faintly eosinophilic and have a granular or laminated texture. Stromal elements are condensed around the tubulocystic structures, showing increased cellularity and decreased collagen as compared to normal ovarian stroma. The stromal cells are uniform with bland, oval to spindle shaped nuclei and a moderate amount of pale eosinophilic cytoplasm, imparting a fibrothecomatous appearance.
In addition to the low-grade component described above, the right ovarian tumor also contains more complex regions with crowding of the tubulocystic structures. Here the cysts demonstrate a marked variability in size as well as architectural irregularities such as layering, budding, and crowding of the epithelium. The epithelial cells have enlarged nuclei, irregular nuclear membranes, and mild to marked nuclear pleomorphism with occasional mitoses present. Interspersed among the tubulocystic structures are a few solid nests and cords of neoplastic epithelial cells and rare single cells. The stroma is similar in appearance to that described in the low grade component. Within the high grade component in the right ovary is a single small focus of invasion.
Immunohistochemical studies showed variable expression of the proliferation marker Ki-67, from < 2% in the low grade areas to 30-40% in the more complex areas.

Diagnosis: Clear cell adenofibroma with borderline features and a single focus (< 5mm) of clear cell adenocarcinoma (right ovary); clear cell adenofibroma (left ovary).

Comments: In the past, histological and epidemiological observation suggested endometriosis as a precursor lesion to clear cell adenocarcinoma, a hypothesis which was subsequently confirmed by molecular genetic evidence (Jiang et al, 1998, Obata et al, 2000, Sato et al, 2000). However, a number of series examining benign and borderline clear cell adenofibromas documented numerous cases of clear cell carcinoma arising in adenofibromas, suggesting the existence of an additional carcinogenetic pathway (Bell et al, 1985, Roth et al, 1984, Sugiyama et al, 1997). In the past year, a number of studies have addressed the potential role of clear cell adenofibroma in the carcinogenesis of clear cell adenocarcinoma, which likely represents a novel pathway distinct from the endometriosis-associated pathway. In 2007, Yamamoto et al. first observed that cases of ovarian clear cell adenocarcinoma associated with an adenofibromatous component manifested different clinicopathologic characteristics from non-adenofibromatous clear cell adenocarcinoma. The adenofibroma-associated carcinomas demonstrated a higher frequency of histologically low-grade tumors, a

lower Ki-67 labeling index, and a better prognosis than non-adenofibromatous carcinomas. Additionally, whereas endometriosis was observed in 68% of the non-adenofibromatous clear cell carcinomas (36 of 53 cases), endometriosis was present in only 15% of the adenofibroma-associated clear cell carcinomas (2 of 14 cases). A separate study (Veras, 2009) also showed that 91% (50 of 55 tumors) of cystic clear cell carcinomas were associated with endometriosis while only 44% (8 of 18 cases) of adenofibroma-associated clear cell carcinomas were associated with endometriosis, but their other observations differed from those of the first group. Specifically, Veras et al. noted that adenofibroma-associated clear cell carcinoma (n=18) was more likely to be diagnosed in advanced stages and to have a poorer outcome than non-adenofibromatous carcinomas. The discrepancy in data might be partially due to the rare incidence of clear cell adenofibromas and inadequate follow-up data for stages higher than stage I. Regardless, we can conclude from both of these studies that adenofibromatous and non-adenofibromatous clear cell carcinomas seem to evolve along different pathways based on the frequency of association with endometriosis, histologic features, stage at presentation, and clinical behavior.

After these initial observations, Yamamoto et al (2008) proceeded to prove that clear cell adenofibroma could be the clonal precursor of clear cell adenocarcinoma. An allelotypic analysis of the two adjacent components from 14 cases found an identical loss of heterozygosity (LOH) pattern at one or more loci in all but one case. Further allelotypic analysis (Yamamoto et al, 2009) comparing adenofibroma-associated clear cell carcinoma to endometriosis-associated carcinoma showed a variable LOH pattern between the two groups, with a significantly increased frequency of LOH at 3p, 5q, and 11q in the endometriosis-associated carcinoma. These data support the presence of two distinct pathways in the carcinogenesis of ovarian clear cell carcinoma. One final study utilizing immunohistochemistry (Yamamoto et al, 2009) examined the expression of $p27^{Kip1}$ – interacting cell-cycle regulatory proteins ($p27^{Kip1}$, Skp2, Cks1, cyclin A, and cyclin E) in benign/borderline adenofibromas, adenofibroma-associated clear cell carcinoma, and endometriosis-associated clear cell carcinoma. Aberrant expression patterns (downregulation of p27, overexpression of Skp2 and cyclin A) were significantly more frequent in the endometriosis-associated clear cell carcinoma than the adenofibromatous carcinoma. This suggests that cell cycle progression is more rapid in the endometriosis-associated carcinoma, which may account for the distinct clinicopathological features of the two clear cell carcinoma subtypes.

The present case illustrates the progression from benign to borderline clear cell adenofibroma as well as the early transformation of borderline clear cell adenofibroma to clear cell adenocarcinoma. Endometriosis was not present in either ovary, supporting the recent distinction between the carcinogenic pathways of adenofibroma-associated clear cell carcinoma and endometriosis-associated clear cell carcinoma of the ovary.

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CASE 12.

Giovanni Falconieri M.D.

Clinical history

A 55 year-old woman undergoes mastectomy for a 6 cm mass. Specimen inspection shows a lobulated, firm tumor that, on cut surface, is white and fasciculated. No further therapy is administered and follow-up at 2 years is negative for tumor recurrence.

Microscopic description

Microscopic examination reveals a cellular tumor featuring a disorderly arrangement of spindle cells. The neoplastic elements have scant stainable cytoplasm, tapered nuclei, and frequent mitoses; they infiltrate the adjacent breast fat in a tentacular fashion. Tumor cells are negative for several antibodies directed against keratins (either low- or high-molecular-weight keratins), desmin, S100 protein, CD34, bcl2, and p63; they are positive for vimentin and actins. Stains for estrogen and progesterone receptors and Her2 are negative.

Diagnosis

High-grade myofibroblastic sarcoma of breast.

Discussion

Sarcomas arising in the breast are rare. The most common is angiosarcoma, especially arising within the background of previous radiation treatment for mammary carcinoma. Regarding sarcomas composed primarily of spindle cells, scattered reports addressing leiomyosarcoma

and peripheral nerve tumors have been published. These tumors appear similar to their counterparts arising elsewhere. Metastases to the breast from mesenchymal tumors arising in the somatic soft parts, including synovial sarcoma, have been reported as well.

Since these tumors are rare, it must be stressed that, before considering a diagnosis of primary mammary sarcoma, a careful case workup is necessary as to rule out the more common sarcomatoid spindle cell carcinoma. Other conditions featuring malignant spindle cells also enter the differential diagnosis and deserve brief consideration.

Spindle cell/metaplastic carcinoma is a highly lethal malignancy portending a poor outcome and requiring prompt, aggressive antitumor treatment. Phenotypic evidence of epithelial differentiation, usually comparable to that of grade III ductal carcinoma, may be recognized only focally or be totally absent. However, biphasic tumors featuring distinct dual neoplastic populations may occasionally be encountered (so-called carcinosarcoma). Metaplastic carcinomas are usually seen in adult women and present with locally advanced disease that may have spread to regional lymph nodes at the time of diagnosis. Characteristically, metaplastic carcinomas are positive for keratins and negative for estrogen and progesterone receptors as well as Her2. As with many triple-negative basal carcinomas, antibodies to p63 and high-molecular-weight keratin may decorate tumor cells.

A subset of metaplastic carcinoma has been recognized, featuring fibroblast-like cells growing in a fibrous-rich ground substance, hence the designation *fibromatosis-like metaplastic carcinoma.* This tumor is composed of spindle cells with mild to moderate nuclear atypia and seems to predominate in older women. The collagenized, scar-like stroma is reminiscent of that seen in ordinary fibromatosis. Unlike the case with metaplastic carcinoma, neither tumor necrosis nor a high level of mitotic activity is generally observed in fibromatosis-like carcinoma. Positive immunoreactivity for keratins is often required to support the diagnosis.

In the case at issue, tumor cells were arranged in a short storiform pattern. Tumor fascicles dissected the adjacent mammary tissue percolating within the breast fat and were composed of uncommitted fibrocyte-like cells immunopositive only for actins and vimentin. The ground substance was scant. Spindle cell carcinoma was ruled out because of the absence of epithelial differentiation, such as duct formation. In addition, no immunoreactivity for keratins or epithelial membrane antigen could be recognized. A relatively long follow-up free of tumor recurrence is unexpected with a metaplastic carcinoma. *Malignant phyllodes tumor* could be excluded based on the gross aspect of the lesion (no leaf-like structures) as well as on the total absence of any residual fibroepithelial lesion. Obviously, overgrowth of the mesenchymal component with cancellation of the phyllodes lobulation could prompt speculation about a possible true sarcoma ex-phyllodes tumor, which cannot be totally excluded. Periductal stromal lesions have recently been described as a distinct spectrum of mesenchymal mammary neoplasms, perhaps representing a *trait d'union* between true stromal sarcomas and phyllodes tumor. Like sarcomas, *periductal stromal tumors* may characteristically exhibit increased cellularity. However, these tumors most often show a cellular arrangement of spindle cells around dilated pseudocystic spaces, with or without hyperplastic changes. Finally, a number of gross and microscopic features are reminiscent of *dermatofibrosarcoma protuberans,* a tumor that often occurs in the trunks of young to adult women and may potentially involve the subcutaneous tissue of the breast. However, the location of the tumor in the mammary tissue proper, the lack of a classic short-storiform "cartwheel" pattern, along with neoplastic cell negativity for CD34 argue against that interpretation.

Suggested readings

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CASE 13.

Giovanni Falconieri M.D.

Clinical history

A quadrant biopsy is performed in a 55-year-old woman who has mammographic evidence of suspicious microcalcifications. Specimen inspection reveals poorly demarcated white–gray consolidations that, on cut surface, express yellowish comedo-like material.

Microscopic description

Breast sections feature gland units irregularly distended by a population of medium-sized to large epithelial cells with highly atypical nuclei. Central necrosis and microcalcifications are noticed. Tumor cells are discohesive, with amphophilic to slightly eosinophilic cytoplasm. No infiltrative component is recognized. Tumor cells are positive for high-molecular-weight keratins as well as estrogen and progesterone receptors; they are negative for E-cadherin. Cells positive for p63 regularly decorate the basal layers of the affected units.

Diagnosis

High-grade pleomorphic lobular carcinoma in situ (PLCIS) with comedonecrosis.

Discussion

In most cases, recognition of classic lobular carcinoma in situ (LCIS) is prompted by a number of histologic and cytologic features, including distention of terminal tubulolobular units by fairly uniform, small to medium-sized epithelial cells. Tumor cells are discohesive and show mild nuclear atypia; mitoses are rare. LCIS is positive for both ER/PR and negative for e-cadherin; it tends to express a greater amount of high-molecular-weight keratins. The diagnosis of LCIS has several clinical implications: notably, it is considered a marker of increased risk for invasive breast cancer, either ipsi- or contralaterally, but its incidental recognition in core needle biopsy specimens is not generally considered an indication for

further surgery. In fact, unlike ductal carcinoma in situ (DCIS), LCIS documented at resection margins is managed conservatively by means of tamoxifen and/or follow up.

Variants of LCIS have been reported, mirroring a number of architectural and/or cytologic changes. Tumor cells may be of larger size, show increased variation of cell shape and size, and may exhibit variable nuclear pleomorphism (e.g., two- to threefold variation in nuclear size with irregularity), an increased nuclear/cytoplasmic ratio, and prominent nucleoli. These lesions are also referred to as pleomorphic LCIS (PLCIS). In addition, tumor-cell necrosis (so-called comedonecrosis) is often encountered. Tumor cells may also feature a relatively abundant, stainable, or "apocrine" cytoplasm (PALCIS).

Because of their different clinical implications, these variants of LCIS must be distinguished from high-grade (or grade III) DCIS, in particular when they are associated with comedonecrosis.

On morphologic grounds, it should be pointed out that PLCIS still maintains the basic microscopic features of conventional lobular neoplasia and that several features militate against DCIS. Although at a first glance necrosis, microcalcification, and high-grade nuclei suggest intraductal carcinoma, discohesion of tumor cells is a clue to lobular neoplasia. On the other hand, comedonecrosis is not a distinctive features of DCIS, since it can be seen in several other proliferative conditions of the breast, such as classic LCIS or florid papillomatosis. Like common LCIS, the high-grade variant is also consistently positive for ER/PR and negative for e-cadherin, whereas a reverse immunostaining pattern is generally expected in high-grade DCIS. These features enable a distinction between the two conditions, although some "overlapping" microscopic entities (i.e., "indefinite" in situ carcinoma) have been reported. Thus there is a phenotype between DCIS and LCIS, including "packing" of tumor cells and heterogeneous e-cadherin staining. It is also possible that some such cases might represent true mixed tumor, thus indicating that the diagnoses of DCIS and LCIS are not mutually exclusive. It is suggested that in situ tumors with a mixed phenotype be treated as DCIS. On the other hand, the management of patients with PLCIS is still controversial, with informed opinions recommending either a conservative approach (along to the lines commonly adopted for classic LCIS) or more effective surgical measures. A number of drawbacks (including the paucity of series, limited data, lack of prospective and clinically validated studies) preclude firm conclusions. In addition it is not known whether the breast cancer risk (level and laterality) associated with this lesion is comparable with that of conventional LCIS. Until the clinicopathologic features of PLCIS are more comprehensively outlined, treatment should be probably more "DCIS-tailored." Furthermore, surgical excision of the entire lesion or a quadrant biopsy is advised in cases of PLCIS diagnosed on core needle biopsy, since there is a significant chance of an associated invasive component.

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SESSION II

CASE 14

Andrew L. Folpe, MD

Sclerosing rhabdomyosarcoma (SRMS) is a very recently described variant of rhabdomyosarcoma, which may closely mimic chondrosarcoma, osteosarcoma, or even angiosarcoma, by virtue of its abundant hyalinized matrix, pseudovascular change, and inconspicuous rhabdomyoblastic differentiation. SRMS was described first by Mentzel and Katenkamp in 2000, in a series of 3 cases (1). We reported 4 additional cases of this rare entity in 2002 (2). Most recently, 14 additional cases of SRMS have been reported in children, in two reports (3, 4).

Microscopically, SRMS are highly infiltrative lesions that display an abundant, hyaline matrix that varies from eosinophilic to basophilic and closely resembles primitive osteoid or chondroid (although calcification and lacunae are absent). This stroma often comprises most of the tumor mass and divides the neoplastic cells into lobules and small nests similar to those seen in primitive chondroid tumors, and single-file arrays reminiscent of those seen in sclerosing osteosarcoma or sclerosing epithelioid fibrosarcoma. The cells of SRMS are primitive appearing, with a minute amount of eosinophilic cytoplasm, irregular nuclear contours with coarse chromatin, and small, occasionally multiple nucleoli. Although a microalveolar pattern is often present, the distinctive large alveolar pattern characteristic of ARMS is not seen. Occasional cases may form anastomosing cords, reminiscent of an

angiosarcoma. Strap-like rhabdomyoblasts, as seen in embryonal RMS (ERMS) are found focally in a minority of cases; multinucleated tumor giant cells and clear cell change, as seen in alveolar RMS (ARMS), are not seen.

By immunohistochemistry, SRMS typically show only focal desmin expression, sometimes with a "dot-like" pattern, but are strongly smooth muscle actin positive (a feature of primitive rhabdomyoblasts). As illustrated by our initial case, it is critical not to ignore this very focal desmin expression or regard it as anomalous. Although desmin expression may be seen in a variety of non-myogenous tumors, including Ewing sarcoma, neuroblastoma, melanoma, tenosynovial giant cell tumor and ossifying fibromyxoid tumor of soft parts, the possibility of true myogenous differentiation should always be excluded with immunostains for myogenin and MyoD1, prior to regarding any desmin expression as anomalous. Myogenin expression is typically seen only focally, in contrast to MyoD1, which is strongly expressed in nearly all tumor cells. Occasional SRMS may express CD99; S100 protein and cytokeratin expression are absent.

It is currently unclear where SRMS belong in the current classification of RMS, although most evidence supports a close relationship with ERMS. We initially considered our cases probable variants of ARMS, because of their occurrence in the extremities of adults, the variably present microalveolar pattern, and the primitive round cell morphology. However, SRMS lack well-formed pseudoalveoli containing discohesive cells and have abundant chondroid or osteoid-like matrix, rather than fibrovascular septae, as seen in ARMS. Additionally, the presence of "strap cells" is typical of ERMS and not a feature of ARMS. The presence of only focal myogenin expression is also much more in keeping with ERMS, in that ARMS are invariably strongly myogenin positive. Finally, the ARMS-associated t (1;12) or t (2;12) fusions have not been identified in any of the small number of SRMS tested.

The differential diagnosis of SRMS includes osteosarcoma, extraskeletal myxoid chondrosarcoma, mesenchymal chondrosarcoma, sclerosing epithelioid fibrosarcoma, and angiosarcoma. Sclerosing OS can be distinguished from sclerosing RMS by virtue of matrix calcification, the frequent presence of osteoclasts, the often epithelioid cytomorphology of the malignant osteoblasts, and the frequent co-existence of other patterns of OS, such as chondroblastic or telangiectatic OS. Extraskeletal myxoid CS typically show growth of bland eosinophilic cells in cords and chains, and lack the densely hyalinized matrix and highly malignant 'small round cells" seen in sclerosing RMS. Mesenchymal CS typically show an admixture of primitive round cell areas with nodules of well-differentiated cartilage, and often display a prominent hemangiopericytoma-like branching vasculature. Well-differentiated cartilage with clear-cut lacunae and branching vasculature are not seen in sclerosing RMS. Those areas of sclerosing RMS that show individual cells and cords of cells embedded in a hyalinized matrix may closely simulate sclerosing epithelioid fibrosarcoma. Unlike RMS, however, SEF invariably show at least focal areas of more typical fibrosarcoma, often resembling low-grade fibromyxoid sarcoma. Lastly, angiosarcoma may be distinguished from pseudovascular sclerosing RMS by virtue of its nested growth and occasional presence of rhabdomyoblasts, as well by the absence of diffuse infiltration of surrounding tissues by anastamosing vascular channels.

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CASE 15

Andrew L. Folpe, MD

Ossifying fibromyxoid tumor of soft parts

OFMT is an extremely rare mesenchymal tumor that may occur in essentially any location, usually in adults. OFMT present as relatively small, painless masses, often with a radiographically apparent shell of bone. Typical OFMT are characterized by a peripheral shell of bone in 70% of cases, lobulated growth, and small, bland cells arranged in cords and nests within a fibromyxoid stroma. The stroma of OFMT varies from highly myxoid to fibrous, and may on occasion hyalinize and calcify. A very characteristic feature of OFMT is its even and regular cell-cell spacing. Mitotic activity is usually very low. S100 protein is expressed by over 70% of typical OFMT and by a smaller percentage of atypical and/or malignant OFMT (see below). A minority of OFMT will show focal expression of cytokeratins, smooth muscle actin or desmin. Some OFMT may also rarely express other markers of putative nerve sheath differentiation, such as CD57 (Leu 7), “neuron-specific” enolase, and glial fibrillary acidic protein.

Malignant OFMT maintain the overall cytoarchitectural features of benign OFMT, but show accentuated lobularity, greatly increased cellularity with nuclear overlapping, coarse chromatin and prominent nucleoli, necrosis, vascular invasion and mitotic activity of > 2/50 HPF (Fig 17-18). Prominent spindling or extensive stromal hyalinization may be present. Bone production may either be absent or may be increased, sometimes within the center of the lesion.

The initial description of OFMT by Enzinger et al emphasized what might be thought of as “typical OFMT” inasmuch as all of the cases were circumscribed, of low cellularity and low nuclear grade, lacked necrosis or vascular space invasion and had mitotic rates of 1-2/ 10HPF (with one exception). Local recurrences occurred in 7 of 41 cases with follow-up information and were generally similar to the primary lesion with the notable exception of 2 cases that showed increased cellularity and mitotic activity. One of these recurrent lesions was described as showing “a transition to a well-differentiated osteosarcoma”. That case was characterized by moderately increased cellularity and cytologic atypia and increased centrally placed hyaline matrix, but maintained the overall cytoarchitectural features of an OFMT. The other patient whose recurrent lesion showed increased cellularity suffered a contralateral soft tissue metastasis.

In the years immediately following that initial publication a number of additional series and cases were reported that for the most part described OFMT with typical histologic features and a benign clinical course. However, unquestionable examples of OFMT were also reported that either appeared histologically malignant or produced metastasis. For example, Yoshida et al first reported a tumor that lacked overtly malignant features but produced local recurrence, distant soft tissue metastasis and death. Kilpatrick and colleagues reported a series of 6 atypical OFMT, one of which appeared overtly malignant, four of which showed lesser degrees of atypicality, and one of which was a histologically typical OFMT that both recurred locally and metastasized to the lungs. Zamecnik et al described 3 histologically malignant tumors in a series of 17 OFMT, 2 of which developed recurrences and one of which metastasized to the lungs. In 2003 we published a series of 70 OFMT, noting local recurrences and metastases in 12% and 4% of "typical" OFMT (those with low nuclear grade, low cellularity and a mitotic rate < 2/50 HPF), as compared with 60% and 60% of "malignant" OFMT (those showing high nuclear grade or a combination of high cellularity and mitotic activity > 2/50 HPF). "Atypical" OFMT, defined as those tumors deviating from "typical" OFMT but not meeting criteria for "malignant" OFMT showed similar outcome to "typical" OFMT. Most recently, Miettinen and Fetsch have examined a very large series (104 cases) of purely typical OFMT (excluding all cases with any atypical features) and noted a local recurrence rate of 22%, but no metastases. Putting all of this together, it would appear that entirely banal-appearing OFMT have an approximately 15% risk of local recurrence and a 5% metastatic risk, supporting their reclassification by the WHO as mesenchymal tumors of intermediate/borderline malignancy. Malignant-appearing OFMT behave as high-grade sarcomas. Within the group of histologically typical OFMT, there are no histologic features that are predictive of metastasis.

The differential diagnosis of OFMT includes epithelioid schwannoma, epithelioid MPNST, mixed tumor/myoepithelioma, extraskeletal myxoid chondrosarcoma, and most importantly, osteosarcoma. Epithelioid schwannomas lack the bone shell and extremely uniform cell-cell spacing seen in OFMT, and often arise adjacent to a nerve. Epithelioid malignant peripheral nerve sheath tumors show much greater cytologic atypia than do OFMT, resembling melanoma. Mixed tumors/ myoepitheliomas do not produce a bone shell, usually show epithelial differentiation, and express epithelial markers, such as cytokeratins much more often than do OFMT. Extraskeletal myxoid chondrosarcomas contain distinctly eosinophilic cells that grow in nests, cords and chains, often with abundant associated hemorrhage and hemosiderin deposition. Osteosarcomas lack a lobular growth pattern, show much greater cytologic atypia and pleomorphism than do even malignant OFMT, and often produce abundant lace-like osteoid, as well as malignant-appearing chondroid matrix. It should be emphasized that malignant OFMT, which may produce an osteosarcoma-like calcified matrix, maintain the overall cytoarchitectural features of typical OFMT and often arise within pre-existing typical OFMT.

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CASE 16

Andrew L. Folpe, MD

Alveolar Soft Part Sarcoma

Historical Aspects

Credit for the original description of ASPS traditionally falls to Christopherson, then a fellow in surgical pathology, working under the direction of Drs. Foote and Stewart. In this seminal series of 12 cases culled from the archives of Memorial Sloan Kettering Cancer Center over a 17 year period, Christopherson and colleagues coined the descriptive term "alveolar soft part sarcoma" for a distinctive and hitherto unrecognized soft tissue tumor. This tumor typically occurred in the extremities of young women, followed a prolonged clinical course with frequent late metastases, and was defined histologically by the presence of an organoid to pseudoalveolar pattern and large, eosinophilic tumor cells. Interestingly, Christopherson et al noted that several examples of ASPS appeared to have been previously published under a variety of other names, including "malignant myoblastoma", "granular cell myoblastoma", and "malignant granular cell myoblastoma". Although Christopherson and co-workers did not, in fact, describe the intracytoplasmic crystalline structures that have come to be one of the hallmarks of ASPS, they did quote an unpublished letter from Pierre Masson at the very end of the Discussion, who noted that "trichrome stains show erythrophylic and cyanophilic inclusions. The latter have in places a crystalline structure". Credit for the seminal description of these crystals thus belongs to Dr. Masson, who also illustrated a PAS stain of them in his 1956 text, *Tumeurs Humaines*.

Apparently unknown to Christopherson and colleagues, ASPS had in fact been described one year previously, by Smetana and Scott in a series of 14 cases retrieved from the archives of the Armed Forces Institute of Pathology, as "malignant tumors of nonchromaffin paraganglia". Careful reading of this study leaves little doubt that the cases reported by these authors were uniformly histologically and clinically identical to those later reported by Christopherson et al. Smetana and Scott chose the term "malignant tumors of nonchromaffin paraganglia" based on the resemblance of their tumors to non-physiologically active paraganglia, postulating that primitive paraganglia-like structures might perhaps normally occur in the somatic soft tissues (a hypothesis since discredited). Smetana and Scott also independently noted the crystals of ASPS, writing that "within the cytoplasm of some of the neoplastic cells were rod-shaped, coarse, basophilic bodies of unknown nature…"

Histopathological Features

ASPS varies little in its appearance from case to case, and is in some respects remarkable among soft tissue neoplasms for the absence of described "variants". As originally described by Christopherson and co-workers (and by Smetana and Scott), ASPS is characterized by uniform, organoid nests of polygonal tumor cells, separated by fibrovascular septa and delicate capillary-sized vascular channels. Within these nests, there is prominent cellular discohesion, leading to the distinctive pseudoalveolar pattern for which it is named. The organoid appearance may be completely lost and the tumor may be composed of sheets of epithelioid cells. Tumors occurring in younger patients and in confined locations, such as the tongue, often show very small nests of cells, closely mimicking true paragangliomas. Intravascular tumor extension is present in most cases. A small minority of tumors show unusual features such as myxoid change, cystic change, hemorrhage, and a prominent lymphocytic infiltrate.

The lesional cells have distinct cell borders and abundant eosinophilic to clear, somewhat granular cytoplasm resulting in an epithelioid appearance. Clear cell change may be very prominent, closely mimicking renal cell carcinoma. Cross striations and cytoplasmic fibrils are absent. A characteristic finding is the presence of PAS-positive, diastase-resistant crystalline structures, which may be rhomboid, rod-like, or spiked, in individual, sheaf-like or stacked configurations. These crystals can also be identified with alcian blue and trichrome histochemical stains. It should be recognized that these crystals are not especially prominent in all cases, and that their identification may require careful searching of multiple sections. The cells of ASPS typically have round, regular, eccentrically placed nuclei with vesicular chromatin and a prominent central nucleolus; multinucleation may be present in a minority of cells. Mitotic activity is usually low and necrosis is infrequent.

Ultrastructural Features

The cells of ASPS typically rest on an incomplete basement membrane and contain only rare, primitive cell-cell junctions. The cytoplasm contains numerous mitochondria and an extensive Golgi complex with adjacent small dense granules. The endoplasmic reticulum is usually, but not always sparse. The distinctive crystals of ASPS are typically found intermingled with these dense granules. They are often bounded by a single membrane and are composed of a periodic latticework of fibers (diameter, 4.5 to 5 nm and periodicity, 10 nm). The composition of these crystals was interpreted as a product similar to renin or aggregates of actin filaments and it was suggested that they could be similar to the inclusions seen in rhabdomyoma or nemaline rod myopathy. However, elegant recent work by Ladanyi and co-workers, including ultrastructural immunohistochemistry, has convincingly demonstrated them to consist of aggregates of the monocarboxylate transporter protein MCT1 and its cellular chaperone, CD147.

Immunohistochemical Findings

Until very recently, immunohistochemistry has played a very limited role in the diagnosis of ASPS. A large number of studies have examined ASPS by immunohistochemical means, both with and without the use of epitope retrieval techniques, often with differing results. In general, ASPS are negative for epithelial markers, such as cytokeratins and epithelial membrane antigen, negative for specific neuroendocrine markers such as chromogranin A and synaptophysin, and negative for specific melanocytic markers, such as HMB45 and Melan-A. Non-specific markers such as neuron-specific enolase and vimentin may be present in roughly 30-50% of cases. There has been a great deal of interest in the expression of muscle-related proteins in ASPS, due to the prevailing theory for many years that ASPS represented an unusual form of myogenic tumor. Antibodies to pan-muscle actins, smooth muscle actins and skeletal muscle actins have been reported to be positive in nearly 50% of ASPS. It should be

noted that pan- and smooth muscle actin expression is by no means specific for myogenous differentiation, and that the ASR-1 antibody to skeletal muscle actins is notoriously difficult to interpret due to high levels of non-specific staining. Desmin expression has been reported in close to 50% of ASPS, although it tends to be present in only a small number of the neoplastic cells. Again, it should be emphasized that desmin expression is not limited to myogenous tumors, and can be seen in a wide variety of other lesions, including melanoma, tenosynovial giant cell tumor, Ewing sarcoma and angiomatoid "malignant" fibrous histiocytoma, among others.

A much more controversial area has been whether ASPS express truly specific markers of skeletal muscle differentiation, such as the myogenic nuclear regulatory proteins MyoD1 and myogenin. In 1991, Rosai et al. reported a case of ASPS, which appeared to show convincing MyoD1 expression by immunofluorescence and Western blot analysis on fresh–frozen tissue. This was followed by the report of Tallini et al. of a MyoD1-positive ASPS, using immunohistochemistry on frozen sections. Regrettably, these two early findings have not been substantiated by any subsequent study. Three separate studies, utilizing commercially available antibodies and modern immunohistochemical techniques, including heat-induced epitope retrieval, have not identified expression of either MyoD1 or myogenin in the over 35 studied cases.

As described in detail in the next section of this review, It has recently been discovered that ASPS are characterized by a tumor specific der(17)t(X;17) (p11;q25) that fuses the *TFE3* gene at Xp11 to the *ASPL* gene at 17q25, creating an ASPL-TFE3 fusion protein. Recently, an antibody directed against the carboxy-terminus of the TFE3 transcription factor has emerged as a highly sensitive and specific marker of ASPS. Although TFE3 appears to be almost universally expressed in normal tissues, this expression is at very low levels, and strong nuclear expression of TFE3 is seen almost exclusively in tumors known to contain TFE3 gene fusions, such as ASPS and rare pediatric renal carcinomas. It must be emphasized that only nuclear expression of TFE3 is of diagnostic value, as cytoplasmic staining (possibly non-specific) is seen in a variety of tumors. The TFE3 antibody binds to the protein downstream of exon 4 and therefore is positive in both fusion variants seen in ASPS (see below). Importantly, however, strong TFE3 is seen in granular cell tumors, a potentially confusing and somewhat ironic finding, given the original classification of ASPS as "malignant granular cell myoblastoma" (malignant granular cell tumor).

Cytogenetic and Molecular Genetic Features

The earliest cytogenetic analysis of ASPS identified multiple structural and numerical abnormalities, strongly suggesting a role for chromosomal abnormalities in the pathogenesis of this tumor. Subsequent karyotypes identified translocations between chromosomes X and 17 and, in 1998, both breakpoints were identified: 17q25 and Xp11.2 (42). Subsequently, Ladanyi et al. characterized the two genes involved in the translocation.

Alveolar soft part sarcoma is characterized by an unbalanced translocation: der(17)t(X:17)(p11;p25). This translocation results in the fusion of a gene of unknown function, *ASPL* (alveolar soft part sarcoma locus), on chromosome 17 to the *TFE3* (transcription factor 3) transcription factor gene on the X chromosome. Consequently, the *ASPL* gene is joined in-frame upstream of either the third or fourth exon of *TFE3*, yielding two fusion variants, type 1 and type 2, respectively (Figure 10). Usually, fusion genes are generated by reciprocal balanced translocations with only one of the 2 chimeric genes being pathogenic. The translocation in ASPS is unusual in that it is unbalanced, although two cases with a reciprocal translocation have been described. It has been suggested that the female

predominance seen in patients with ASPS is due to the presence of two X chromosomes in these patients, increasing their chances of a translocation involving this chromosome.

Clinical Features and Behavior

ASPS is extremely rare, and is generally thought to account for less than 1% of soft tissue tumors overall. ASPS has, however accounted for roughly 10% of cases reported from large sarcoma referral centers, in both adults and children. Approximately 60% of ASPS occur in women, although this female sex predilection may be less pronounced in pediatric patients. The tumor typically occurs in the deep soft tissues, most often in the buttock and thigh, with a smaller number of cases involving other soft tissue locations such as the arm, chest and retroperitoneal tissues. In children, a substantial percentage of cases occur in the head and neck, often in the orbit or tongue. ASPS have been reported in a very wide variety of locations, including the bladder, stomach, gynecologic tract and bone. Most often ASPS present as painless masses, which may be highly vascular on imaging studies.

ASPS behave as relatively indolent, but relentless sarcomas, characterized by late metastases and an extended clinical course: survival rates of 77% at 2 years, 60% at 5 years, 38% at 10 years, and only 15% at 20 years were reported by Lieberman and co-workers. Another large series from the MD Anderson Cancer Center noted a 5-year disease free survival of 71% in patients presenting with localized disease, as compared with only 20% in patients presenting with metastases. The prognosis for pediatric patients with ASPS may be considerably better, with Pappo et al reporting disease free survival in 9 of 11 cases (with relatively short follow-up duration), and Casanova et al reporting 100% survival at >5-year follow-up duration in 12 patients with localized disease at presentation. Lingual and orbital tumors also have very high survival rates, likely reflecting a combination of small size at time of diagnosis and younger patient age.

There are no histopathological features that are predictive of prognosis in patients with ASPS, and these tumors should not be graded under either the NCI or French grading schema. Features associated with improved prognosis include younger age at diagnosis, small tumor size, and the presence of localized disease. ASPS most frequently metastasize to lungs, brain and bone. Metastases, sometimes to unusual locations such as the breast, may be the presenting symptom in some patients with ASPS. Adjuvant chemotherapy does not appear to be effective in the treatment of ASPS, although there may be some role for adjuvant radiotherapy in reducing the risk for local recurrences.

Line of Differentiation

The “cell of origin”, or better “line of differentiation” taken by ASPS has been the subject of considerable speculation and controversy over the past 50 years. As noted above, ASPS were originally regarded as malignant variants of “granular cell myoblastoma”, a term that encompasses the entity now known as granular cell tumor. The speculation that ASPS represented unusual malignant tumors of non-chromaffin paraganglia has subsequently been disproved by a number of ultrastructural, histochemical and immunohistochemical studies, documenting the complete absence of neuroendocrine differentiation in ASPS. Similarly, the suggestion that ASPS were “malignant angioreninomas” has been disproved by negative immunohistochemical studies for renin, as well as by the absence of clinical signs of hyperreninism in patients with ASPS. Perhaps the longest lived hypothesis has been that ASPS are an unusual myogenous tumor, based on some ultrastructural similarity of component of the ASPS crystal to actin filaments, overlap between the appearance of these crystals and those seen in nemaline myopathy and rhabdomyoma, and variable expression of muscle-related proteins, such as actins and desmin. However, as discussed above (Immunohistochemical Findings), the specificity and significance of these

immunohistochemical findings have been questioned, and there is not convincing evidence of expression of truly muscle-specific proteins in ASPS.

In light of the recent discovery that ASPS is part of the family of translocation-associated sarcomas, there exists an additional hypothesis- namely that ASPS is displaying a "scrambled phenotype", without a normal counterpart. This would seem to be a compelling hypothesis, given the lack of resemblance of ASPS to any known structure, its resolute defiance of all attempts to categorize it, and our increasing understanding that a subset of human neoplasms, such as perivascular epithelioid cell neoplasms, likely lack normal counterparts. Alternatively, it cannot be entirely excluded that ASPS recapitulates the phenotype of a yet-to-be discovered, extremely rare, normal cell of unknown function.

Differential Diagnosis

The differential diagnosis of ASPS is fairly broad, and revolves around neoplasms that may show nested/organoid patterns of growth and cells with abundant eosinophilic cytoplasm. Renal cell carcinomas, adrenal cortical carcinomas, and hepatocellular carcinomas may mimic ASPS by virtue of their abundant eosinophilic to clear cytoplasm. Immunohistochemical demonstration of strong cytokeratin expression, expression of site-associated markers (e.g., "renal cell carcinoma antigen" in renal cell carcinoma, Melan-A cross-reactivity in adrenal cortical carcinoma, HepPar1 in hepatocellular carcinoma), and absence of TFE3 expression should assist greatly in this differential diagnosis. Pediatric renal cell carcinomas with *TFE3* gene fusions may show absent cytokeratin expression and TFE3 expression; clinical correlation as to the presence of a renal mass is critical in this situation. Malignant melanoma may simulate ASPS, but should be easily separated with immunohistochemistry for S100 protein, HMB45 and Melan A. Paragangliomas show strong chromogranin A and synaptophysin expression, unlike ASPS. Alveolar rhabdomyosarcoma, despite its somewhat similar name, is an entirely different appearing "small blue round cell tumor", which strongly express desmin, and myogenin nuclear regulatory proteins. Perivascular epithelioid cell neoplasms, such as epithelioid angiomyolipoma, co-express smooth muscle actin and melanocytic markers, and are almost always TFE3-negative. Granular cell tumors lack the striking cytologic atypia seen in ASPS, in most instances, and show strong S100 protein expression. TFE3, however, may be expressed by granular cell tumors, a potential pitfall.

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CASE 17.

Michele Bisceglia M.D.

Clinical history

A 26 year-old male, who had sustained progressive fatigue, muscle weakness, diffuse bone pain, and vertebral fractures for 3 years and was wheel-chair bound for 1 year, was admitted to our hospital in February 2007 for endocrinologic investigation. He had been hospitalized elsewhere because of hypophosphatemic rickets, obesity, diabetes mellitus type II, arterial hypertension, and hypogonadotropic hypogonadism in 2004 and 2005. He was prescribed testosterone, growth hormone, 1,25-dehydroxyvitamin D (aka 1-25-dehydroxy cholecalciferol or calcitriol, the active form of vitamin D3 / cholecalciferol) and vitamin D2 (ergocalciferol) without any clinico-radiologic benefit on his mineral bone metabolism. The patient had no family history of rickets or bone disease.
Laboratory investigation in February 2007 confirmed previously documented hypophosphatemia (1.26 mg/dL, reference range 2.7 - 4.5), hyperphosphaturia as indicated by decreased maximum transport of phosphate in renal proximal tubules ($TmPO_4$/GFR, 1.7; normal range 2.5 - 4.2), high serum total alkaline phosphatase (304 u/L, reference range 40 - 129), normocalcemia (calcium-albumin adjusted: 9 mg/dL, reference range 8.1 - 10.4), and – additionally - slightly elevated serum level of parathormone (73.3 pg/mL, normal range 10 - 65). Despite medical treatment, serum level of calcifediol (aka calcidiol, 25-hydroxycalciferol or 25-hydroxivitamin D, the best indicator of blood vitamin D status) was very low (9.9 ng/mL, reference range > 32). Testosterone serum level was marginally low (3.05 ng/mL, reference range 3 - 9). Spine roentgenograms demonstrated osteopenia, thoracolumbar vertebral fractures. Bone scintiscan revealed costochondral uptake suggestive of rachitic rosary. Whole body octreotide scan and computerized tomography (CT) scan did not show any additional lesions. The patient was discharged home receiving phosphate, $1,25(HO)_2$-vitamin D, and calcium supplementation. He was re-admitted six months later. At this time, repeat total body scintigraphy with octreotide and total body CT scan disclosed small, low signal intensity in the heel of his right foot. Fluorodeoxyglucose positron emission tomography (^{18}F-FDG-PET) scan revealed high uptake in the soft tissue of the right calcaneal region. Ultrasonogram and magnetic resonance imaging (MRI) showed a 2.5 cm solid lesion in the subcutaneous panniculus of the right heel. The lesion was excised in September 2007. A preoperative blood sample from median cubital vein to determine serum level of fibroblast growth factor 23 (FGF-23) was submitted to the clinical laboratory. The soft tissue mass was sent to the anatomic pathology laboratory.

Pathologic Findings

A well demarcated 2.5 cm gritty soft tissue mass was surrounded by grossly unremarkable subcutaneous adipose tissue. Histopathologically, the lesion was made up of oval to short spindle-cells set in a collagenous matrix with diffuse lattice-like calcific deposits, focally reminiscent of a chondromyxoid lesion, and exhibited a rich capillary network, microhemorrhages, clusters of multinucleated giant cells, and foci of osseous metaplasia. Cell

pleomorphism was not a feature. Mitotic figures were scant (< 1/10HPF). The surgical margins were uninvolved.

Diagnosis
Phosphaturic mesenchymal tumor, mixed connective tissue type.

Follow-up
Just three days after excision of the right heel tumor, both his phosphatemia (3.38 mg/dL) and the ratio of maximum rate of renal tubular reabsorption of phosphate to glomerular filtration rate ($TmPO_4$/GFR, 2.8) normalized. His serum level of fibroblast growth factor 23 (FGF-23) was highly elevated preoperatively (100.7 pg/mL; reference range 10-50), but dropped to normal values (15.5 pg/mL) within 3 days, His postoperative course was uncomplicated and the patient was discharged 8 days after excision of a phosphaturic mesenchymal tumor. Regarding his bone mineralization status, he promptly and completely recovered.

Discussion

Phosphaturic mesenchymal tumor (PMT) is an uncommon tumor causing oncogenic osteomalacia by virtue of overexpressing fibroblast growth factor–23 (FGF-23), a protein capable of inhibiting renal tubular phosphate transport, that is the most important of the four known phospaturic factors of the "phosphatonin" family, which also includes the secreted frizzled related protein 4 (sFRP-4), the fibroblast growth factor 7 (FGF-7), and the matrix extracellular phosphoglycoprotein (MEPE), all acting upon the kidney to reduce phosphate reabsorption (1-7).
Although the first description reporting a case of osteomalacia in a patient affected by a (bone) tumor had been already described by McCance in late forties (Q J Med 1947;16:33-47) and the causal role for a tumor in this clinicopathologic syndrome was hypothesized by Prader et al in late fifties (Helv Pediatr Acta 1959;14:554-565) and subsequently confirmed in seventies by Evans and Azzopardi (Lancet 1972;1:353-354) and by Olefsky et al (New Engl J Med 1972;286:740-745), the term PMT was first coined in 1987 by Weidner and Santa Cruz (8). PMT has been reported under several names by referring pathologists both before and after 1987, with hemangiopericytoma being the most common rendered diagnosis. According to a 2004 review paper on this issue by Folpe et al, who revised the original pathological diagnoses, a total of 109 cases of PMT had been published up to 2002, to which they added 32 new cases on their own (9). Subsequently, based on a computerized world literature search (PubMed/Medline), from 2002 to the end of March 2010, using [oncocytic osteomalacia], [tumor-induced osteomalacia], and [phosphaturic mesenchymal tumor] as search terms, we found 108 additional cases of PMTs.

Usually patients with this tumor complain of a long history of bone pain, spontaneous fractures, and muscle weakness, dating back to several years (up to 15-20). Often they are so severely affected to be unable to walk and wheel-chair bound. The typical laboratory findings secondary to tumor-induced inhibition of renal phosphate reabsorption and consequent phosphate loss are hypophosphatemia and hyperphosphaturia, which finally result in an inadequate mineralization of osteoid in mature bone, the metabolic disorder known as *oncogenic osteomalacia/rickets* (1-5, 8, 10-11). Often patients have also low serum levels of 1,25-dihydroxyvitamin D3, probably due to the inhibiting effect by the phosphaturic factor on the renal 25-hydroxyvitamin D-1-alpha-hydroxylase, the enzyme converting 25-hydroxyvitamin D3 to the active metabolite 1,25-dihydroxyvitamin D3. However oncogenic osteomalacia is vitamin D resistant. Serum alkaline phosphatase is elevated. Other serum chemical analyses usually show normal serum calcium, normal parathormone and normal calcitonin (osteocalcin) levels, normocalciuria and normal kidney function. However

parathormone may occasionally be elevated, suggesting the development of a secondary hyperparathyroidism as a reaction to a long-term therapy with phosphate and vitamin D supplementation, occasionally progressing to a tertiary hyperparathyroidism, requiring parathyroidectomy. Hypophosphatemic rickets and osteomalacia are detected by skeletal radiographs and bone scintigraphy. By conventional imaging techniques (CT and MRI scans) usually but not always a tumor is discovered if accurately searched for, and recently a PET-CT scan has emerged as the most effective means of tumor detection even in cases in which other imaging studies have failed (12-13). Somatostatin receptor imaging has been recently proved to improve the detection of such tumors, based on the postulate that such tumors do express somatostatin receptors (14-15). Measurement of FGF-23 serum level is a helpful diagnostic clinical tool in tumor-induced osteomalacia, and the detection of high serum level in regional venous blood sampling may aid even in tumor localization (16-17).

PMT is usually located in soft tissue, but intraosseus as well as sinonasal locations are also on record. In Folpe et al's own series of PMTs 18 out of 32 total cases occurred in soft tissue, 9 in bone and 2 in paranasal sinuses, while from the 109 reviewed cases by the same authors as many 41 were soft tissue lesions, 59 bone lesions and 9 nasal/sinusal (9). In the most recent above said literature review for the years 2002-2010, 58 cases occurred in soft tissue, 24 in bone, 12 in nasal/paranasal cavities, 4 were adjacent to or involved the central nervous system coverings (2 intracranial; 2 intraspinal), and 3 were visceral (1 each in tongue, liver and uterus) out of 101 total cases for which the location was ascertained. Patients are usually in their adulthood, but pediatric cases are also on record (18). Age range was 5 to 63 years in the series of 17 cases reviewed by Weidner and Santa Cruz, and 9 to 77 years in the original series of Folpe et al. Out of 108 total cases found during the years 2002-2010, 2 occurred in pediatric age (1 case each at 2 and 15 years). Any site can be affected with the lower extremities as the most common (40-50% of cases), followed by the head and neck area (15-20%), trunk (15-20%) and upper extremities (around 10%) (19). Foot was affected in 4 of 32 total cases in Folpe's et al own series and in 6 of 109 cases reviewed up to 2002 by the same authors (9), and in 8 of 97 cases which were found in the literature in the 2002-2010 period. Tumor size is variable, ranging from 1 cm to 15 cm. The median size for soft tissue locations was of 5.6 cm in Folpe et al's series. In soft tissue grossly the tumor is quite circumscribed, of firm consistency and not infrequently endowed with a partial shell of metaplastic bone. On sectioning microcysts or hemorrhagic foci can also be seen.

At histology the most common morphological pattern of PMT is the so-called *mixed connective tissue type*, which was seen in 20 out of 21 of soft tissue cases in Folpe et al's original series and in 36 cases revised by Folpe et al from the available literature out of 41 PMTs of soft tissue previously published with sufficient histological illustrations (9). Thus the *mixed connective tissue pattern* (8,9,10), is to be intended as the prototype of the morphology of PMT, being characterized by a distinctive oval to bland spindle cell proliferation, embedded in a myxoid or myxochondroid matrix, with smudgy appearance and peppered by distinctive flocculent calcifications. A prominent fibrohistiocytic reaction, hemorrhages, hemosiderin deposits and osteoclast-like giant cells are usually seen. Giant cells may appear in groups, so at times vaguely recalling a giant cell tumor. A well-developed and variously sized vasculature is a constant feature, which in contrast to hemangioma and hemangiopericytoma, has the immunoprofile of lymphatic vessels as this was demonstrated using antibodies to lymphatic endothelial cell antigens (e.g. lymphatic vessel endothelial hyaluronan receptor 1 /LYVE-1) (20). Microcysts, osteoid-like matrix, woven metaplastic bone, adipocytic differentiation and sometimes some perivascular myoid changes are all in the spectrum of this polymorphic pattern. Conventional hemangiopericytoma has also been reported by Folpe et al as the causal tumor of oncogenic osteomalacia in one case.

In bone, the *mixed connective tissue variant* is also the most frequent histological pattern of PMT, having been recognized by Folpe et al in 25 out of 39 cases which had appeared in the literature with sufficient illustrations/data (9). Other histological variants/patterns occurring in bone (but not in soft tissue) are the *osteoblastoma-like tumor*, the *non-ossifying fibroma-like tumor* and the *ossifying fibroma-like tumor* (8-10), which on aggregate have been diagnosed by Weidner and Santa Cruz in 7 of 8 cases of bone PMTs of their revised series (8) and in 14 of 39 cases of bone PMT revised from the literature by Folpe et al (9). Other occasional different osteomalacia associated-tumor types seen in bone cases of PMT are enchondroma, hemangioma and osteosarcoma.
In sinonasal locations PMT usually exhibits a morphological pattern recalling the sinonasal-type hemangiopericytoma, however the classic mixed connective tissue type has also been recently reported (21-22).

Although PMT of mixed connective tissue type is mostly benign, with bland to low-grade cell morphology, absent necrosis, low mitotic index, and absent atypical mitotic figures, at times it is histologically malignant both in soft tissue and bone locations (*malignant connective tissue variant*) also with clinical recurrence and/or metastatic disease (pulmonary metastases) (9). The frequence of histologically malignant PMT is around 4-10% as assessed by Folpe et al with regards to both their own series of cases and cases from the literature (9). In our most recent literature review and based on the original diagnosis rendered by reporting authors the rate of frequency for malignant PMTs was 1%.

Immunohistochemically tumor cells are negative for neural, epithelial, vascular, neuroendocrine and hemato-lymphoid markers, including CD34. Muscle markers, including desmin, are negative, except alpha-smooth muscle actin, which occasionally has been found focally positive. Tumor cells are positive for vimentin, a feature which is common to any mesenchymal tumor, but are specifically immunoreactive with FGF-23 antibody (9, 11, 23), matrix extracellular phosphoglycoprotein (MEPE) (9), and dentin matrix protein 1 (DMP -1) (24). Expression by tumor of FGF-23 was also confirmed by RT-PCR both on frozen tissue (in 2 of 2 cases in which fresh tissue was available) and on paraffin embedded tumor tissue (in 16 of 17 cases). Few cases have been studied by electron microscopy with tumor cells showing an organelle-poor cytoplasm, devoid of any specific fine structure, thus appearing as primitive mesenchymal cells. However in 2 cases some suggestive neuroendocrine dense core granules were noted (21,25), and this raised the hypothesis that PMT might be a tumor of neural or neuroendocrine differentiation.

The differential diagnosis concerning with the clinicopathologic entity of oncogenic osteomalacia has many folds: the laboratory findings, the defective skeletal mineralization, the skeletal deformities (if any), the clinical discovery of a tumor, and the histopathological diagnosis. Serum and urinary laboratory findings, along with the defective skeletal mineralization should suggest in differential both X-linked hypophosphatemic rickets (XLH - Gene map locus Xp22.2-p22.1) (26) and autosomal dominant hypophosphatemic rickets (ADHR - Gene map locus 12p13.3) (27), two conditions apparently due to deranged genetic mechanisms. XLH is caused by inactivating mutations involving the PHEX gene, which encodes a membrane-bound endopeptidase, thus likely causing ineffective degradation of FGF-23 and resulting in increased FGF-23 serum levels. ADHR is associated with mutations of the gene encoding FGF-23, which leads to stabilization of protein and protects it from degradation. However, these two genetic syndromes have both an inheritance pattern and clinical onset in early childhood, characteristic enough to be distinguished from oncogenic osteomalacia.

If a tumor is discovered in a patient presenting with hypophosphatemic osteomalacia associated with skeletal deformities, then fibrous dysplasia of bone and/or McCune-Albright

syndrome should be considered in differential since in around 50% of these clinicopathologic conditions renal phosphate wasting occurs (3,28-29), and serum elevated FGF-23 levels have also been documented (29). Neurofibromatosis (3,9) is another systemic tumor syndrome occasionally found associated with hypophosphatemic osteomalacia due to deranged phosphatonin pathway.

Based on ASBMR criteria (including typical biochemical profile of hypophosphatemic osteomalacia, clinical symptoms of deranged skeletal mineralization, absence of personal or familial history of hypophosphatemic disorders in childhood, normal height at adult age, absence of skeletal deformities, previous documentation of normal fasting phosphate serum levels, exclusion of acquired Fanconi syndrome, and absence of FGF-23 gene mutation) a tumor-induced osteomalacia diagnosis (aka, oncogenic osteomalacia) can be firmly established even in absence of tumor discovery (30). Based on data resulting from our 2002-2010 review of the literature, in 7 of 107 total cases the tumor was not found and in several the tumor diagnosis was made after repeat hospitalization and despite careful clinicoradiological search.

Occasionally, non-mesenchymal tumors, such as breast, prostate and lung carcinomas, and multiple myeloma may be associated with oncogenic osteomalacia (3,9), and these also have to be excluded from the diagnosis referring to genuine PMT.

Histopathologically and especially in soft tissue, the differential diagnosis revolves around the mixed connective tissue variant and in this context includes special variants of benign fibrous histiocytoma, soft tissue hemangiopericytoma, soft tissue chondroma, soft tissue giant cell tumor, mesenchymal chondrosarcoma, extraskeletal osteosarcoma. The awareness of the PMT of the mixed connective tissue variant as a discrete histological entity should assist in the correct diagnosis.

Occasionally PMT of mixed connective tissue type without oncogenic osteomalacia can be encountered, probably due to secretion of inactive or insufficient FGF-23. Three of such cases out of 32 total cases have been included in Folpe's et al own series (9). A case has also been published in which osteomalacia appeared one year after discovery of a tumor which was treated by partial excision (31).

PMT is usually a benign tumor, and completed surgical removal is the treatment of choice which by definition is dramatically curative for the vitamin D resistant tumor-induced osteomalacia/rickets. In patients in whom the tumor location was not assessed or the surgical treatment is impossible, pharmacological treatment with phosphate and calcitriol is indicated, but the therapeutical benefits are dubious. Suggestion also has been given that oncogenic osteomalacia can be treated with subcutaneous administration of somatostatin analogues (32), if the tumor expresses somatostatin receptors: however this therapeutic option has subsequently been strongly questioned by others. Finally it is of extremely interest to note FGF-23 has currently emerged as a tumor biomarker to assist patient management, since its failure to fall within normal range after surgical intervention should suggest an incomplete tumor removal. In malignant cases measurement of FGF-23 serum level is also of assistance in the patient's follow-up, since its increase should suggest local recurrence and/or metastatic disease.

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Case 18.

Ivan Damjanov

Clinical History

- **45-year-old man**
- **Anterior chest pain for approximately 6 months**
- **X-ray revealed a 2nd rib mass measuring 7.1 x 4.6 cm**
- **The rib was resected**
- **Follow up (2 years): No recurrence of tumor**

CLEAR CELL CHRONDROSARCOMA
Mayo Clinic Data (LE Wold, et al. Atlas of Orthopedic Pathology, Elsevier, 2008)

CLEAR CELL CHONDROSARCOMA
RADIOLOGIC FINDINGS

Small tumor appears as sharply demarcated lucency and shows sclerosis at the periphery
Expansion of the bone, calcifications (25%)
Larger tumors show marked cortical destruction and appear obviously malignant
Dd: Chondroblastoma, chondrosarcoma,giant cell tumor

Mayo Clinic (LE Wold, et al.
Atlas of Orthopedic Pathology, Elsevier, 2008)

CLEAR CELL CHONDROSARCOMA

HISTOPATHOLOGY
-Irregularly lobulated
-Foci of cartilage admixed to clear cells
-Round clear cells with vesicular nuclei and sharp cytoplasmic borders
-Bone trabecullae in tumor
-Multinucleated osteoclasts
-Conventional chondrosarcoma in 50%

Differential diagnosis

Chondroblastoma
Osteoblastoma
Aneurysmal bone tumor
Chondrosarcoma
Chondroblastic osteosarcoma

TREATMENT
Surgical resection
Radiation therapy not recommended

PROGNOSIS
Overall favorable
Late recurrence even 10-15 after initial surgery
Bjornson et al (Cancer 1998): 7/48 died of tumor

FINAL DIAGNOSIS
Clear cell chondrosarcoma of the rib

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Patient alive and without tumor 4 years after onset of symptoms

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CASE 19

Kumarasen Cooper M.D.

SCLEROSING ANGIOMATOID NODULAR TRANSFORMATION

Clinical History

A 36-year-old woman presented with a two week history of worsening left upper quadrant abdominal pain.

Radiologic Imaging

CT scan revealed a 7.5 cm, solid, well-circumscribed mass arising from the inferior aspect of the spleen. Following contrast injection, the mass did not show enhancement characteristics to that seen in more common benign solid splenic masses such as hemangioma and hamartoma. In fact, the mass was almost isodense with normal spleen (except for a central unenhanced central stellate area).

Gross Specimen

A solitary unencapsulated but well-circumscribed mass (6.0 x 5.5 x 5.0 cm) with multiple red-brown nodules (ranging from 0.5-2.0 cm) with an adjacent rim of normal spleen. The nodules were separated by dense bands of fibrous stroma that coalesced to form a dense central stellate scar.

Microscopic Examination

The mass comprised multiple angiomatoid nodules within fibrous stroma and vascular slit-like spaces. The nodules were characterized by aggregates of plump endothelial cells and pericytes lining prominent slit-like vascular spaces in a sieve-like arrangement, with extravasated erythrocytes. The endothelial cells exhibited minimal cytologic atypia with rare mitotic activity.

The intervening collagenous stroma contained a variable number of reactive myofibroblasts, scattered lymphocytes and plasma cells, and hemosiderin laden macrophages.

Diagnosis

SCLEROSING ANGIOMATOID NODULAR TRANSFORMATION (SANT)

Discussion

SANT is a rare benign vascular lesion, first fully characterized by Martel et al in a report of 25 cases in 2004 (with over 50 cases now described in the literature). As the descriptive name implies, the lesion is a non-neoplastic,

nodular, vascular proliferation of the red pulp with prominent sclerosis, and splenectomy being curative. Although the exact nature of the lesion is uncertain, it has been postulated that SANT may represent a splenic hamartoma that has undergone sclerosis, a transformative process in response to a stromal proliferation, or a response to an organized hematoma: all involving splenic red pulp.

The recapitulation of an admixture of blood vessels in the angiomatoid nodules of SANT, similar to normal red pulp, is highlighted by the demonstration of an identical immunophenotypic characterization of the blood vessels:

* capillary-like vessels (CD34+, CD31+, CD8-) (predominant component)
* small vein-like structures (CD34-, CD31+, CD8-)
* few open sinusoidal channels (CD34-, CD31+, CD8+)

Nevertheless, the gross and morphological characteristics are fairly characteristic to identify SANT and immunophenotypic analysis is not routinely necessary for the diagnosis.

The differential diagnosis of SANT includes splenic hamartoma, inflammatory myofibroblastic tumor, littoral cell angioma, and splenic hemangioendothelioma. Splenic hamartoma is a tumor-like lesion composed of disorganized mature red pulp elements, usually found incidentally. The slit-like vascular channels lined by plump endothelial cells resemble splenic sinuses with an absence of normal red pulp cords and lymphoid elements. In contrast, SANT is characterized by a mixture of sinusoids, capillary and vein-like vessels, essentially comprising red pulp tissue. Although postulated that SANT may represent a peculiar form of splenic hamartoma, the latter do not have angiomatoid nodules with a mixture of sinusoids, vein-like and capillary-like vessels.

Inflammatory myofibroblastic tumor (IMT) of the spleen is composed of spindle cells (fibroblasts/myofibroblasts) and associated with a mixed inflammatory (lymphocytes/plasma cells) and a variably hypocellular fibrocollagenous stroma. Although the internodular areas of SANT resemble IMT, the latter lacks the angiomatoid nodules. Hence, some authors recommend a careful examination for angiomatoid nodules in all suspected cases of splenic IMT.

Littoral cell angioma (LCA) is a distinctive vascular neoplasm of the spleen with immunophenotypic features reminiscent of sinus lining (littoral) cells of splenic red pulp. LCA is characterized by multiple nodules involving red pulp comprising variably sized vascular channels lined by tall endothelial cells with vesicular nuclei and small nucleoli (CD31/CD8/CD21+). Papillary fronds, cellular vacuolization and hemophagocytosis, along with aggregates of eosinophilic globules may also be seen in LCA.

True splenic hemangioendotheliomas are rare and controversial, showing a range of morphologic appearances characterized by ill-defined vascular spaces, epithelioid or spindle cell morphology with minimal cytologic atypia and low mitotic activity.

Whilst the etiology of SANT is still uncertain, recent evidence has highlighted a possible link to IgG4-related sclerosing lesions (expanding the spectrum of diseases such as autoimmune pancreatitis, retroperitoneal fibrosis, cholangitis, Kuttner's tumor, etc). Occasional cases of SANT have demonstrated significantly higher IgG4/IgG ratios in SANT (than in control spleens). Nevertheless, further investigation is warranted before any conclusion can be made.

In summary, SANT is a recently recognized non-neoplastic vascular lesion of the spleen, characterized by numerous angiomatoid nodules surrounded by variable fibrosclerotic stroma and a lymphoplasmacytic cellular infiltrate. It appears to be entirely benign with splenectomy being curative in reported cases.

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CASE 20.

Michele Bisceglia, M.D.

Clinical History and Pathological Features

Intraoperative consultation

A 38-year old female with “ascites” and a presumptive diagnosis of non-Hodgkin lymphoma underwent laparoscopic retroperitoneal paraaortic lymphadenectomy in June 2004. A piecemeal excised 3.0 cm in aggregate mass of soft tissue was submitted fresh for intraoperative consultation and tissue triage.

Frozen sections revealed total effacement of the lymph node architecture and complete replacement by a bland-appearing leiomyomatous-like proliferation. In places, there were slightly ectatic lymphatic channels as well as scattered, small, round foci of residual lymphoid tissue. The accumulated peritoneal fluid was described as chylous. Both, the histopathological findings and clinical information suggested ***the possibility of lymphangioleiomyomatosis involving retroperitoneal lymph nodes***. Most of the fragments were immersed in 10%

buffered formalin for paraffin embedding and standard processing, small tissue fragments were fixed in Karnovski solution and processed for electron microscopy.

Permanent sections

H&E stained permanent sections confirmed a spindle cell proliferation with myoid features exhibiting a distinctive pericytomatous-arrangement characterized by spindle cell bundles distributed around an arborizing network of endothelium-lined slit-like spaces. Immunohistochemically, the myoid cells were diffusely reactive for vimentin, alpha-smooth muscle actin and desmin. Scattered cells lying in minute clusters or singly were immunoreactive for HMB-45 and melan-A. Additional immunomarkers, such S-100 protein, EMA, cytokeratins, and CD34 all were negative. CD34 highlighted the rich capillary network of lymphatic vessels alternating with the spindle cell fascicles. Based on the coexpression of both smooth muscle markers and melanocytic markers, the myo-melanocytic nature of the spindle cell proliferation mainly composing the lesion was ascertained. Nuclear immunostaining test for estrogen and progesterone receptors was also positive in 40% and 60% of the myo-melanocytic cells, respectively.

Ultrastructural examination

The myoid cells shared features of both smooth muscle and melanocytes. Their cytoplasms displayed a hybrid phenotype containing intracytoplasmic wisps of actin-like microfilaments in subplasmalemmal localization with dense bodies, and membrane bound granules of variable electron density corresponding to stage 1 and 2 premelanosomes and stage 3 melanosomes with a variety of configurations.

Diagnosis

Lymphangioleiomyomatosis involving the lymph node – likely systemic – (exclude tuberous sclerosis complex).

Comment

The clinical charts of this patient were reviewed. Her signs and symptoms of lymphangioleiomyomatosis, that had eluded her clinicians for about 9 years, became obvious in view of her newly established diagnosis. The patient had experienced shortness of breath, and its onset coincided with her first pregnancy in 1995. Her dyspnea subsided after delivery. Exacerbation of difficulty breathing became manifest four years later, during her second pregnancy. Therefore, in 1999 she underwent pulmonary function testing for worsening of her respiratory symptoms, which revealed significant airflow limitation. Concomitantly, allergic testing was positive for Dermatophagoides pteronissymus and Dermatophagoides farinae. With a clinical diagnosis of chronic asthmatiform obstructive pulmonary disease, the patient was treated accordingly with bronchodilators, corticosteroids, and antibiotics. In January 2003 spirometry demonstrated reduced forced expiratory volume (FEV_1) and forced vital capacity (FVC), increased total lung capacity (TLC), increased residual volume (RV), and increased RV/TLC ratio. Computed tomography (CT) scanning revealed diffuse bilateral cystic changes of lung parenchyma, changes that were interpreted as centrilobular pulmonary emphysema. In May 2004 she was hospitalized because of severe dyspnea, cough, and chest pain at our institution. Auscultation revealed reduction of breath sounds. Routine chest X-ray demonstrated hyperinflation. A right pleural effusion was evacuated by repeat thoracenteses. The pleural fluid was described as chylus (opalescent-white). Its chylous nature was proven by biochemical analyses (triglyceride level 1401 mg/ml). Other chemical analyses performed on the chyle were total protein (4.89 g/dl), albumin (2.98g/dl), glucose (108 mg/dl), cholesterol (91 mg/dl). Blood gas analyses documented reduction in diffusing capacity: pH 7.4; pO2=47.9 mmHg; pCO_2= 37.5 mmHg. High resolution chest CT scan (HRCT) confirmed previously documented findings of diffuse, homogeneous, small (< 1.0 cm diameter) thin-walled cysts. No significant mediastinal or hilar lymphadenopathy was identified. CT scan of

the abdomen showed multiple enlarged periaortic and pelvic lymph nodes. A periaortic lymph node was biopsied in June 2004. Sections from this lymphadenectomy specimen were used for your review.
No skin, heart, brain, and/or intraabdominal visceral sign or pathological manifestation suggesting tuberous sclerosis complex was discovered, and the definitive diagnosis was that of ***"sporadic lymphangioleiomyomatosis" (not associated with angiomyolipoma)***.

Follow-up
Administration of Tamoxifen and luteinizing hormone-releasing hormone analogs (LHRH) resulted in minimal transient clinical benefits. Less than 2 years after the diagnosis, the patient died while in waiting list for lung transplantation. Autopsy was not performed.

Discussion

Lymphangioleiomyomatosis (LAM) (OMIM # 606690) is a systemic condition, affecting almost exclusively women in their reproductive age. It is characterized by an abnormal proliferation of myoid cells (so-called LAM cells) in the lungs as well as in the axial lymphatics and lymph nodes of the thorax and retroperitoneum (1). The disorderly growth of cells with smooth muscle phenotype results in progressive obstruction of airways and lymphatics. LAM most often occurs as a *sporadic disease*, but also occurs in women with tuberous sclerosis complex (TSC) (*syndromic or TSC-related LAM*). There are no pathologic differences between sporadic and syndromic (TSC-related) LAM.

Sporadic LAM is a rare disease with an estimated prevalence of approximately 1 to 2 cases per million women in US and among populations of Caucasian descent (2-3), and is even more rare among Asian and African individuals. Syndromic LAM affects 4-5% of women with TSC (4), but subclinical involvement is much more frequent than thought (up to 50% [5]). TSC, which occurs up to 1 in 6,000 live births (6), is an autosomal dominantly inherited systemic malformation syndrome, linked to TSC1 and TSC2 tumor suppressor genes, mapped on chromosome 9q (9q34) and chromosome 16p (16p13.3) respectively, with the former encoding hamartin and the latter - which accounts for the two thirds of mutations - encoding tuberin.

TSC diagnosis is primarily clinical [OMIM # 191100], based on the evidence of *major clinical features* (facial angiofibromas, ungual or periungual fibromas, hypomelanotic macules, shagreen patches, retinal hamartomas and retinal astrocytomas, cortical tubers, subependymal nodules and subependymal giant cell astrocytomas, cardiac rhabdomyomas, lymphangioleiomyomatosis, and renal angiomyolipomas) and *minor clinical features* (enamel dental pits, hamartomatous rectal polyps, bone cysts, cerebral white matter migration lines, gingival fibromas, retinal achromic patches, confetti skin lesions, multiple renal cysts, and "nonrenal hamartomas" (7-8). The category of "non-renal hamartomas" includes extrarenal angiomyolipomas, pulmonary and extrapulmonary clear cell sugar tumors of visceral organs and somatic soft tissue, and multifocal micronodular pneumocyte hyperplasia (9).

Histogenesis from epithelioid perivascular cell (10) has been proposed for renal and extrarenal angiomyolipomas, pulmonary and extrapulmonary LAM, pulmonary and extrapulmonary clear cell sugar tumors, which thus at times are collectively called PEC-omas (11-12).

Regarding TSC diagnosis and LAM we would like to emphasize the following: i. a definitive clinical diagnosis of TSC now requires two or more distinct types of lesions. ii. multiple lesions of the same type (e.g., multiple angiomyolipomas) in the same organ system are counted as one (6-7); iii. in this context and from the clinical point of view, LAM and renal (as well as extrarenal) angiomyolipoma have to be considered as the same lesion and when concomitantly present to be counted as one; iv. foci of LAM have been reported in renal

angiomyolipoma (13-14); v. visceral involvement other than lung (e.g., uterus) has been described in TSC-related LAM (15); vi. visceral circumscribed TSC-related LAM (aka, nodular variant of LAM) has also been observed (e.g., in the kidney concomitantly with classic angiomyolipoma) (14); vii. renal angiomyolipoma is the most frequent sign of TSC and is often (50-60%) found also in association with sporadic LAM (2,16-17); viii. LAM associated with angiomyolipoma does not equate TSC.

Although – according to the diagnostic criteria outlined above - LAM is not diagnostic on its own for TSC, it is considered *per se* by some authors to be an incomplete expression (*forme fruste*) of this condition (18). From the genetic standpoint, syndromic LAM patients harbour germline mutations, but (according to the Knudson theory) the disease is usually caused by a second somatic cellular hit, which inactivates the remaining normal allele ("loss of heterozygosity" resulting in "two hit" $TSC^{-/-}$ cells). The second hit is not only new in each of several tumors occurring in TSC but may involve TSC1 or TSC2 gene, independently from the germline $TSC1^{-/+}$ or $TSC2^{-/+}$ mutant allele (mechanism of trans-heterozygosity). Analogously, in sporadic LAM patients, who by definition do not have either TSC1 or TSC2 germline mutations, molecular analyses also have found somatic mutations of TSC2 gene in lung and kidney ($TSC2^{+/+}$ / $TSC2^{+/-}$ mosaicism) and loss of heterozygosity in $TSC2^{-/-}$ LAM cells (18-19). TSC1 and TSC2 are tumor suppressor genes and their encoded proteins downregulate cell growth and proliferation by inhibiting mTOR (mammalian target of rapamycin), a ubiquitous serine-threonine kinase (20). Inactivating genetic mutations of TSC1 and/or TSC2 intimately involve the regulation of protein synthesis, cell growth, and cell proliferation (20).

Some other studies have demonstrated that pulmonary LAM cells are the same cells of lymph nodal LAM and renal angiomyolipoma, thus suggesting the possibility that LAM cells are capable to migrate via lymphatics (3), and pulmonary LAM is thought to represent "metastatic" disease from other sites (renal angiomyolipomas, and lymph node and lymphatics LAM) (3). This hypothesis has been supported by the demonstration that LAM patients with lung involvement have circulating LAM cells in peripheral blood (3) and that recurrent pulmonary LAM after lung transplantation derive from native LAM (21-22).

In descending order of frequency, in sporadic LAM, thoracic (mediastinal and hilar pulmonary), intraabdominal, and cervical lymph nodes can be affected with or without lung involvement (23).
Lymph node involvement is called extrapulmonary LAM, and extrapulmonary LAM often preceeds lung involvement (24), another point in favour of the capacity of LAM cells to migrate from periphery to lung. When a lymph node is involved in isolation, then the term lymphangioleiomyoma is used (23).

The clinical diagnosis of pulmonary LAM, in both sporadic and syndromic (TSC-related) forms, is based on HRCT which documents diffuse bilateral lung cystic changes. Lung cysts in conjunction with the evidence of renal angiomyolipoma are considered diagnostic of LAM, and lung biopsy is not needed in these circumstances (3). With the evidence of CT findings suggestive of LAM, but renal angiomyolipomas are not detected, then lung biopsy is recommended to establish the diagnosis: open biopsy is preferable in order to obtain sufficient amount of tissue, but transbronchial lung biopsy also can yeld diagnostic tissue (25).

Pulmonary LAM is a chronic relentless progressive and fatal disease, in both sporadic and syndromic forms, sometimes spanning decades, although occasionally it may rapidly progresses (3). Pulmonary LAM is the 3rd cause of death in TSC-related form, after renal disease and brain tumors (2). It is a disease of women in childbearing age, although occasional premenarchal and postmenopausal cases (with hormonal manipulation), and extraordinary cases in males with TSC are also on record. Estrogens play a central role in disease

progression (3, 26). It is widely accepted that the disease is exacerbated by estrogen administration, while antiestrogens and progestins abate it, a fact which has been also noted in analogous experimental models (27). The main symptom of pulmonary LAM is shortness of breath in a patient with cystic airspaces on HRCT (honeycomb changes in full blown disease), usually misinterpreted as emphysema, and quantitative HRCT can give appropriate indices to assess disease severity in these subjects (28). The diagnosis of pulmonary (systemic) LAM should be strongly suspected in any woman of chidbearing age, who presents with emphysema, recurrent pneumothorax, and chylous effusions. Cyst formation in the lung is likely due to a combination of mechanisms, such as air entrapment due to interstitial nodular LAM cell proliferations, elastase and alpha-1-antitrypsin imbalance leading to elastic fibre degradation. The disease course is associated with several complications, such as spontaneous pneumothorax (50%), chylothorax (30%), chyloperitoneum (10%), chyluria (2%), hemoptysis. Chylous effusions and spillage are due to structurally abnormal lymphatics, involved by LAM cell proliferation, and LAM cell proliferation is known to elicit abundant lymphangiogenesis (29). Meningiomas has been seen in around 3% of LAM cases in both sporadic and syndromic forms, some in patients who had received progestins (30).

The histological diagnosis relies upon documentation of LAM cells proliferations in any site of involvement. LAM cells are typically immunopositive for both smooth muscle antigens and HMB-45, one of the well-known melanoma-associated antigen. Frequent estrogen and progesterone receptor immunoreactivity is usually documented in LAM cells both in the lung and lymph node (26-27). Lymph node involvement by LAM is well illustrated by the case presented herein. Lung involvement has been the subject of many papers and is well treated in textbooks on pulmonary diseases (1, 31), to which the readers is invited to refer. Histology of lung biopsy shows cystic changes and spindle to epithelioid LAM cells infiltrates peripherally located as plaque-nodules at the borders of the cysts or protruding as polyps and papillations inside the cysts. Larger myoid nodules and polypoid lesions of LAM cell proliferations can be easily differentiated from benign metastasizing leiomyoma, since the absence of cystic lesions in the latter. For prognostic purposes a LAM histological score (LHS) has been proposed, which is based on the percentage of lung tissue involvement by cystic lesions and LAM cells infiltrates. This scoring system is graded as follows: LHS1= <25%; LHS2= 25% to 50%; LHS3= >50%) (1, 32).
Additionally, in the lung, multifocal micronodular pneumocyte hyperplasia is noted, which is a hamartomatous lesion which is seen in descending order of frequency in TSC patients with and without LAM, and in sporadic LAM (9), to be distinguished from atypical adenomatous alveolar hyperplasia. Rarely multifocal micronodular pneumocyte hyperplasia may also be seen in non-TSC/non-LAM patients as a sole manifestation (9).

In conclusion LAM is a rare and often unrecognized disease, for which early diagnosis is auspicable. Early therapeutic intervention may improve the symptomatology and slow the disease progression. The mainstay of treatment is based on hormonal manipulation, such as oophorectomy, progestin therapy (medroxyprogesterone acetate), tamoxifen, LHRH analogs. Sirolimus, an immunosuppressive drug, acting as mTOR inhibitor, has recently been also proposed (33).

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CASE 21

Thomas Krausz M.D.

Clinical History

55-year-old male with a history of deep-seated left calf mass (> 10 cm in diameter on imaging). Open biopsies were performed and frozen section requested. Both the frozen

section and the paraffin section caused diagnostic difficulty. The submitted slide is from the biopsy material. Subsequently the tumor was excised and definitive diagnosis made.

Pathology

The biopsy fragments show a neoplasm mostly composed of rounded-epithelioid tumor cells arranged in cords, clusters and sheets in a distinctly collagenous hyalinized stroma. The tumor cells exhibit vesicular, round to oval nuclei with finely dispersed chromatin and small indistinct nucleoli. The cytoplasm varies from clear to pale-eosinophilic. Mitotic activity is low (less than 1 per 20 high power fields).

I found the diagnosis very difficult in this case and immunohistochemical studies did not help me to reach a firm conclusion, which is annoying. So, what did I consider? Sclerosing epithelioid fibrosarcoma (not exactly right); peculiar burnt-out solitary fibrous tumor with mostly epithelioid cells (CD34 expression was only focal); epithelioid hemangioepithelioma; and soft tissue myoepithelioma. Fortunately, the diagnostic dilemma resolved following excision of the tumor.

The resection specimen weighed 250 g and consisted of two partially joined, sharply circumscribed, ovoid, yellow-tan masses. The larger mass measured 13 cm and the smaller 9 cm in greatest dimension. The cut surface of the larger mass demonstrated rubbery, tan white tissue, with about 10% necrosis and a hemorrhagic area consistent with biopsy site. The cut surface of the smaller mass was only focally rubbery being of a mostly softer fleshy consistency and with about 20% necrosis.

Microscopically, sections from the larger mass were very similar to the biopsy but there was more obvious vascularity including HPC-like vessels with focally hyalinized walls. Also there were spindle cell areas, which, together with the vessels and collagen, now were clearly supporting the diagnosis of solitary fibrous tumor (SFT).

The small mass had a minor component of typical SFT while in most part there was much more cellularity, less collagenous matrix and up to 10 mitoses per 10 HPFs. And most interestingly, the epithelioid component seen in the biopsy and larger mass was not present in the smaller mass.

Diagnosis: Malignant solitary fibrous tumor

Comments

The diagnosis of classic SFT is rather straightforward. However, it is my experience that on smaller biopsy fragments and sometimes even on resection specimens the diagnosis of malignant SFT is problematic. This is because of the rather heterogenous phenotype of the tumor cells from spindled to epithelioid to pleomorphic combined with the sometimes confusing immuoprofile. While the CD34 immunoreactivity is usually present in the “benign” classic SFTs it is often lost in large part in the histologically malignant tumors. In addition, aberrant expression of keratin, desmin and S100 may be focally observed. The greatest help in diagnosing histologically malignant SFTs is residual or focal conventional component with increased mitoses.

However, there are rare examples of malignant SFTs which deviate greatly from all conventional features of structure and cytology of SFT. There is an excellent discussion of this topic by Mosquera and Fletcher in 2009. They presented 8 cases of malignant SFT with a discrete anaplastic component and put the question “Is this dedifferentiated SFT?”

I selected this case for this seminar because I can recall at least 3 other cases in which I failed to recognize malignant SFT on biopsy material, but I am still learning! We pathologists need to be familiar with the expanding morphologic spectrum of SFT, which I will illustrate in my presentation.

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CASE 22

Markku Miettinen, M.D.

History:
23-year-old man. Swelling and pain left lower leg below the knee, from where an 11x7x5 cm tumor was excised. On sectioning, the tumor was white and focally mucoid.

Diagnosis:
Low-grade fibromyxoid sarcoma

Specific notes of the case

This case is a typical example of low-grade fibromyxoid sarcoma. Characteristic features include low-grade atypia, collagen-rich, variably myxoid matrix, and low mitotic activity. Hypervascularity is notable, often with perivascular hypercellularity. Immunophenotype is nonspecific, but EMA-positivity is commonly reported. Definitive diagnostic test is demonstration of specific fusion transcripts (see below), but FUS-gene rearrangement can also be demonstrated by FISH probes, and this is a practical although not disease specific test (also seen in myxoid liposarcoma, among others).

General discussion of the entity

Harry Evans in 1987 described this clinicopathologically distinctive low-grade sarcoma in 3 patients in 1987 and in 1993 in a larger series.[1] The tumor occurs predominantly in young and middle aged adults between the age of 25 and 45 and also in children. Male predominance has been reported in some series. The tumor is usually intramuscular and is often relatively large, but some examples have been smaller, including those that have involved superficial soft tissues. The most common sites of presentation are the thigh and buttocks, inguinal area, shoulder region, and chest wall. This tumor has also been reported in the retroperitoneum and mesentery.[1-7]

The clinical course is typically slow. Local recurrences may occur during a long time span, up to 50 years. Lung metastases developed in two early series in 7/12 and 1/11 cases, often after a long period of repeated local recurrences. [1,2] We have seen cases in which pulmonary metastases developed before detection of a large, occult primary soft tissue tumor involving the buttock or abdominal soft tissues. In many cases, the patients live long even after development of pulmonary metastases undergoing multiple thoracotomies for recurrent pulmonary metastases.

The tumor size usually is in a range of 3-10 cm, but can be larger than 15 cm. Grossly the tumor is firm and rubbery, but it may be have a mildly mucoid appearance on sectioning. Although this tumor may appear well-circumscribed, it typically microscopically infiltrates skeletal muscle.

Histologically the tumor is often multinodular. The nodules typically show a myxoid character, while between them there are dense fibrous areas. The cellular nodules are hypervascular and contain mildly atypical fibroblasts in a fibromyxoid matrix. Some tumors are more evenly collagenous and have fibromatosis or neurofibroma-like appearance when wavy nuclei and collagen fibers are present. Some cases contain perivascular cellular foci. In many areas, the tumor shows a swirling or storiform appearance. Mitotic activity is low, but focal necrosis may be present.

In some cases, the tumor may undergo transformation to a higher histologic grade with sheets or streaks of ovoid cells, and this appearance may closely resemble sclerosing epithelioid fibrosarcoma. A more cellular, epithelioid appearance is often seen in the pulmonary metastases. [1,3]

A histologic variant of LGFMS has been described as hyalinizing spindle cell tumor with giant rosettes. [3,7,8] The reported cases predominantly occurred in young adults (mean age 38 years) intramuscularly in the proximal extremities showing similar clinicopathologic features as the main variant. This tumor contains peculiar giant rosettes formed by epithelioid fibroblasts with a core of dense, hyalinized collagen. Otherwise the histologic features are similar to those of LGFMS.

LGFMS is vimentin-positive and shows no specific differentiation markers. However, focal positivity for EMA has been a common feature, and smooth muscle actin and CD34-positivity an occasional finding. These tumors are negative for desmin, S100 protein, and keratins.[3,7]

Differential diagnosis

Prior to its description, many examples of LGFMSs were undoubtedly diagnosed as benign tumors, with diagnoses ranging from fibroma or neurofibroma variants to fibromatosis and deep fibrous histiocytoma. A higher cellularity, multinodular, fibromyxoid character should allow one to distinguish LGFMS from benign fibroblastic tumors.

Low-grade myxofibrosarcoma (low-grade myxoid MFH) differs from low-grade fibromyxoid sarcoma by a more developed vascular pattern, more myxoid matrix, presence of greater, at least focal nuclear atypia, and tendency for perivascular hypercellular zones. [9] Desmoid is less cellular, and shows spindled cells with lesser atypia in a more uniformly collagenous matrix.

Genetics

The t(7;16)(q34;p11) translocation is a typical sole cytogenetic finding in both the classical and the hyaline rosette variants. [7,8] The corresponding molecular genetic event is FUS-CREB3L2 gene (previously called BBF2H7) fusion. [10] An alternative gene fusion FUS-CREB3L1 has been reported in a minority of cases. The latter change has not been observed at the cytogenetic level, but the expected translocation would be t(11;16)(p11;p11). [11]

CREB3L2 and CREB3L1 are closely related transcription factors that shuttle between intracellular membranes and nucleus and are activated by intramembrane proteolysis. [12]

PCR-based fusion transcript assay[13] and interphase FISH for FUS gene rearrangement can be used in the differential diagnosis of LGFMS and other fibroblastic tumors, but the same gene is also rearranged in myxoid liposarcoma translocation. [14]

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CASE 23

Markku Miettinen, M.D.

History:
31 year-old man. A large, 13 cm pelvic-abdominal mass is excised. Biopsy suggests sarcoma of unspecified type.

Diagnosis:
Dendritic reticulum cell sarcoma/tumor

Specific discussion of the case: This is a histologically typical but immunophenotypically somewhat unusual case. The tumor appears to arise from Castleman disease, a previously known occurrence. Large tumor size, presence of necrosis and atypia indicate malignant potential, although this is difficult to quantify given the relative lack of reference information on this very rare entity. Typical immunophenotypical features included positivity for CD21, whereas S100 positivity and desmin, although rarely reported in this entity, were detected in this case. We had problems in arriving at a specific diagnosis of needle core biopsies, and considered un-specified sarcoma. However, in the excision specimen, diagnosis was more straightforward based on histologic clues: origin in Castleman disease, storiform/concentric pattern, and intermingling lymphocytes.

General discussion of the entity:
This rare tumor was originally reported by Monda and Rosai in cervical lymph nodes.[1] It is a neoplastic counterpart of dendritic reticulum cells located in the nodal germinal centers, and has also been referred to as dendritic reticulum cell sarcoma.

DRCT occurs in adults of all ages and equally in men and women, and median age is around 40 years. Most examples involve lymph nodes, tonsil or spleen, but some involve extranodal tissues in the body cavities: mediastinum and retroperitoneum, or gastrointestinal tract. The examples in the neck are usually smaller < 5 cm, whereas those in the body cavities are often large > 10 cm. Local recurrence and metastasis has been reported in up to 30-40% of patients.[1-7]

Histologically DRCT is composed of syncytial sheets of spindled oval cells, often in as storiform pattern and sometimes concentrically arranged around blood vessels. The tumor cells typically intermingle with lymphocytes, which is a good clue for this diagnosis. Pools of amorhpous material (quite similar to what is seen in intestinal GISTs) correspond to prominent interdigitating cell processes. Mitotic activity varies, but is 0-5 per 10 HPFs in the majority of cases, and this case falls into this range also. In the current cases, the presence of tumor necrosis and large tumor size >10 cm are indications to suggest malignant potential. In the present case, adjacent elements of Castleman disease were present suggesting origin from Castleman disease, as reported in some cases previously.

Immunohistochemically typical is expression of antigens of dendritic reticulum cells: CD21, and CD35, and variably of CD23. Newer markers expressed in DRCT although not specific for it include fascin, clusterin[8], and podoplanin. [9] In addition, desmoplakin and EMA-positivity is common, and S100 protein positivity occasional. These tumors are typically negative for keratins, CD1A, S100 protein, and desmin. However, this case was unusual in that in was positive for desmin and S100 protein, in addition to markers of dendritic reticulum cells (CD21 positive, CD35 negative). However, the tumor was KIT-negative, in contrast to GIST.

Electron microscopy demonstrates prominent cell processes, and desmosomal junctions are present between some processes. In this case, it was not done. No specific genetic testing is available for this entity.

Differential diagnosis

Abdominal mesenchymal tumors that especially enter into differential diagnosis of abdominal dendritic reticulum cell tumors especially include gastrointestinal stromal tumor and dedifferentiated liposarcoma. Both can have some similarities. For example, small intestinal GISTs that frequently form apparent extragastrointestinal masses, also contain prominent cell processes. KIT-negativity and expression of markers of dendritic reticulum sarcoma helps to distinguish this tumor from GIST.

Dedifferentiated liposarcoma, and especially its variant with meningothelial-like whorls, has some superficial similarity to dendritic reticulum cell sarcoma. Thorough sampling and discovery of well-differentiated liposarcoma component helps to diagnose dedifferentiated liposarcoma. Also useful is nuclear MDM2 expression, seen in a proportion of well-differentiated and dedifferentiated liposarcoma cells.

Distinction from lymphoma is usually straightforward: lack of expression T- B- and activated lymphoid cell specific markers.

Follicular dendritic reticulum cell tumor

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CASE 24.

Hugo Dominguez M.D.

CLINICAL HISTORY.

A 31 year-old male, In May 2009 (four months before admission) developed abdominal and lumbar pain, physical examination disclosed a large palpable intra-abdominal tumor. A CT-scan demonstrated tumor in relationship with ascending colon, and liver nodules suggestive of metastases.

Laparotomy findings were: Intra-abdominal tumor located at mesocolon (15 x 12 cm), and multiple peritoneal implants, with adherences to hepatic capsule and hepato-duodenal ligament.

In October 2009 chemotherapy treatment was initiated, and completed 4 cycles. In March 2010, a PET disclosed a residual para-rectal mass 3.1 cm in diameter.

HISTOLOGICAL FINDINGS.

The neoplasm is composed of small round cells arranged in solid sheaths and trabecular structures, with zones of geographic necrosis. The cell groups are surrounded by loose stroma with a basophilic myxoid appearance. The cells have a high nuclear/cytoplasmic ratio, the nuclei are hyperchromatic, slightly lobulated, they have inconspicuous nucleoli, and 20 mitoses per 10 HPF.

IMMUNOHISTOCHEMISTRY

Positive for Bcl-2, CK-AE1-AE3, CK 8, CK18 (paranuclear dots), Vimentin, CD99 (weak), EMA (Weak). Negative for**:** Actin, Desmin, WT1, CK20, CK7, Calretinin, CA125, CD56, and C-Kit

CYTOGENETICS (Performed at MD Anderson Cancer Center): t(11;22)(p13;q12).

Diagnosis: Desmoplastic Small Round Cell Tumor (DSRCT) with myxoid stroma

DISCUSSION. Desmoplastic Small Round Cell Tumor (*synonyms: intra-abdominal desmoplastic small cell tumor, desmoplastic small cell tumor with divergent differentiation, desmoplastic primitive neuroectodermal tumor*) is a recently described entity that occurs in children and young adults with a male predominance. It occurs in many locations but the majority present as a rapidly growing tumor in or near the abdominal cavity. It is a locally recurrent high grade neoplasm with a poor prognosis in spite of diverse chemotherapy regimes.

Histologically DSRCT is composed of nests, trabeculae and small sheaths surrounded by abundant loose or fibrous stroma, and necrosis is a constant feature. The neoplastic cells are intermediate in size and contain scant cytoplasm, occasionally abundant with rhabdoid appearance. The nuclei are round, hyperchromatic, with inconspicuous nucleoli. The mitotic rate is high. Ultrastructural studies have described primitive cells with small junctions, intermediate filaments and occasional cell processes, intracellular lumens, and dense core granules.

The immunophenotype of DSRCT is: positive for Vimentin (paranuclear 90%), EMA (90%), WT1 (90%), and Desmin (paranuclear 80%). CD99 is seen in 20% of cases. Negative for Actin, S100, Synaptofisin, HMB45, Neurofilament, CD45.
By cytogenetics DSRCT demonstrates a t(11;22)(p13;q12) that produces a fusion of *EWS/FLI1* genes, instead of p24;q11 observed in classic PNET, that produces *EWS/WT1*.

CASE 25

Eduardo Zambrano M.D.

Clinical history
An 81-year-old male with history of localized prostate cancer diagnosed in 2002 (Stage I; pT1C, N0, cM0; Gleason 6/7; PSA 13.8 at diagnosis) presented in March 2008 with right hip pain. His prostate cancer was treated with external beam radiation to 75.6 Gy completed in 5/2002. The patient had noted back pain for several months before his visit, and later developed pain in the lateral aspect of his right hip and groin and shooting pain down his thigh. MRI of the hip was performed, and demonstrated abnormalities to the right superior pubic ramus, extending into the ischium and into the anterior and medial aspect of the acetabulum. A hypointense T1 weighted signal replaced bone, with significant enhancement and heterogeneous T2 weighted signal. A soft tissue mass outside of the bone with some brighter T2 weighted signal was also seen. There was extension into the adductors. A whole body scan indicated increased uptake in the right acetabulum and medial portion of the right ischial pubic ramus. The possibility of a blastic metastatic lesion from his previous prostate cancer was considered (PSA was 0.24 ng/mL). A CT-guided core needle biopsy of the right ischial pubic ramus lesion was performed, with subsequent resection of the affected area following histopathological diagnosis.

Pathologic findings
The resected gross specimen consisted predominantly of red/brown skeletal muscle (14 x 12.5 x 12.5 cm) with an adherent portion of transected pelvis (11.5 x 9.0 x 2.8 cm). The specimen was sectioned from anterior to posterior to reveal an 8.1 x 7.5 x 5.2 cm tan/white, lobulated, firm, irregular mass grossly involving bone and invading into the surrounding muscle. Histological sections submitted after prolonged decalcification showed pre-existing bone with a sparse trabecular framework inundated and replaced by sheets of malignant chondrocytes and osteoblasts with areas of malignant bone formation. The lobules of malignant cartilage showed prominent cellular aggregation and spindling at the periphery. All margins were negative.

Diagnosis: POST-RADIATION CHONDROBLASTIC OSTEOSARCOMA.

Discussion
Osteosarcomas are highly aggressive malignant tumors of mesenchymal origin, in which the malignant cells produce osteoid. After myeloma, osteosarcoma is the most common primary malignant bone tumor. It has a bimodal age distribution, with most patients being less than 20 years old at diagnosis, and a smaller second peak in the elderly. Males are slightly more affected than females (1.6:1). Most osteosarcomas arise sporadically, but some are associated with other conditions, such as Paget's disease, fibrous dysplasia, bone infarcts and history of prior irradiation.

The incidence of post-radiation sarcoma ranges from 0.03% to 0.8% of all patients treated with external beam radiotherapy. Osteosarcoma is one of the most frequent secondary malignant neoplasms that occur within the radiation field, accounting for 17 to 38% of all post-radiation sarcomas. Most osteosarcomas arise from bone, but of all radiation-induced sarcomas, 13% are reported to be extraskeletal osteosarcomas, and between 3.8% to 10% of all extraskeletal osteosarcomas are reported to be radiation-induced. The remaining histological types of post-radiation sarcomas usually correspond to undifferentiated pleomorphic sarcomas (malignant fibrous histiocytoma), fibrosarcoma, MPNST, chondrosarcoma, angiosarcoma or Ewing sarcoma, among others.

In order to diagnose post-radiation sarcoma, several conditions must be met: 1) History of irradiation to the area involved by the sarcoma; 2) Latency period between the irradiation and the development of sarcoma; 3) Verification by histology of the sarcoma, which should have significant differences from the original neoplasm for which radiation was given; 4) Patients with familial cancer syndromes, such as Li-Fraumeni or Rothmund-Thompson, should be excluded. The mean doses that have been reported to be associated with the development of post-radiation osteosarcomas vary from 43 to 64 Gy. Doses below 10 Gy are associated with negligible risk. The mean latency period reported for radiation-induced sarcomas is 10-15 years, but it has been difficult to reach a consensus on how short this period might be and still support the diagnosis of radiation-induced malignancy. A 3-4 year latency period seems to be realistic, but there have been reports of even shorter latency periods, with the shortest being of only 3 months. Some factors may contribute to shorten the latency period, including: high doses of radiotherapy, concurrent administration of chemotherapy (especially alkylating agents), and radiation given to pediatric patients.

Post-radiation osteosarcoma has a predilection for the shoulder and pelvic girdles, which mainly reflects the use of radiotherapy for the treatment of breast and gynecologic cancers, respectively. The histologic characteristics of post-radiation osteosarcoma are no different from sporadic osteosarcoma. In most series, the most common type is fibroblastic (30-38%), followed by osteoblastic (18-34%), and chondroblastic (12-28%) types. Mixed histologies are less frequent. Most lesions tend to be high-grade (grade 3 or 4). There does not appear to be an association between the radiation type (orthovoltage or megavoltage) and the histologic type of osteosarcoma.

Four tumor suppressor genes implicated in the development of osteosarcomas in humans have been mapped to chromosomes 3q, 13q (*Rb1* gene), 17p (*TP53* gene), and 18q (osteosarcoma tumor suppressor gene *OSTS*, associated with osteosarcoma and familial Paget's disease). Genetic differences between individuals may explain why patients receiving comparable doses of radiation have different outcomes with regards to osteosarcoma development. Individuals exposed to radiation who have a greater genetic susceptibility could be at higher risk of developing post-radiation osteosarcomas.

Treatment of radiation-induced osteosarcoma includes chemotherapy and wide surgical resection of the mass. When the location is favorable for a complete surgical resection, the prognosis is the same as for sporadic osteosarcoma. However, given the predilection for axial locations, the overall prognosis of post-radiation osteosarcoma is generally worse than for conventional osteosarcoma.

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CASE 26

Eduardo Zambrano, M.D.

Clinical history

27 year-old male with some swelling on the posterior aspect of his left arm for a couple of years, felt to be occupation-related. Following physical therapy the mass reportedly disappeared. However, few months prior to his presentation he developed swelling over the medial aspect of his proximal left arm. The lesion was excised at an outside institution without prior MRI, and was presumed to be a benign cyst. Following excision, the patient developed recurring cyst formation, which was repeatedly aspirated. He subsequently had an MRI and a CT-guided biopsy of the more solid aspect of the lesion, which was interpreted at a different institution as a low grade sarcoma, and the patient was referred to our medical center. An MRI demonstrated within the left triceps a 3.0 x 3.0 x 4.7 cm heterogeneous, solid, lobulated, enhancing mass with an adjacent 5.3 x 5.2 x 4.6 cm fluid collection. The lesion was re-excised.

Pathologic findings

The resected specimen consisted of a portion of red-brown muscle (9.8 x 8.4 x 5.2 cm), with overlying ellipse of tan-brown skin (7.8 x 2.4 cm). The specimen was serially sectioned to reveal a mass consisting of two portions. The first was a 5.2 x 5.0 x 4.4 cm cystic area with smooth lining, filled with red serous fluid. The second was a 3.8 x 3.1 x 2.7 cm well-circumscribed nodule with a white fibrous capsule directly abutting the cystic portion of the mass. Histological sections showed a relatively well-circumscribed nodule consisting of a population of histiocytoid to myoid/spindled cells. The cells were arranged in a slightly lobular architecture, with large angiomatoid cystic spaces and abundant hemosiderin deposits. Frequent large atypical cells with convoluted pleomorphic nuclei and occasional nuclear pseudoinclusions were present. Occasional mitoses were noted. Most of the tumor was surrounded by a thick, fibrous rind. A dense inflammatory infiltrate predominantly consisting of mature-appearing lymphocytes and plasma cells was present in some areas. Immunohistochemical stains demonstrated strong and diffuse staining for vimentin and patchy but strong staining with desmin in the spindled cell population. Other immunohistiochemical markers performed were negative, including SMA, S100, ALK1, cytokeratins, CD34 and EMA. Split signal fluorescent in-situ hybridization for EWS was positive.

Diagnosis: ANGIOMATOID FIBROUS HISTIOCYTOMA.

Discussion

Angiomatoid fibrous histiocytoma (AFH) represents a low grade mesenchymal neoplasm of uncertain differentiation. It was originally defined as a subtype of malignant fibrous histiocytoma (MFH); however, the term ‘AFH’ is currently preferred due to its generally favorable prognosis. It occurs predominantly in the deep dermis and subcutis of extremities of

adolescents and young adults. Clinically, patients may experience systemic symptoms such as fever, anemia and weight loss.

Microscopically, AFH is characterized by pseudovascular, blood-filled cystic spaces surrounded by a multinodular proliferation of spindle and/or plump histiocytoid cells. A dense chronic inflammatory lymphoplasmacytic cuff with germinal centers may be present surrounding the lesion, sometimes giving the impression of lymph node involved by metastatic disease. Some cases of AFH display striking pleomorphism and mitotic activity, a feature that does not correlate with aggressive behavior. However, when these features are present, a diagnosis of high grade malignancy may be erroneously entertained. Immunohistochemically, the tumor cells are often positive for vimentin, desmin, EMA, CD68 and CD99.

Genetic studies of fusion genes in AFH have revealed *EWSR1–CREB1* fusion gene resulting from t(2;22)(q33;q12), *EWSR1–ATF1* fusion gene resulting from t(12;22)(q13;q12), and *FUS–ATF1* fusion gene from t(12;16)(q13;p11). Interestingly, some of these fusion genes, such as *EWSR1–CREB1* and *EWSR1–ATF1*, have also been detected in clear-cell sarcoma of soft tissue and gastrointestinal tract, which is an aggressive soft-tissue sarcoma, morphologically and clinically distinct from AFH. Other sarcomas known to harbor rearrangements of the *EWSR1* and *ATF1* genes include Ewing sarcoma, extraskeletal myxoid chondrosarcoma, desmoplastic small round cell tumor, myxoid liposarcoma and low-grade fibromyxoid sarcoma.

The recurrence rate of AFH is between 2% and 11%, and the metastatic rate is <1%. Rare deaths from late distant metastases have been reported. There are no known clinical, morphological or genetic factors that predict aggressive behavior. Complete surgical excision without adjuvant therapy is the recommended treatment for these tumors.

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CASE 27.

Giovanni Falconieri M.D.

Clinical history

A 75-year-old man is admitted to the hospital following the incidental discovery of a mass in the anterior mediastinum. The patient is also diabetic and hypertensive. Thoracotomy is performed and the mediastinal tumor is only partially resected. It is a multinodular, 8- by 6-cm yellow mass showing white–gray and hemorrhagic areas. Postoperative treatment includes radiation therapy. Nevertheless, 18 months after surgery, the patient developed local recurrence and died of acute heart failure 2 years after diagnosis.

Microscopic description

The tumor is composed of atypical spindle cells in a myxoid stroma featuring a delicate capillary network. Large, often multinucleated atypical cells showing clear cytoplasmic vacuoles are noticed as well. Several areas of necrosis are encountered, and the tumor periphery features a rim of fibrous tissue adjacent to normal mediastinal fat. Remnants of thymus may be observed. In addition, there are focal hypercellular areas showing poorly differentiated and pleomorphic cells. Tumor cells are positive for S100 and vimentin. CD34 decorates the vascular component but is not found in the neoplastic population. Other stains—including keratins, EMA, actins, and desmin—are negative.

Diagnosis: Myxoid liposarcoma of the anterior mediastinum with a focal dedifferentiated component.

Discussion

The mediastinum is a unique compartment featuring diverse anatomic structures that may give rise to a wide spectrum of tumors. Mesenchymal neoplasms are uncommon but not exceptional and may encompass a broad microscopic spectrum. Microscopically comparable mediastinal–soft tissue tumors differ from their somatic soft tissue equivalents, often being recognized either incidentally or at a late clinical stage, depending on the degree of compression or invasion of intrathoracic organs. Synovial sarcoma and liposarcoma are considered the most frequent intrathoracic mesenchymal malignancies.

Liposarcomas may occur either in the anterior or the posterior mediastinum. Tumors of the anterior mediastinum may grow in intimate association with the thymus. Data from larger series indicate that mediastinal liposarcomas affect mainly adults, although they may be sporadically seen in pediatric patients. Presenting symptoms are related to compression exerted by tumor on the adjacent anatomic structures; they include shortness of breath as well as cough or palpitation. Interestingly, 11 out of the 28 cases described by Klimstra et al. were asymptomatic and/or discovered incidentally. Like lipomas, liposarcomas have also been described in obese patients with systemic lipomatosis. Microscopically, mediastinal liposarcomas are similar to liposarcoma occurring in other locations, such as the deep somatic soft tissue and retroperitoneum. Well-differentiated and myxoid liposarcomas are the most frequent histiotypes. Complete resection is the primary treatment. Local recurrence is frequent; however, distant metastases are not common. As stated above, it may be difficult to differentiate liposarcomas from lipomas, especially in well-differentiated forms; only long-term follow-up will uncover the biologic potential of these tumors. Recently, investigations have been carried out on the amplification of oncogenes MDM2 (murine double-minute type 2) and CDK4 (cyclin-dependent kinase 4) on chromosome 12 q13-15. In particular, FISH analysis of MDM-2 and CDK-2 has proven highly sensitive and specific as an added means to segregate lipoma-like liposarcoma within well-differentiated adipocytic tumors. Under these premises, a reappraisal of well-differentiated fatty tumors of the mediastinum may well be helpful in segregating lipoma into a fair diagnostic category, along to the lines recently pursued for retroperitoneal lipoma. Likewise, the same molecular approach may be

successfully used to recognize poorly differentiated pleomorphic liposarcoma with little or no phenotypic evidence of lipoblastic differentiation.

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SESSION III

CASE 28

Kumarasen Cooper M.D.

PLEXIFORM ANGIOMYXOID MYOFIBROBLASTIC TUMOR OF THE STOMACH (PLEXIFORM FIBROMYXOMA)

Clinical History

A 64-year-old man presented with epigastric pain and was found to have a submucosal antral mass on upper endoscopy. Initial endoscopic biopsies

revealed hyperplastic changes of the antral mucosa and a subsequent laparoscopic excision of the gastric mass was performed.

Gross Specimen

A well-circumscribed tumor measuring 4.0 x 2.0 cm with a tan to pale yellow, whorled cut surface. Although firm, a mucoid to gelatinous appearance was evident.

Microscopic Examination

On low power magnification, the tumor revealed a characteristic plexiform or multinodular growth pattern involving both the submucosa and muscularis propria of the antral (gastric) wall. The nodules ranged in size from 1 to 7 mm and comprised ovoid to spindle cells embedded in an abundant intercellular fibromyxoid stroma/matrix and highly vascularized stroma. The latter was composed of a prominent arborizing capillary network. The tumor cells had a pale cytoplasm with indistinct cell borders surrounded by an inconspicuous or delicate network of collagen matrix. Although mild nuclear atypia (enlargement) was evident, no mitotic activity was seen. The periphery of the tumor was ill-defined and appeared infiltrative.

Immunohistochemistry

The tumor cells are diffusely positive for muscle specific actin and alpha-smooth muscle actin, consistent with a myofibroblastic immunophenotype. Importantly, S-100, EMA, CD34, C-KIT, DOG1 and β-catenin were negative.

Follow-Up

The patient is alive and well approximately three years following surgery with no evidence of recurrent tumor.

Diagnosis

PLEXIFORM ANGIOMYXOID MYOFIBROBLASTIC TUMOR OF THE STOMACH (Plexiform Fibromyxoma)

Discussion

Plexiform angiomyxoid myofibroblastic tumor (PAMT) of the stomach was first described in a report of two cases by Takahashi et al from Japan in 2007. Since then, 16 cases have been described in the literature to date, including a contribution of 12 cases from Miettinen et al in 2009, proposing the term "plexiform fibromyxoma" for this peculiar and distinctive multinodular tumor of the stomach.

PAMT has a predilection for young and middle-aged adults, but may occur in children and older adults (as in the present case). Median tumor size varies from 3.6-5.5 cm with an exclusive location in the gastric antrum. GI bleeding is the most common clinical presentation with pain, pyloric obstruction and rarely perforation. Nevertheless, PAMT appears to be a benign neoplasm on the basis of follow-up data. With the limited number of reports, complete local excision and follow-up is warranted until further information on the behavior of this tumor is forthcoming. Even though extragastric extension has been demonstrated in some tumors, a more conservative surgical approach than partial

gastrectomy may be appropriate, especially in smaller tumors.

The majority of mesenchymal tumors of the stomach are gastrointestinal stromal tumors (GISTs) which need to be differentiated from smooth muscle tumors (both leiomyoma and leiomyosarcoma), nerve sheath tumors (schwannoma and perineurioma), desmoid fibromatosis, solitary fibrous tumor (SFT), inflammatory fibroid polyp, and inflammatory myofibroblastic tumor.

PAMTs appear to be morphologically distinctive with a remarkable plexiform growth pattern, bland ovoid spindle cells embedded in an abundant fibromyxoid stroma with rich capillary network. PAMTs are consistently immunonegative for C-KIT (CD117), DOG1, PDGFRA and CD34, with approximately 8 examined cases negative for KIT or PDGFRA activating mutations, comfortably ruling out potential confusion with GIST in the differential diagnosis. Miettinen estimates a ratio of 150 GIST:1 PAMT, highlighting the rarity of this neoplasm. Only rare GISTs have a prominent myxoid stroma, which appear more commonly in epithelioid GIST.

The immunohistochemistry of PAMT seems to support a myofibroblastic-fibroblastic phenotype (MSA+, SMA+, desmin-), although both Takahashi et al and Yoshida et al suggest that a subset of PAMTs may possess features of smooth muscle differentiation, both on morphology (blunt-ended nuclei and long eosinophilic cytoplasm) and immunohistochemistry (SMA/MSA+ dense cytoplasm in contrast to "tram-track" pattern seen in myofibroblasts, and focal desmin immunoreactivity). Nevertheless, myxoid leiomyomas as a differential diagnosis (reported in the esophagus) typically contain areas of solid fascicular pattern and cytoplasmic eosinophilia and are diffusely positive for desmin. Presently, most authors accept (for now) PAMT to demonstrate myofibroblastic differentiation, confirmed on ultrastructural examination.

Nerve sheath tumors (both cellular schwannoma and rarely plexiform neurofibroma) can easily be ruled out based on morphology and diffuse S-100 immunoreactivity (the latter consistently negative in PAMT). Desmoid fibromatosis may have foci of myxoid stroma, but typically show long fascicular arrangements of spindle cells with intermixed dense collagen. Further, most desmoid fibromatoses show positive immunoreaction with β-catenin in the nuclei of tumor cells. SFT may also occasionally show myxoid change, but the alternating hypocellular and hypercellular areas, dense keloid-type collagen, hemangiopericytomatous vascularity and CD34/CD99 immunoreactivity are distinctive.

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CASE 29

Kumarasen Cooper M.D.

RECURRENT RHABDOID GIST OF THE SMALL INTESTINE

Clinical History

A 5.0 cm abdominal tumor was resected from a 62-year-old woman in 2008.

Past History, Radiology and Gross Specimen

· 2002: Her past history was significant in that a 17.0 cm mass arising from her small bowel was resected in 2002, along with peritoneal metastases. Following a diagnosis of malignant GIST, the patient was treated with Imatinib (Gleevec) for one year, with no evidence of disease in the subsequent three years.
· 2006: In 2006, tumor recurrence necessitated another course of Imatinib. However, progression of the tumor (whilst on Imatinib) resulted in a second resection of a 7.0 cm tumor.
· 2008: Unfortunately, the tumor again recurred in 2008. Following treatment with both Imatinib and a second tyrosine kinase inhibitor, Sunitinib, a third resection was performed (5.0 cm) representing the current submitted slide/section.

Microscopic Examination

· 2002: Hypercellular, moderately pleomorphic, spindled and epithelioid cells arranged in fascicles (unusual for small intestine GIST, which usually shows an organoid architecture). A minor component (5-10%) of the tumor demonstrated a distinct rhabdoid morphology, as evidenced by large polygonal cells with eccentric hyperchromatic nuclei and dense eosinophilic cytoplasm. Necrosis was present, as were numerous mitoses (20/10 high power fields). The tumor cells were immunoreactive with C-KIT, CD34, (DOG-1) and S-100 focally. Desmin, α-SMA, pan-cytokeratin and HMB-45 were negative.
· 2006: The recurrent GIST (7.0 cm) was similar in its spindled appearance to the original tumor, however the epithelioid component was hypocellular and hyalinized, consistent with post-chemotherapy (Imatinib) therapy. Interestingly, rhabdoid cells were not seen, although this may reflect a sampling bias.
· 2008: This second recurrence (5.0 cm), representing the submitted slide, was entirely composed of rhabdoid cells without evidence of the spindled/epithelioid morphology of the previous tumors. Repeated immunohistochemistry demonstrated a

strong, diffuse immunoreactivity with C-KIT, (DOG-1) and CD34, whilst S-100 was negative.

Diagnosis
RECURRENT GIST WITH RHABDOID DIFFERENTIATION OF THE SMALL INTESTINE

Follow-Up
Unfortunately the patient died shortly after the second recurrence was resected.

Discussion
To date there have been 51 cases of rhabdoid GIST reported in the literature. All of these cases arose in the stomach and were frequently admixed with spindled/epithelioid morphology. Although the primary (2002) and first recurrent tumor (2006) in our patient had a predominantly spindled/epithelioid morphology, the pure rhabdoid GIST (second recurrence, 2008) following multiple resections and treatment with tyrosine kinase inhibitors (both Imatinib and Sunitinib) represents the first case (to our knowledge) arising in the small intestine.

Further, whilst follow-up was not documented in all 51 cases of gastric rhabdoid GIST, none showed evidence of malignancy (recurrence or metastatic disease). The present case arising in the small intestine was malignant at presentation (both size and mitoses with peritoneal metastases), even though only about 5-10% of the morphology comprised rhabdoid features. This begs the question whether rhabdoid morphology in small intestinal GIST is indicative of an aggressive behavior? Clearly a greater number of cases, along with follow-up, is necessary to answer this question.

The most common mutation described in approximately 60% of rhabdoid GISTs (gastric/epithelioid) is the PDGFRA mutation (substitution of aspartic acid for valine at codon 842) with a documented poor response to Imatinib. The only KIT mutations identified in rhabdoid GISTs are exon 11 mutations (in-frame deletions clustering between codons 550 and 561). These tend to be the most common mutations seen in all KIT mutated GISTs regardless of phenotype/location. The submitted case harbored an exon 11 KIT mutation (in all three tumors: 2002, 2006 and 2008) resulting in the insertion of leucine and lysine between codons 579 and 580. This is an exceedingly rare mutation in any GIST and is the first described in rhabdoid GIST. Codon 558 insertions are almost exclusively found in epithelioid GIST accounting for <1% of all KIT mutations, and define a subset of tumors that often prove to be malignant. However, the prognostic implication outside of the 558 insertion is not known. We postulate that the 579-580 leucine-lysine insertion in the present rhabdoid GIST may represent a rare but aggressive mutation and may even correlate with rhabdoid morphology. Again, more cases are required to fully understand the full implications of this mutation. In addition, the present case acquired a secondary exon 17 KIT mutation in the 2006 recurrence, which has been shown to confer resistance to both Imatinib and Sunitinib therapy. This mutation was NOT demonstrated in the 2002 tumor; however, surprisingly, was also NOT present in the 2008 recurrence.

In general, KIT exon 11 mutations are usually susceptible to Imatinib therapy. However, most small intestinal GISTs show KIT mutations involving exons 9, 13 and 17, with exon 9 responding poorly to Imatinib.

Recognizing the differential diagnosis of intra-abdominal rhabdoid tumors is essential to target appropriate immunohistochemistry:

- Metastatic melanoma (S-100, HMB-45, Mart-1 and C-KIT)
- Clear cell sarcoma (as for melanoma and t12;22)
- Leiomyosarcoma (desmin, actin, C-KIT negative)
- Angiosarcoma (CD31, CD34, FVIII)
- Mesothelioma (pan-cytokeratin, calretinin, D2-40, CK5/6)
- MPNST (40% S-100/GFAP; C-KIT negative)

[cid:205274218@27042010-1E29]

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Guidelines for Risk Assessment of Primary Gastrointestinal Stromal Tumor

Tumor Parameters Risk of Progressive Disease (%)		
Mitotic Index	Size, cm	Stomach Duodenum Jejunum/Ileum Rectum
<5 per 50 HPFs	<2	None (0) None (0) None (0)
	>2 but <5	Very low (1.9) Low (8.3) Low (4.3) Low (8.5)
	>5 but <10	Low (3.6) Insufficient data Moderate (24)
	>10	Insufficient data Moderate (10) High (34)

High (52)
High (57)
>5 per 50 HPFs <2 None
Insufficient data High
High
(54)
>2 but <5 Moderate
(16) High (50)
High (73)
High
(52)
>5 but <10 High (55)

Insufficient data High
(85)
Insufficient data
>10 High
(86) High
(86) High
(90)
High (71)

* Based on 5 mm2 field (old microscope requires 50 HPF): new microscope 20 HPF
** Defined as recurrence, metastasis or tumor-related death

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CASE 30

P. E. Wakely, Jr., M.D.

Case History: A 68-year old man presented with a 3 month history of progressive dysphagia. After extensive testing a 3rd chest CT scan showed a pedunculated heterogeneous esophageal mass that was removed.

Pathologic Findings: The specimen consisted of a firm, club-shaped 15.0 cm mass with a 2.3 cm stalk. A smooth granular surface was tan-pink, and the distal end had a 2.6 cm ulcer. Cut sections showed a central core consisting of various-sized lobules of fibroadipose tissue with small hemorrhagic foci. Non-keratinizing squamous epithelium surfaced the polyp. The core contained fibroadipose tissue with foci of chronic inflammation. Scattered polygonal or stellate-shaped lipoblasts characterized by markedly hyperchromatic, multilobular nuclei and multinucleated cells were randomly scattered within the fibroadipose tissue. Also present were single and loose aggregates of large rounded and elongated cells containing abundant eosinophilic cytoplasm typical of rhabdomyomatous differentiation. Many of these displayed elongated strap-like rectangular shapes with multiple nuclei arranged in tandem. Immunohistology results: positive nuclear staining of lipoblasts with MDM2 and CDK4; rhabdomyomatous cells strongly positive with myoglobin, myogenin, HHF-35 actin, and vimentin. Spindle cell areas were positive with CD34 and vimentin. Mitoses were scarce, and none were atypical. Proliferation index marker (Ki-67 stain) showed <5% positively stained cells. S-100 was positive only in adipose tissue. No staining occurred with CD117, HMB-45, SMA, or cytokeratin AE1/AE3. FISH analysis using the LSI MDM2 DNA probe demonstrated amplification of the *MDM2* gene locus at 12q14-q15 in 64% of the interphase cells.

Diagnosis: **RHABDOMYOMATOUS WELL-DIFFERENTIATED LIPOSARCOMA ARISING IN GIANT FIBROVASCULAR POLYP OF ESOPHAGUS.**

Discussion: Large pedunculated polyps (>5 cm. in dimension) of the esophagus are rare. At least 111 cases were reported as of 2006. Over 90% are giant fibrovascular polyps (GFVP) as described by Stout et al. GFVP is typically seen in middle aged men (age range 18 mos. - 88 yrs.). 70% present with progressive dysphagia and weight loss. About 25%, have a more dramatic presentation that involves regurgitation of the polyp into the oral cavity with respiratory compromise, or even asphyxiation due to glottic obstruction.

Pathogenesis of esophageal GFVP likely derives from an outpouching of loose submucosal tissue into the lumen of the cervical esophagus where more than 80% of GFVP

originate. Normal peristaltic action, and tractional forces produce by the polyp's own weight eventually lead to "giant" proportions, and assumes a club or sausage shape. The upper esophagus is where most benign neoplasms or tumor-like lesions arise including leiomyoma, hemangioma, inflammatory pseudotumor, granular cell tumor, and schwannoma. Malignant polyps such as liposarcoma, leiomyosarcoma, and spindle cell carcinoma more often arise in the mid-distal esophagus.

An admixture of different tissues explains the polyp's heterogeneity using MRI and CT scan. Since any one of these components may predominate, a varied nomenclature is applied to GFVP including lipoma, fibromyxoma, fibroma, hamartoma, fibrolipoma, and fibroepithelial polyp. The WHO recommends the term *fibrovascular polyp* for any lesion with these previously described characteristics. Caceres et al found 4 cases (3.6%) with malignant transformation – primarily squamous cell carcinoma or liposarcoma. We initially interpreted the lipoblasts in our case incorrectly as "atypical" stromal cells. Positive immunohistology for MDM2 and CDK4 along with confirmatory FISH analysis demonstrating amplification of the *MDM2* locus confirmed the diagnosis of liposarcoma.

GFVP can position itself against the esophageal wall and the small stalk may be overlooked. Barium swallow may be interpreted as normal because contrast is allowed to pass smoothly down the esophagus without getting around the polyp to demonstrate its intraluminal location. Esophagoscopy may be misinterpreted as an intramural mass or extrinsic compression by a thoracic mass, or may even be reported as normal.

Treatment of choice for GFVP is local surgical resection. Rarely would a patient need an esophagectomy; however, this has been performed in some studies.

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CASE 31

David Ben-Dor M.D.

PANCREATIC ACINAR CELL CARCINOMA

History:
an 83 year old man underwent a Whipple procedure for removal of a "duodenal mass".

Gross description:
opening of the duodenum revealed a large bulbous mass extending from the pancreas and protruding into the lumen. No tumor was described on the mucosa. Sectioning of the pancreas revealed a mass measuring 17x10x9 cm which was well circumscribed and composed of solid tan tissue with areas of necrosis.

Histology: the tumor is composed of relatively monomorphic cells containing ovoid nuclei with prominent nucleoli and eosinophilic cytoplasm arranged in nests and sheets. The tumor extends through the duodenal wall. Immunohistochemically focal positivity is seen with trypsin and chymotrypsin, while neuroendocrine stains and stains for particular hormones were negative. Metastatic tumor was found in 1 lymph node. Pancreatic parenchyma from outside the mass shows atrophic changes.

Diagnosis: pancreatic acinar cell carcinoma

Discussion: While single case reports on this tumor date from as early as 1947 (see references in Klimstra et al), a large series was published in 1992 (Klimstra et al, Am J Surg Pathol 16(9): 815-837, 1992). More recently this topic was summarized very nicely in the recently published AFIP fascicle on pancreatic tumors (series IV no. 6 edited by Hruban, Pitman, and Klimstra- chapter 9 pp. 191-215), and I will base the below more or less liberally on these sources .

As implied in the name this neoplasm is composed of cells which show acinar differentiation- namely, the production of zymogen granules containing pancreatic exocrine enzymes. Almost all acinar cell tumors are malignant- a benign variant, acinar cell cystadenoma, seems to be quite rare, with only a few cases reported. Acinar cell carcinomas comprise 1-2% of adult pancreatic neoplasms and while about 15% of pediatric pancreatic tumors are of this type, due to the greater number of pancreatic neoplasms in adults only about 6% of cases of this entity are found in children. The incidence in adults peaks in the seventh decade and most patients are male; it is rare between the ages of 20-40. This tumor usually presents with non-specific symptoms- weight loss, abdominal pain, nausea and vomiting. Jaundice, a classical feature of biliary obstruction, is not common in patients with this tumor which usually does not involve the major duct. However when hepatic metastase are present some patients present dramatically with a unique syndrome - the lipase hypersecretion syndrome, characterized by the appearance of subcutaneous fat necrosis, polyarthralgia, and eosinophilia, and engendered by the leakage of pancreatic enzymes into the blood. Grossly, though this tumor can appear in any portion of the pancreas, about half of the cases in the Klimstra series were found in the head; most are greater than 10 cm., well circumscribed (though penetration

into adjacent structures may be seen in some cases), and solid, though necrosis may be prominent in the larger masses. Histologically they are cellular and usually with relatively sparse fibrous stroma. Despite the gross impression on microscopic examination penetration of the capsule is common. The tumor cells are most commonly arranged in two patterns- solid or acinar. In the latter small lumens may be seen- an important feature for identifying this tumor, while the former is composed of sheets, cords, and nests without lumens (however even in cases predominantly of solid type foci of acinar differentiation can be found even if focally). More rarely the tumors form glands with dilated lumens or trabeculae. The amount of cytoplasm and of d-PAS positive granules correlates with cellular differentiation type, both being more prominent and obvious in the acinar type (though the granules may be only focally found in the solid variant, they are almost always found if looked for, especially at the interface with the stroma or capsule), showing in places preservation of the apical cytoplasmic polarity of non-neoplastic acini. The nuclei are situated in the basilar portion of the cells in the acinar variant creating a palisade arrangement, and centrally in the solid type, (though even in the latter type palisading can be found at the tumor –stromal interface), and are generally uniform with prominent nucleoli (an important feature for identifying this tumor, especially the solid variant). Rarely the cytoplasm may be oncocytic due to the presence of numerous mitochondria, or the nucleus may be pushed to the periphery of the cell by the granular cytoplasm, mimicking signet ring cells (though lacking mucin of course).
Confirmation of acinar differentiation can be made with immunohistochemical stains, with antibodies to, trypsin, chymotrypsin and lipase most often positive (up to 95% for the first two, and 80% for the latter, as per table 9-2 in the fascicle) this again varying with degree of acinar differentiation (and is focal in the solid type); positivity for amylase is rare. Scattered neuroendocrine cells (less than 25% of total) can be demonstrated with synaptophysin or chromogranin in up to 54% of the cases. Ultrastructural examination reveals electron dense zymogen granules and a second type of granule highly characteristic of pancreatic acinar differentiation- the "irregular fibrillary granule"- which is not found in normal acinar cells.. As denoted by the name, these show a variety of shapes and are composed of fibrillary material. Aspirates of this tumor are characterized by irregularly shaped groups and sheets of bland cells with coarsely granular cytoplasm and uniform nuclei with prominent nucleoli. Stripped nuclei seen in the background are most helpful in the differential diagnosis with non-neoplastic acini.
As concerns differential diagnosis, acinar cell tumors can be easily confused with *pancreatic neuroendocrine neoplasms*. This issue can be resolved using immunohistochemical staining which in the case of acinar carcinoma will show at least a preponderance of cells positive for the markers discussed above, with the latter showing neuroendocrine differentiation solely. On morphological grounds acinar cell carcinoma is favored by the presence of widespread acinar differentiation, basal nuclear polarization, eosinophilic granular cytoplasm, prominent nucleoli, and easily found mitoses, while neuroendocrine tumors will demonstrate salt and pepper nucleoli which are centrally situated, trabecular or gyriform disposition of the cells, and hyalinized or amyloid stroma. Another tumor which might be confused with acinar cell carcinoma is *pancreatoblastoma,* which may demonstrate extensive acinar differentiation. This entity however is characteristically found in the first decade and shows squamoid differentiation, a feature not described in acinar cell carcinoma. *Solid-pseudopapillary tumors* of the pancreas may show sheets of uniform cells mimicking the solid areas of acinar cell carcinoma; these tumors however usually appear in female patients in their twenties and show degenerative pseudopapillary changes not seen in the latter. Immunohistochemically these tumors are also distinct, as the former show positivity for CD10 and nuclear positivity for beta-catenin.

Despite their relatively tame appearance acinic cell carcinomas are known to be aggressive. Metastases are found in more than half the patients at the onset and appear subsequently in an additional 25% during the course of the disease. Median survival is 1.14 years and five year survival is 25%. Presentation with the lipase hypersecretion syndrome is particularly ominous prognostically, as it is seen in the presence of liver metastases.

While scattered neuroendocrine cells are often found as mentioned previously, neoplasms of acinar cells containing a more significant population of neuroendocrine cells, greater than 25%, are defined as mixed acinar-endocrine carcinomas (Klimstra et al, Am J Surg Pathol 18(8): 765-778, 1994). These rare lesions are often not easily distinguishable histologically from the conventional type of acinar carcinoma; the acinar and endocrine components are intimately admixed and can often be separated only by immunohistochemical markers for neuroendocrine differentiation, and expression of specific hormones is rare. The acinar component is still predominant and identified by the same markers as mentioned above. Clinically these tumors behave similarly to the pure acinar type.

In the same vein, a very recent publication (Stelow et al, Am J Surg Pathol 34(4): 510-518, 2010) describes 11 tumors showing both ductal and acinar differentiation. Five of these cases consisted of typical acinar carcinoma cells on a background of extracellular mucin secretion, with evidence of intracytoplasmic mucin including signet ring cells. The other six cases showed areas of typical acinar carcinoma mixed with others showing the morphology of conventional invasive ductal carcinoma. Mucin was demonstrated histochemically in these tumors, and immunohistochemistry showed positivity for trypsin, and markers for ductal differentiation (including CK19, CEA, CA19.9). In most instances where staining was performed for both mucin and trypsin the staining patterns were exclusive; however a few cells were found to be positive for both. In addition three cases also showed greater than 25% positivity for neuroendocrine markers, diagnosed as "mixed acinar endocrine ductal carcinomas". High grade pancreatic intraepithelial neoplasia was found in 30% of the cases, giving rise to speculation about the possibility of the ductal and acinar components arising from a joint intraductal precursor.

In fact an intraductal type of acinar carcinoma has recently been described by a group of authors, including Dr Volkan Adsay, representing various institutions (Basturk et al, Am J Surg Pathol 31: 363-370, 2007). This paper described 7 cases of acinar carcinoma with either nodular and or papillary-cystic morphology, all with evidence of intraductal origin. Histologically the tumors showed features typical of acinar differentiation which was confirmed histochemically with PAS stains and immunohistochemically with stains for trypsin. Some of these tumors showed a minor neuroendocrine component evident on histological examination. These tumors were smaller than usual and the clinical course appears to be less aggressive than in typical acinar cell carcinoma, with metastasis found in only one case.

Very rare cases of acinar cell cystadenocarcinoma containing cysts lined by neoplastic acinar cells as described above have also been reported. The clinical course is similar to that of the usual type of tumor.

CASE 32

David Ben-Dor M.D.

MEST-K

History and Gross Description:

A 53 year old woman presented with flank pain and microscopic hematuria. Imaging studies revealed a large mass in the right kidney. Nephrectomy was performed which revealed a 9 cm. mass arising in the upper pole with a smooth surface and well demarcated from the surrounding parenchyma which was itself unremarkable. The cut surface showed multiple cysts of varying sizes, ranging from 0.2-2.5 cm. admixed with solid areas.

Histological description:
The cysts are lined by bland epithelium in places hobnail like and in places flattened. The septae and solid areas are composed to varying degrees (depending on sampling) by dense fibrous tissue, and spindle cell stroma. The latter particularly adjacent to the epithelial cysts can be wavy resembling ovarian stroma. These foci would be positive for estrogen and progesterone receptors.

Diagnosis: Mixed epithelial and stromal tumor of kidney (MEST)

Discussion: this term was first proposed by Michal Michal in 1998 (Pathology Res. Prac. 194: 445-448) for a renal lesion he described in a 48 year old woman. That particular case was grossly described as solid and well circumscribed and histologically consisted of an epithelial proliferation of variably sized ducts, the larger ones lined by hobnail cells, and a spindle cell stromal proliferation with ovarian features. It was emphasized that the stromal proliferation was always in tandem with the epithelial component and did not aggregate on its own. No other histological features of ovarian differentiation were found. The stromal cells were found to be positive for actin and desmin as is seen in normal and neoplastic ovarian stroma as well as in certain hepatobiliary lesions which also contain this type of stroma (evaluation for female hormone receptors was not reported or performed on this case). The issue of possible homology with other cystic lesions previously reported in children (cystic nephroma (CN), solitary multilocular cyst of the kidney) was brought up but not confirmed in this paper.

Subsequently, in a letter to the editor of the same journal (Pathology Research and Practice, 196: 275-276, 2000) Michal commented on the fact that after publishing their original case report he and his colleagues encountered other papers reporting on cases of fibrocystic lesions in the kidney similar to that one. He stressed the following attributes shared by these cases: they have ovarian type spindle cell stroma (which may become fibrotic) also showing myoid differentiation, hobnail epithelial cells, are benign, occur in adult women (as do other tumors of the hepatobiliary tract and pancreas which also contain ovarian like stroma). The author contended that these tumors, though previously reported under different names (adult type of mesoblastic nephroma, cystic hamartoma of the pelvis, adult type of cystic nephroma (as differentiated from the pediatric type), and solid and cystic biphasic tumor of the kidney) actually can be considered to represent a single entity. As the type and degree of proliferation of the epithelial and stromal components may vary between cases, they were given different names by different authors, depending on whether there was more or less stroma or to what degree the epithelial structures were dilated.

At the same time, in 2000, Volkan Adsay and other noted experts on renal tumors (John Eble, John Srigley, Edward Jones, and David Grignon representing four major institutions) culled from their files 12 cases "characterized grossly by a mixture of solid and cystic areas and histologically consisting of a mixture of spindle cell stroma and varied epithelial elements" which they also called "mixed epithelial and stormal tumors of the kidney" (borrowing Michal's term) and reporting on them in an article published in the American Journal of Surgical Pathology (24: 958-970). 11 of the patients were women, 7 of whom had been

taking long term oral estrogens; the male patient had been receiving estrogens for prostatic adenocarcinoma. The lesions were grossly described as showing varying amounts of cystic and solid areas. The epithelial component showed varying degrees of complexity but cysts lined by hobnail cells were commonly seen. The stroma which tended to condense about the epithelial elements was described as being predominantly "fibrotic and paucicellular" in three cases and "markedly cellular with a fascicular arrangement" in four, with smooth muscle features noted. Comment on a varying resemblance of the stromal elements to leiomyoma, ovarian stroma, or solitary fibrous tumor was made. The stromal cells were positive for muscle markers (desmin and actin) and also for estrogen and progesterone receptors. Though recognizing the similarity of the lesions they described to those previously described by others as Michal noted in his letter, one of the diagnostic terms previously used, "adult type of mesoblastic nephroma", was not considered appropriate, as mesoblastic nephroma is a lesion of infancy and the proliferating element is stromal, with the epithelium being entrapped and not part of the pathological process (as it is in MEST). They ventured the theory that the exposure to estrogens describe in these patients could lead to proliferation of the "periductal fetal mesenchyme" which in term stimulates the activity of the epithelial component. In agreement with Michal homology to similar lesions in the liver and pancreas was noted by these authors.

Afterwards, in 2004, an additional group of cases was reported on by a group including Michal, Saul Suster, and Michele Bisceglia (Virchows Archiv 445: 359-367). This group of patients included 22 women and 2 men. Grossly the tumors were described as predominantly cystic and the histological features for the tumors in women were as previously described, with the presence of ovarian type stroma positive for estrogen and progesterone receptors emphasized. Unique in their cases was the presence of mullerian features in the epithelial component of some cases, which showed endometrioid, clear cell, squamous, and transitional differentiation, with one showing areas of mullerian adenofibroma/adenosarcoma. They drew a differentiation between the female and male cases, as the latter showed only thin walled cysts without ovarian stroma and no hobnail cells. They felt that the male cases would be better considered separately and termed "cystic nephroma" with the term MEST best reserved for the female cases. The term cystic nephroma was in fact borrowed from pediatric pathology to describe fibrous and cystic lesions composed only of differentiated tissues with an apparent resemblance to some of the lesions being discussed here. According to the authors the pediatric lesions would be best considered together with cystic partially differentiated nephroblastoma, and they felt that lesions in women previously called cystic nephroma should be reclassified as MEST.

Studies from more recent years have concerned themselves with the relationship between MEST and cystic nephroma. In one such paper from 2007 (Turbiner et al, American Journal of Surgical Pathology 31: 489-500), 34 cases from various institutions were compared. 20 of the cases were classified as CN and 14 as MEST, which were differentiated by the thickness of the septae (as proposed earlier by Eble and Bonsib in 1998- see Semin. Diagn. Pathol. 15: 2-20), the former diagnosis made only in cases where the septae were less than 5 mm. All cases of MEST were women while two of the CN cases were male. While the age range of the patients showed a relatively wide span (from the third to the seventh decade), it was centered in the early fifties (as was seen in earlier studies). Based on analysis of various histological parameters it was concluded that these two purported separate entities represent an overlapping spectrum. On the whole the CN group tended to show a lesser stromal epithelial ratio than the MEST group, with larger cysts in the former and smaller glands and phyllodes type structures in the latter, but these differences did not reach statistical

significance. The stroma tended to be more hypercellular and showed more stromal condensation in MEST and was more collagenized and hypocellular in CN. While ovarian stroma was seen in both types, it was more often seen in the MEST cases where it was more copious (the presence or absence of ovarian stroma in the male patients was not specifically stated). In keeping with advances in immunohistochemistry since the earlier publication, in this study the stroma was found to be positive for CD10, inhibin, and calretinin, in proportion to the presence of ovarian stroma, with inhibin and also calretinin staining the luteinized cells. A marked difference was noted in the degree of positivity for ER and PR between the groups, with this feature being present to a significantly greater degree in MEST than in CN. The authors suggested that MEST may undergo progressive fibrosis and transform into CN (though the age demographics as far as median and mean ages were similar in both groups, and the patient with the highest age- 84- actually belonged in the MEST category) and proposed that they be considered as belonging to one entity, for which they coined the term "renal epithelial and stroma tumor" (REST). In a letter to the editor, Zhou (Am J Surg Pathol 34: 127, 2010) pointed out that in his material published earlier in 2009 (Am J Surg Pathol 33: 72-80, 2009) ovarian stroma was usually found in lesions from premenopausal women whereas those in postmenopausal women were preponderantly fibrotic.

Mai et al (Pathology 39: 235-240, 2007) make an interesting suggestion regarding the nature of the fibrotic/hypocellular stroma in MEST. They make the analogy between it and endometriotic or endocervical stroma, considering it another form of expression of mullerian differentiation, and not just a regressive phenomenon, thus broadening the spectrum of phenotypes seen in this lesion. Their 14 patients included two men. Ovarian stroma was seen only in the women. Positivity for estrogen and progesterone receptors was widespread in the stroma of the tumors in women, both in the areas resembling ovarian stroma and also in the more fibrotic tissue. Estrogen and progesterone receptor positivity was also found in the tumors from male patients, but to a lesser extent. They relate the presence of mullerian tissues to the common origin of the ovaries and kidneys from the urogenital ridge. Interestingly a few cells positive for ER/PR were also found in non-lesional renal tissues. In their view the stromal cells are the neoplastic elements in this condition and they in turn induce proliferation of entrapped epithelial structures.

With these claims in mind Tickoo et al (Modern Pathology 21: 60-65, 2008) have published some provocative observations, namely the presence of estrogen/progesterone positive cells in non-neoplastic stroma close to cystic dilated structures in kidneys removed for obstructive nephropathy or containing cysts of other etiologies. Very interestingly, most of the patients were males!! Histologically, the stroma was described as variably composed of "short spindled to stellate cells superficially resembling stroma of basal endometrium or endometrial polyps, paucicellular stroma with plump nuclei and fibrotic background (superficially resembling endocervical stroma), to densely cellular stroma with wavy nuclei resembling ovarian type stroma". They express their belief that these types of non-ovarian stroma (homologous to that seen in other parts of the female genital tract) are more common in MEST than the perception reinforced in the literature which stresses the ovarian type without openly acknowledging the others (though illustrated in the various reports). They also report the presence of ER/PR positive cells in otherwise non-lesional tissues of the specimens they studied, and rarely in the controls. In their view the stromal proliferation in this setting is secondary to the epithelial changes. Perhaps patients who develop renal lesions with mullerian stroma- either neoplastic in MEST or non-neoplastic in cystic kidneys- have a propensity to do so which originates in particular developmental events occurring in the urogenital ridge in embryonic life.

In passing, ovarian or mullerian type stroma has also been documented in the recently described angiomyolipomas with epithelial cysts (see above reviews for references). While

MEST is benign and cured by excision, it should be kept in mind that rarely malignancy is seen in the stroma. In fact, one case of MEST submitted in the past to the AMR seminar was thought by some other members to be malignant. Thus the diagnosis of typical MEST as a benign lesion must be made only upon careful evaluation of the various components of the lesions.
I would like to close with an editorial comment made by Dr Ferran Algaba referring to the review by Montironi et al (European Urology 54: 1237-1246): "All the above leads us to reflect on what a pathologic classification is. The pathologic classifications of the tumors are based on the organization of the different tumor masses according to common morphologic characteristics. The purpose of these classifications is to include in a single term lesions with similar biology and, therefore, similar prognosis and treatment. *However, by not knowing the etiopathogeny of the majority of neoplasias, the classification criteria are morphologic and immunophenotypical appearances that suggest similarities to us*, for which reason they must be reviewed and modified periodically according to the clinical therapeutic evolution of the established groups, trying to find those characteristics that *actually express a common and useful character*. Only close intercommunication among pathologists, urologists, oncologists, and biologists will make these classifications the most accurate and truthful possible".

CASE 33

David Ben-Dor M.D.

Mucinous tubular and spindle cell carcinoma of the kidney:

Gross and histological description:
The specimen consisted of a kidney measuring 13x7 x4 cm. with a well circumscribed mass at the upper pole measuring 4x3 cm showing abundant necrosis and hemorrhage.
Histological examination shows a proliferation of tubules with open or gaping lumens filled with mucin staining positively with alcian blue. The tubules are lined by rather monomorphic cuboidal cells containing uniform round nuclei. These cells are positive for CK7, vimentin, and AMACR.

Diagnosis: mucinous and tubular spindle cell carcinoma of the kidney.

This tumor which shows a unique combination of features – tubules lined by cuboidal cells with low grade nuclei, spindle cells of similar nuclear features, and mucin production- was recently acknowledged to be a distinct entity in the latest WHO classification of renal tumors (2003).

As reviewed by MacLennan and Bostwick (Clin Lab Med. 25: 393-406, 2005), Farrow delineated, first in an abstract in 1994 and later (together
with others) in publication form (MacLennan G., Farrow G., and Bostwick D.- Urology 50:679-684, 1997), a group of renal tumors characterized by mucin production and tubulo-cystic architecture which he considered to be low grade variants of the usually more aggressive (morphologically and clinically) tumors of collecting duct origin. In retrospect, MacLennan and Bostwick pointed out that these lesions actually consisted of two separate entities: one, with duct-like or cyst-like structures (now known as tubulo-cystic carcinoma), and the other composed of anastomosing tubular structures with abundant background mucin

(mucinous and tubular spindle cell carcinoma). In most of the patients there was no disease progression.

Additional series of mucinous tubular and spindle cell carcinoma were subsequently published in turn. In 2001 Parwani et al (Hum Pathol 32: 506-512) discussed four cases of what they termed "low-grade myxoid renal epithelial neoplasms with distal nephron differentiation", all in women, with solid well circumscribed renal masses histologically characterized by bland, low cuboidal cells forming curved or straight interconnecting, and elongated tubules on a myxoid at times "bubbly" background. In places the tumor cells were spindled. Immunohistochemically the tumors were positive for high molecular weight keratin, indicative of collecting tubule differentiation (though negative staining for ulex europaeus agglutinin did not favor that possibility), and negative for CD15, a proximal tubule marker. The possibility of origin from the loop of Henle was also discussed but could not be proven. The feeling of the authors was that the tumor was probably of "generic" distal nephron origin. None of these patients' tumors spread or recurred.

In the following year Hes et al (Histopathology 41: 549-555) reported on an additional 11 cases (this group evenly divided between men and women, at variance with other reports) of what they termed "spindle and cuboidal renal cell carcinoma". They noted transitions between the spindle and cuboidal cell components, both of which were low grade. Three patients had in addition nephrolithiasis and one patient also had renal cell carcinoma. These authors also considered derivation of the tumor from the loop of Henle ("loopoma") but offered no immunohistochemical support for that concept. Metanephric adenoma, which is EMA negative (in contrast to this tumor) and lacks spindle cells, and sarcomatoid renal cell carcinoma, which unlike this case shows features of a high grade tumor, were included in the differential diagnosis. There was no disease progression in seven of the patients.

Also in 2002 Rakozy et al (Mod Pathol. 15: 1162-1171) reported on 4 cases, again involving only women. In their histological description they also noted the presence of macrophages. Their immunohistochemical results- including positivity for EMA and peanut agglutinin- were interpreted as favoring collecting duct origin (though as in the Parwani paper, ulex, also a collecting duct marker, was negative), though a low grade variant. They also performed genetic studies whose results- losses of multiple chromosomes- indicated collecting duct origin, with some affinity to the genetic derangements found in chromophobe renal cell carcinoma, considered as well to be a non-aggressive tumor of collecting duct origin. As in the other series these patients did well, with no metastatic disease or death from disease, but with multiple recurrences in one patient.

More recently Fine et al (Am J Surg Pathol 30: 1554-1560, 2006) described 17 cases (13 of which were female) including some with only scant mucin ("mucin poor"), thus broadening the histological spectrum of this entity, and called attention to the fact that areas of tubular predominance without mucin which they found in their cases should not lead to confusion with the solid type of papillary carcinoma. This confusion could be reinforced by the presence of rare foci of papillae or papillations (without stromal cores) and foamy macrophages in mucinous spindle cell and tubular carcinoma. However they concluded that papillary carcinoma and mucinous and spindle cell carcinomas were *not* related, since the reported characteristic genetic profile of the former (gains of chromosomes 7 and 17) was not found in the latter.

Argani et al (Am J Surg Pathol. 32: 1353-1359) described five cases diagnosed as renal papillary carcinoma, all in males, containing low grade spindle cells and angulated or curvilinear tubules, similar to mucinous and spindle cell tumors, but without mucin. Papillae were only focally found. Genetic analysis showed trisomies of chromosomes 7 and 17, typical for papillary carcinoma, in most of the cases. These cases demonstrated to the authors that papillary carcinomas can have some morphological resemblance with the mucinous and spindle cell type, but that despite that these two tumors are separate unrelated entities. These cases also show that spindle cell differentiation of low grade type may be seen in the context of papillary carcinomas and is not necessarily indicative of sarcomatoid transformation.

Shen et al (Ann Diag Pathol 11: 13-21, 2007) studied 12 cases (female predominance of 9:3) of mucinous tubular and spindle cell carcinoma immunohistochemically and stressed the similarities with papillary carcinomas, as both stained positively for proximal tubule markers: AMACR, RCCma, and CD15, and for CK7, which stains the distal tubule as well as papillary carcinoma. Unlike Argani et al these authors feel that the mucinous and spindle cell tumors *are* a variant of papillary carcinoma both deriving from the proximal tubule; though the former lack the characteristic 7, 17 trisomies of the latter, they both share in common losses of other chromosomes. Another group of authors (Paner et al, Am J Surg Pathol 30: 13-19, 2006) found a similar amount of overlap in the immunohistochemical staining properties of both tumors, including positivity for CK7, EMA, and especially for AMACR in both papillary and mucinous tubular and spindle cell carcinomas, with the major difference being that papillary carcinomas are mostly positive for CD10 and the mucinous tubular and spindle cell carcinomas are negative. They expressed caution in the use of immunohistochemistry in the differential diagnosis between these entities; however they stopped short of deducing from this that the latter is a subset of the former. These authors also stressed the similarity in immunohistochemical properties of the between the tubular cells and spindle cells, deducing that both are ontogenically similar.

In summary: mucinous tubular and spindle cell carcinomas are clinically and morphologically low grade neoplasms with a distinctive histological appearance found mostly in women. Latest immunohistochemical evidence would indicate origin from the proximal convoluted tubule. These tumors share immunohistochemical properties with papillary carcinomas and both tumors can focally show histological features more characteristic of the other, but they genetically they are for the most part (but not entirely) disparate. Whether or not mucinous spindle cell and spindle cell carcinoma is a subset of papillary carcinoma is currently being debated in the literature.

CASE 34

N. Volkan Adsay, MD

History

46 year old female presented with abdominal pain, vomiting, steatorrhea and weight loss. CT scan showed a complex 10 cm solid and cystic mass in the body of the pancreas The patient underwent pancreatectomy.

Macroscopic findings

A 10 cm, well circumscribed and seemingly encapsulated mass with both cystic and solid components was identified. The solid component was 5.5 cm in largest diameter and had tan, fleshy appearance with foci of nodular excrescences.

Microscopic findings
The sections from the solid areas revealed well-demarcated nodular growth pattern characteristic of intraductal neoplasms. The nodules were composed of tightly packed small tubular (acinar like) units lined by predominantly cuboidal cells without any mucin production. The nuclei were round to oval and markedly atypical, though relatively monotonous. Mitotic figures were readily identifiable. Necrosis was noted. In scattered foci, constituting approximately 20% of the tumor, there were widely separated glandular elements with irregular contours and cytomorphology different than that of the rest of the tumor, embedded in sclerotic stroma. These foci represented invasive carcinoma.

Diagnosis: INTRADUCTAL TUBULOPAPILLARY CARCINOMA OF THE PANCREAS, ASSOCIATED WITH INVASIVE CARCINOMA OF TUBULAR TYPE.

Discussion
In the pancreas, *intraductal neoplasms* are defined as mass-forming preinvasive neoplasms that form grossly visible (typically > 1.0 cm) tumors. They are to be distinguished from PanINs which are incidental/microscopic ("flat", non-mass-forming) forms of dysplasia.

Two distinct types of intraductal neoplasms are recognized in the upcoming WHO classification. 1) Intraductal papillary mucinous neoplasms, which are composed of mucinous type cells and are often associated with prominent mucin production. 2) Intraductal tubulopapillary neoplasms which are characterized by a distinctive tubular pattern of non-mucinous cells.

Intraductal tubulopapillary neoplasm (ITPN), is the category designation for the group of tumors that were originally reported as intraductal tubular carcinoma by Tajiri et al in a study of 4 cases. It is a new addition to the challenging differential diagnosis of pancreatic tumors fundamentally characterized by intraductal growth. Rare cases also show focal tubulopapillary configuration and therefore in the upcoming WHO-2010 blue book, this tumor type will be recognized as a distinct category under the heading of *intraductal tubulopapillary neoplasm.* We have recently analyzed 18 examples of this entity in multiinstutional collaborative study (Klimstra and Adsay et al), which represents the largest series analyzed to date. The following discussion is based on our current experience with these tumors.

Clinical features
There appears to be no gender predominance and mean age is mid-50's (range, 25-72). The main presenting symptoms include abdominal pain, vomiting, steatorrhea and weight loss. Interestingly, jaundice is very uncommon, which goes along with the intraductal (slow-growing) nature of these neoplasms.

Radiologically, the tumors typically show solid and cystic areas, and intraductal nature is often appreciable by expert radiologists in many cases, and a pre-operative diagnosis of "IPMN" (the better known category in intraductal neoplasms) is typically rendered in such cases. The tumors are more commonly located in the head of the pancreas (54%), and more rarely, in the tail (15%), however, a substantial number of cases exhibit diffuse involvement (31%).

Macroscopic features

These tumors are often large at the time of diagnosis from 2.5-15 cm (mean = 7 cm), similar to the IPMN but typically larger than usual ductal adenocarcinomas, and reveal both solid and cystic areas. Solid areas can be soft in some cases. They often have recognizable intraductal component, although the ducts can be filled, expanded and replaced by tumor to an extent that the native ductal system is often not recognizable in the vicinity of the tumor.

Microscopic features

The common characteristic, present in all cases, is the presence of smooth-contoured nodules which is often the only reflection of the intraductal nature of these tumors, because native ducts are often filled and replaced entirely by the tumor and thus their ductal nature is no longer recognizable on microscopic sections, unless there is a remnant of a retained ductal epithelium.

Typically the nodules are composed of tightly-packed small tubular units, which on occasion, may solidify into sheet like configuration in some areas. The solid pattern may be quite prominent in some cases. Rare examples show tubulopapillary configuration rather than pure tubular pattern, however, in our opinion, if a case shows significant tubulopapillary pattern, the possibility of pancreatobiliary type IPMN ought to be considered more highly. Comedo-like necrosis is noted in rare cases. Occasionally, spindle cell change has been observed. Also uncommon is the presence of cystic ducts devoid of any significant intraluminal polypoid proliferations. In rare examples, an intervening desmoplastic-like stroma is observed *within* the nodules, separating out the tubules, and creating an invasive appearance, which if taken out of context, can easily be misinterpreted as invasive carcinoma.

The cells are, by definition, of non mucinous type. They are typically cuboidal and relatively uniform; however, they are fairly large and atypical such that all the reported cases thus far have been classified as “carcinoma”. Mitotic activity is often high; it is not uncommon to see multiple mitotic figures in one high power field. The nucleoli are often prominent. As such, the overall cytomorphology closely resembles that of acinar cell carcinomas. In rare cases, apical snouts and even amorphous acidophilic secretions are seen; however, typically there is no evidence of zymogenic type granularity, and no crystals of acinar differentiation are noted.

Invasion is difficult to assess in these tumors, because of the relative complexity of the intraductal process. About a third of the cases we analyzed had areas that we interpreted as true invasion; although more than half of these were very limited in amount, showing minimal invasion to the periductal areas, which we classified as “microinvasion”. This particular case under discussion, however, had substantial amount of invasive carcinoma in some areas. Vascular and perineural invasion is exceedingly uncommon in these tumors. No extension beyond the pancreas has been noted.

Immunophenotype

Cytokeratin profile of ITPNs is similar to pancreatic ductal neoplasia and other foregut epithelial tumors: PanCK is expressed in all, and CK7 (85%) and CK19 (85%) are common as well, while CK20 is mostly negative (7%). Expression of mucin related oncoproteins is also fairly similar to that of other pancreatic ductal neoplasia, but perhaps to a lesser degree (CA19-9 in 95 %, B72.3 in 40, mCEA in 40, CA125 in 15 %). MUC expression profile speaks for a gastro-pancreatic rather than intestinal lineage: MUC1 is expressed 90 %, mostly in the apical membrane of the cells lining the tubules, MUC6 is positive in 60%, while MUC2, MUC4 and MUC5AC are negative. Most importantly, these tumors are negative for endocrine and acinar markers.

Molecular findings
Molecular alterations detected in ITPNs are in keeping with their intraductal (indolent) nature; these tumors appear to be genetically more stable than ordinary ductal adenocarcinomas. P53 is overexpressed in 20% and p16 in 50%. DPC4 is retained in more than 90% of the cases.

Differential diagnosis
At the clinical level, the main differential diagnosis of ITPN is with IPMN and ordinary ductal adenocarcinoma. At the microscopic level, in contrast, the most challenging issue is to distinguish these tumors from acinar cell carcinomas, which can also show nodular, tubular and even intraductal growth patterns. ITPNs and acinar cell carcinomas (ACC) can be very similar morphologically. The findings that may help in their distinction are the following: Apical acidophilic zymogenic-type granules, if present, are characteristic of ACC and not evident in ITPN. Nucleoli can be present in ITPNs, but are seldom as prominent as they are in ACC. Similarly, secretory material can be seen in ITPNs; however, these do not show the characteristics of enzymatic-type secretions or intraluminal crystals of ACC. Finally, immunohistochemical stain for trypsin may be necessary in some cases.

The other important differential is with pancreatobiliary variant of IPMN. In addition to the papillary configuration of cuboidal and mucin-poor cells, the similarities between these two entities also extend to include their immunophenotype (showing MUC1, and the pyoloropancreatic differentiation marker, MUC6+, while being negative for intestinal differentiation markers). In our opinion, an intraductal neoplasm with predominantly papillary pattern and this immunophenotype ought to be classified as a PB-type IPMN rather than a papilla-predominant ITPN.

The rare stroma-rich variants of ITPN also fall into the differential diagnosis of invasive ductal adenocarcinoma, especially if the process is examined out of context under high power. However, low power examination is usually diagnostic, illustrating the nodular and well-circumscribed nature of the process overall.

Clinical course
There is only limited data available regarding the prognosis of these tumors. In the group of patients we analyzed, 60% were alive and well at 5 yrs. Remaining patients had recurrence or metastasis to either lymph nodes or liver, and 15% died of their disease within 3 years. No correlation was found between invasion and prognosis, presumably relating to the sampling phenomenon. Interestingly, one patient had completion pancreatectomy with suspect findings, which proved to be non-neoplastic duct ectasia. This data suggests that these tumors are malignant, but often with protracted clinical course.

Reporting of ITPN
It is recommended that intraductal neoplasms are reported in a similar fashion as they would be reported in other organs. For example, our preferred approach for this particular case would be the following:

Pancreas; pancreatectomy:
- Invasive carcinoma of tubular type (estimated, 2 cm) arising in an intraductal tubulopapillary neoplasm (estimated, 8 cm) with extensive carcinoma in-situ.
- No vascular or perineural invasion is identified.
- All 12 lymph nodes are negative for carcinoma.

- Pancreatic resection margins are negative for in-situ or invasive carcinoma; however the tumor bulges into free surfaces of the pancreas.

Comment: The limited data on the prognosis of such tumors suggest that they have a more protracted clinical course than the conventional ductal adenocarcinomas of the pancreas. Because this patient has a substantial invasive component, consultation with oncology and possibility of chemotherapy ought to be considered.

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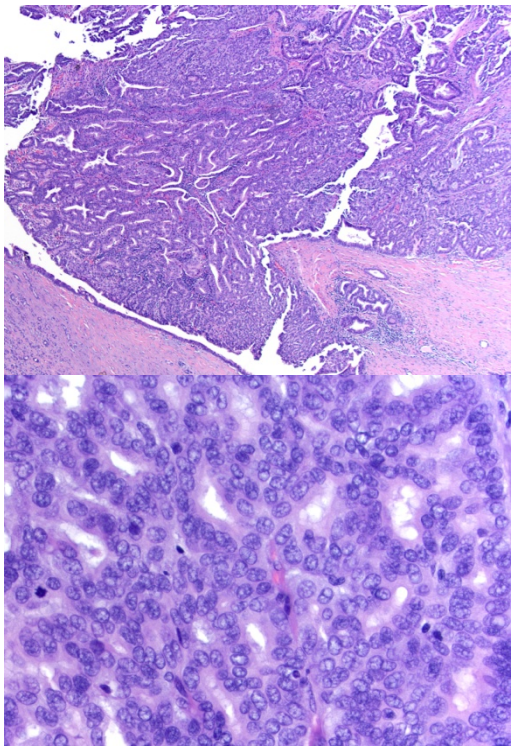

CASE 35

N. Volkan Adsay, MD

History: 56-year old male with complaints of abdominal pain, jaundice and weight loss was found to have an ill-defined mass involving the pancreatic head. Pancreatoduodenectomy was performed with the clinical diagnosis of pancreas cancer.

Macroscopic features: The pancreatic head had been transformed into a firm uniform mass. Cut sections revealed that pancreatic parenchyma was replaced by white, fibrous tissue without a discrete mass formation, and the pancreatic duct was mildly narrowed.

Microscopic features: Microscopic sections revealed dense lymphoplasmacytic infiltrates in a perilobular and periductal distribution with involvement of the epithelium in some ducts. This was associated with interstitial fibrosis surrounding the inflamed ducts replacing much of the acinar parenchyma. There was also a fibroblastic/myofibroblastic proliferation, focally exhibiting a vague storiform pattern. Small veins with aggregates of lymphoid cells in the wall and undermining the endothelium were also noted. Immunohistochemical studies revelaed >20 IgG4-positive plasma cells per HPF.

DIAGNOSIS: LYMPHOPLASMACYTIC SCLEROSING PANCREATITIS (IGG-4 RELATED AUTOIMMUNE PANCREATITIS)

Discussion:
This case illustrates an important and challenging group of lesions that occur within the pancreas: Pancreatic pseudotumors. For cases in which a benign process, usually inflammatory, forms a mass that clinically mimics pancreas or periampullary cancers, we employ the term ***pseudotumoral pancreatitis.*** This is *not* a specific entity but a term that we use simply for the "mass-forming" examples of chronic pancreatitis that are mistaken clinically for carcinoma. In our experience and also in others', 5-6% of pancreatectomies performed with the intention of removing "pancreas cancer" prove to be pseudotumoral pancreatitis after histopathologic examination. This phenomenon can occur in any etiopathogenetic subgroup of chronic pancreatitis, however, there are certain entities that are much more prone to lead to such a pseudotumor formation and these will be discussed in detail below: 1) autoimmune pancreatitis, 2) paraduodenal pancreatitis and 3) developmental disorders.

It should be kept in mind that before a case can be classified as pseudotumoral pancreatitis, extensive sampling of the specimen and careful examination to rule out carcinoma is a must, because any of the findings described below can be mimicked by peri-tumoral pancreatitis.

1. Autoimmune Pancreatitis (AIP)

A.AIP of lymphoplasmacytic sclerosing pancreatitis (LPSP) type

Lymphoplasmacytic sclerosing pancreatitis (LPSP) is the most distinctive and most common subset of autoimmune injury occurring in the pancreas. The delineation of LPSP brought to light an entity that is now recognized as *IgG-related sclerosing diseases* which can involve, in

addition to pancreas, several other organs including biliary tract (PSC type picture in 60%) salivary glands (Sjogren in 15%), thyroid (10%) and others. What used to be known as idiopathic fibrosclerosing diseases (orbital pseudotumor, Riedel struma, sclerosing mesenteritis and others) are also now known to occur as a part of the IgG4-related sclorsing diseases and can be seen in association with LPSP. Among patients with LPSP, however, autoimmune disorders are identified in 15-20 %, discovered in some at the time of operation, and in others, during subsequent investigation.

Jaundice, presumably due to the sclerotic changes involving the bile duct is the most common presentation finding of LPSP, seen in >70% of the cases. Approximately 25 % of patients present with abdominal pain or discomfort without jaundice. In about 80 % of the cases, the lesion is located in the head of the organ, and also involves the bile-duct.

In addition to serum IgG4 levels, which is specific when it is higher than >135 mg/dL, "sausage-like enlargement" of the pancreas, and the "halo" formation (“capsule-like low density rim”) around the lesion by imaging studies are helpful clues to the diagnosis but are not always definitive in making the distinction from adenocarcinoma. Unfortunately, diagnosis with FNA is also often very difficult and prone to misdiagnosis due to the atypical appearance of the inflamed tissue. In one study, the best criterion of FNA was found to be the presence of stromal fragments with embedded lymphocytes greater than 30 per 60x; however, this was noted in only about a third of LPSP cases, and could also be seen in adenocarcinomas in rare occasions (12%) too. For this reason, many patients still undergo pancreatoduodenectomy operation, because it is difficult to completely exclude the possibility of carcinoma in a given patient. In our experience, more than half of the cases that we refer as "pseudotumoral pancreatitis" are AIP/LPSP. The process often also involves the common bile duct, leading to jaundice and further complicating the differential diagnosis from adenocarcinoma.

Macroscopically, LPSP forms firm fibrotic masses and can mimic adenocarcinoma. The main feature that helps distinguish LPSP from carcinoma is the more milky color of the fibrosis and preservation of pancreatic contours.

Characteristic morphologic features of LPSP at microscopic level include dense lymphoplasmacytic infiltrate, delicate-wavy sclerosis and storiform myofibroblastic proliferation, the latter creating a picture that can be mistaken as “inflammatory pseudotumor” (or inflammatory myofibroblastic tumor). The inflammation can be predominantly periolobular, but in many cases it is also periductal. Compared to the degree of inflammation in the periductal tissue, intraepithelial lymphoplasmacytic infiltration is fairly minimal, though identifiable by careful inspection in most cases. Inflammation is typically accompanied by the distinctive sclerosis of the ducts. Another hallmark of this entity is inflammation of medium-sized veins (periphelibitis), while the arteries are usually spared. In some cases, the inflammation is lymphocyte rich, and may even have lymphoid follicles. In pancreatoduodenectomy specimens, the common bile duct is also often involved by the inflammation, and in fact, may prove to show the most intense periductal inflammation, including follicule formation.

Participation of IgG4-positive plasma cells in the inflammation is one of the hallmarks of LPSP, and this can be highlighted by immunohistochemistry. IgG4 immunopositive plasma cells are usually prominent, however, there is no agreement among authors as to which cut-off is to be employed as the minimum criterion for diagnosis of LPSP: While some believe

10/HPF is adequate for this diagnosis, others believe a higher threshold is necessary and employ 40/HPF.

In terms of treatment, steroid administration appears to be highly effective in controlling the process in most cases. Also, since about 1/4th is associated with established autoimmune conditions (PSC, Sjogren's, others), it is recommended to investigate the patients to determine this possibility.

B.Subtypes of "autoimmune pancreatitis"<u>other than</u> LPSP

In addition to LPSP, other patterns caused by autoimmune injury in the pancreas have been recognized although there are uncertainties regarding the minimum criteria or terminology of these rarer types.

AIP with "GELs" (granulocytic epithelial lesions): Intraepithelial neutrophils can be encountered in some cases with "autoimmune pancreatitis" and appears to be more common in patients with ulcerative colitis as well. These patients are predominantly males. IgG4 may be minimal or negative in these patients, both in serum or in the tissue.

AIP of arteritic type: Rarely, patients present with a pancreatic pseudotumor in which arteritis as the salient or sole finding of an autoimmune process, while lacking the characteristic injury patterns of LPSP or gel-forming AIP. This group has yet to be fully characterized. Some cases occur in the background of SLE.

2. Paraduodenal pancreatitis

The second most common cause of pseudotumoral pancreatitis is paraduodenal pancreatitis. In fact, in our experience, nowadays, this is a more common source of misdiagnosis for cancer in the pancreas field clinically than even AIP.

Paraduoenal pancreatitis is the name we employ for a distinctive variant of pancreatitis that occurs on the duodenal wall and the adjacent pancreatic tissue, and often centered in and around the accessory duct and accessory ampulla. This process also used to be known as *groove pancreatitis or cystic dystrophy of heterotopic pancreas*. It is characterized by an exuberant myofibroblastic proliferation, often in a fascicular arrangement, leads to narrowing of duodenal lumen and scarred appearance of duodenal wall such that the duodenal mucosa often acquires a nodularity or cobblestoning type appearance. Duodenal mucosa is also often thickened with Brunner's gland hyperplasia, in addition to the myoid proliferation. Upon sectioning, the duodenal wall shows trabeculated appearance of the duodenal musculature, accompanied by cystic change of variable sizes. In some cases, cyst formation can be prominent and measure up to several centimeters ("paraduodenal wall cyst").

Under microscopic examination, often, there are round (well-circumscribed), small lobules of pancreatic tissue scattered amidst the myoid stromal proliferation, hence the name *myoadenomatosis* previously for this entity. Another designation given to this process in the literature is *cystic dystrophy of heterotopic pancreas*, because there is usually cystic changes (either dilated ducts or pseudocysts), surrounded by lobules of pancreatic tissue, located on the duodenal wall. These ducts may contain inspissated secretory material. Some cysts are devoid of epithelium, but instead lined by more cellular fibroblastic reaction. On occasion, the lining fibroblasts may appear epithelioid and raise the concern of a sarcomatoid carcinoma.

Cyst contents may extravasate and lead to foreign-body giant cell reaction and induce eosinophilia in the stroma.

Most patients with paraduodenal pancreatitis preoperatively receive the diagnosis of either periampullary or pancreatic cancer. It displays several stigmata of cancers, including the highly infiltrative appearance, dilatation of the common bile duct, “invasion” and narrowing of portal and mesenteric vessels etc. In our experience, our MRI experts find the “tubulocystic” type changes in the trace of accessory duct and duodenal wall (in the accessory ampullary region) to be highly specific for this entity.

The reasons why this process develops especially around the accessory ampulla or accessory duct are not known. In some cases, pancreas divisum (persistence of embryologic-type, dorsal-ventral separated drainage systems) is suspected. One possibility we consider is the occlusion of a functionally overactive accessory duct by yet unknown mechanisms, perhaps partially triggered by alcohol abuse. The morphologic findings (both macroscopic and microscopic), however, are quite distinctive. Moreover, this process appears to have some consistent clinical associations. The vast majority of the patients are relatively young males (around 40’s), often with a history of alcohol abuse, and some with history of hypertension.

3.Other (developmental) tumor-like lesions

There are other tumor-like lesions in the pancreas. So-called adenomyoma of the ampulla may also mimic carcinoma. This is a difficult phenomenon to define. In some studies 60% of autopsy cases were found to have ampullary “adenomyoma”. Some people employ 5 mm thickness of ampullary musculature as the criteria to diagnose this entity. Regardless, in some patients with obstructive jaundice and suspected “tumor” in the ampulla, the only pathologic finding identified proves to be a thickened ampulla.
Another example is lipomatous pseudohypertrophy, which is replacement of pancreatic parenchyma with adipose tissue. Ectopic spleen also may also present as a mass in the pancreas. Recently reported pancreatic hamartomas also form tumors in this organ.
We have also seen examples of pseudoaneurysm of splenic artery presenting as pancreatic masses. Clinical correlation is the only means to establish this diagnosis.

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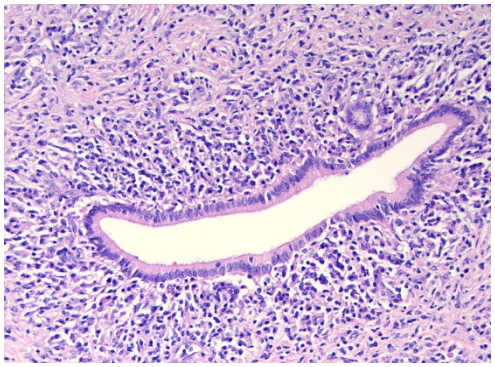

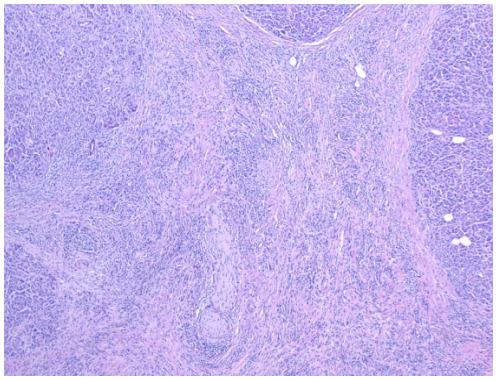

CASE 36

N. Volkan Adsay, MD

Clinical History:
A 59-year-old-female patient underwent laparotomy with the clinical diagnosis of acute appendicitis. During the operation, in addition to appendectomy for a relatively unremarkable appendix, multiple peritoneal nodules were identified and biopsied. Gross examination of the appendix revealed a mildly distorted appendix covered with tan-grey exudates. No tumor or discrete nodules were appreciated macroscopically.

Microscopic findings:
On low-power examination, a subtle infiltration involving the full-thickness of the appendiceal wall but sparing the mucosa was appreciated. There was no significant desmoplastic reaction. On close-up view, the infiltration was composed of well-formed, small, round glandular elements that are widely separated. Individual glands were made up of well organized goblet cells (with abundant intracytoplasmic mucin) with their nuclei compressed at the periphery, creating a picture virtually indistinguishable from a normal crypt. In some areas these crypt-like structures had more compressed, irregular pattern, and focally, cytologic atypia was substantial.

Diagnosis: ADENOCARCINOMA EX (DEDIFFERENTIATED) GOBLET CELL CARCINOID OF THE APPENDIX

Discussion:

Goblet cell carcinoid, conventional type
Goblet cell carcinoid (GCC) has been reported under different names including, adenocarcinoid, mucinous carcinoid, intermediate type of carcinoid, crypt cell carcinoma, amphicrine (endo-exocrine) neoplasia, composite tumor and microglandular carcinoma.

GCC accounts for less than 5% of primary tumors of the appendix. Although GCCs are classified under the generic category of “carcinoids” (or neuroendocrine neoplasia), it is becoming increasingly clear that they ought to be regarded somewhat separately from

ordinary carcinoids. They appear to be amphicrine tumors, presumably arising from pluripotent stem cells with divergent neuroendocrine and mucinous differentiation.

Mean age of the patients with GCCs is mid-50's (as opposed to mid-30's for classical carcinoids). Clinical diagnosis of GCC is seldom made preoperatively. Most of the patients present with signs and symptoms of an acute appendicitis due to luminal obstruction. The tumor cells proliferate sparsely and do not form nodules. The appendiceal wall thickens diffusely with fibrous proliferation, leading to contraction of the appendiceal lumen, which is the cause of the appendicitis. Other manifestations include asymptomatic patients, intussusception, gastrointestinal bleeding, increasing abdominal girth, chronic intermittent lower abdominal pain, and secondary genitourinary complications.

Macroscopically, in most cases, there is no well-defined mass. There maybe mucoid induration without dilatation of the lumen.

Classical GCC has a very distinctive morphology. The hallmark of this tumor is widely separated well-formed and round glandular units intervened by smooth muscle or stroma without any significant desmoplastic reaction. The glands themselves have a very distinctive appearance that closely resemble colonic crypts, composed entirely of goblet-like cells with their nuclei strikingly well polarized at the periphery. Paneth cells can be seen. Rare examples have conventional (nested) carcinoid component.

Classical GCC is characterized by predominantly submucosal growth. Extension into the muscle and serosa is common, but the mucosa is characteristically spared, except for the areas of apparent connection between tumor nest and the base of the crypts. Diffuse infiltration into the peri-appendiceal fat and perineural invasion is seen in most cases[1].

Dual (amphicrine) phenotype of the classical GCC is evidenced both at ultrastructural and immunohistochemical levels, exhibiting both abundant intracellular mucin as well as neurosecretory granules which can be highlighted by chromgranin and synaptophysin stains.

The distinctive pathologic features of GCC are also translated into in its clinical behavior. In contrast with appendiceal carcinoids that metastasize in < 5% of cases, GCCs metastasize in 15 to 30% of cases. The most common route of metastasis is through lymphatic vessels, trans-coelomic and intraperitoneal invasion whereas hematogenous metastasis to the liver or other distant organs is rare. The ovary is the most common target site of metastasis of GCC followed by abdominal carcinomatosis.

High-grade ("mixed", "ex", "dedifferentiated") version of GCC

It is becoming increasingly clear that GCCs come to clinical attention in two highly different settings: 1) Tumors localized to the appendix, clinically presenting with appendicitis type picture. Recent literature indicates that such cases have fairly high cure rate and indolent behavior with protracted clinical course. 2) Advanced tumors presenting with "peritoneal carcinomatosis" type picture, typically involving peritoneal surfaces, ovaries and the uterus (occurring predominantly in females). This dichotomy is also reflected on the conflicting data regarding the biology of these tumors, especially studied by GI versus GYN pathologists. This second (high stage) group was the subject of a recent study published in the Am J Surg Pathol, October 2007, by the GYN pathology groups of Harvard and Johns Hopkins Universities under the heading of "*ovarian metastases of appendiceal tumors with goblet cell carcinoid-like and signet ring cell patterns: a report of 30 cases*". GI pathology groups, on

the other hand, are exposed to both the conventional and high-grade examples of this entity. Accordingly, in a recent analysis, the GI pathology group from Memorial Sloan-Kettering proposed to classify the spectrum of GCCs into A, B and C categories (see reference by Tang et al). Typical GCCs were designated as group A, and distinguished from "adenocarcinoma ex GCC" on the basis of the histologic features of the tumor at the primary site. The adenocarcinoma ex GCC group was further divided into signet ring cell type (group B) and poorly differentiated adenocarcinoma type (group C). It was noted that groups B and C have fairly aggressive clinical behavior.

The findings in these two studies are accordance with our experience with these tumors presented in abstract form at the USCAP 2010 meeting (Mod Path; 23(1):137A, 2010) under the heading of "*dedifferentiated goblet cell carcinoid (adenocarcinoma ex GCC) is a morphologically distinctive and aggressive neoplasm with peritoneal dissemination: An analysis of 35 cases*". All these tumors exhibited, in addition to conventional GCC pattern, some areas with high-grade cytologic features and/or mixed patterns including *cord-like infiltration, signet-ring cells in cords or individual cells, non-mucinous microglandular pattern, intestinal pattern or extravasated mucin*. In our experience, these high-stage/high-grade tumors with peritoneal carcinomatosis were found in both studies to have a median survival of <2 years.

Management of GCCs

There are no established guidelines for the management of such tumors. In the light of these recent data, appendectomy appears to be curative in most but not all appendix-confined tumors. However, even before the high-stage/high-grade category was fully appreciated, some authors were already recommending right hemicolectomy when one or more of the following criteria are noted: 1) cellular undifferentiation; 2) increased mitotic activity; 3) involvement of the base of the appendix with cecal wall infiltration; 4) lymph node metastasis; and 5) tumor size greater than 2 cm. It is clear that the stage, for which there are different proposals (ENETs, AJCC/TNM etc), will also have an important role in the management of these tumors. Recent data (studies mentioned above) indicates that high-grade features may warrant not only more aggressive surgery but also careful evaluation of the patients for metastases. In addition to right hemicolectomy, some authors also advocate bilateral oophorectomy in female patients because of the possibility of ovarian metastasis. Conversely, due to the tendency of GCCs to spread to the ovaries, it is recommended that an appendectomy be routinely performed in patients with "Krukenberg's tumor" of the ovary where no gross obvious primary neoplasm is found.

It is also important for surgical pathologists to remember the distinctive morphologic features of GCC, because it may help identify the primary in patients with widely metastatic disease.

BEHAVIOR OF NETS: TERMINOLOGY AND CLASSIFICATION ISSUES

In general, GAP-NETs ought to be regarded as low-grade indolent malignant neoplasia. Having said that, it is also clear that very small and incidental examples, especially in certain clinical settings, may represent **precursors or evolving malignancies**, i.e., GI-pancreatic counterparts of pulmonary *tumorlets*. In fact, some authors do regard such lesions conceptually as dysplastic or "Tis" type lesions. In particular, the incidental lesions detected in patients with MEN (in the pancreas) or autoimmune gastritis (in the stomach; enterochromaffin-like cell lesions) are good examples of the "precursor" (Tis) NET phenomenon.

The **primary location** of the tumor seems to be an important determinant of the outcome as well. This should not be surprising since adenocarcinoma of colon is very different than adenocarcinoma of stomach, and there is no reason why NETs of these two sites can be expected to be similar. Most appendicial classical carcinoids are asymptomatic (%2 of the overall autopsies), and similarly, most rectal carcinoids are also non-metastatic.

However, part of the more "benign behavior" of NETs of some regions such as appendix and rectum may also be related to the **stage** at which these tumors are detected. Most are detected incidentally and are typically small (< 0.5 cm) at the time of diagnosis. The fact that both appendix and rectum are narrow zones of the GI tract and thus lead to symptoms and early detection of the lesions may be a factor. In support of this impression, rectal and appendiceal carcinoids with the size of >2cm have a much higher metastatic risk.

The challenges in determining the behavior of NETs have also led to conflicting and problematic terminology. The terminology problem is best examplified in pancreatic NETs. In the WHO 2004 classification, lesions restricted to the pancreas are called "well differentiated endocrine *tumor*" (WHO-1). Those with invasion and/or metastasis are called "well differentiated endocrine *carcinoma*" (WHO-2). Lesions with >10 mitosis/ 10 HPF are classified as "poorly differentiated endocrine carcinoma" (WHO-3). WHO-1 (well differentiated endocrine tumor) category is further classified within itself as WHO-1A ("benign behavior") and WHO-1B ("uncertain behavior") based on tumor size >2 cm, or mitosis> 2/ 10 HPF or vascular/neural invasion.

In our opinion, this approach has various problems:

1- It might give the impression that there is a continuum between WHO-2 and WHO-3, whereas WHO-3 (poorly differentiated neuroendocrine ca) is an exceedingly uncommon tumor and is an independent entity with a very different biology than the WHO-2.
2- The most important issue is the inconsistency between the subgroup designations and the prognosis. The cases included in the WHO-1A ("benign behavior") category shows recurrence and metastasis in %5 of the cases. More importantly, those included in the WHO-1B ("uncertain behavior") category has recurrence and metastasis in up to %40. And furthermore, %20 of these so-called "uncertain behavior" tumors lead to the demise of the patients within 5-10 years.
3- This WHO 2004 terminology has complete reliance on clinical information, which is often unknown to the pathologist at the time of diagnosis. For example a metastasis unbeknownst to the pathologist changes the diagnosis from"tumor with benign behavior" into a "carcinoma".
4- Divergent from conventional oncologic pathologic principles, in the WHO 2004, stage and grade had been combined such that a category is defined both by size (stage parameter), mitotic activity (grade parameter) and several other prognosticators including vascular invasion, all jumbled up together. To avoid the confusion, a new staging system has been proposed by ENETS (European Neuroendocrine Tumor Society, 2006), but it remains to be seen whether it is clinically relevant and will find wide recognition. 7th edition of TNM/AJCC (2010), in the meantime, advocates using the staging parameters of pancreatic adenocarcinoma.

Upcoming WHO blue book

Some of the problematic aspects of NETs (carcinoid, PEN) will partially be addressed in the upcoming WHO:

1. The whole spectrum will be referred under one name: NET.
2. The spectrum will be graded as NET 1, NET 2, and NET 3 based on either mitotic activity (per 10 HPF) or Ki-67 index (percent of cells): NET-1 as $\leq$ 2, NET-2 as 2-20, and NET-3 as >20. This will be utilized regardless of the location.
3. Staging parameters will be separated out and will be reported separately. ENETs stage and TNM/AJCC staging, however, have substantial differences, and therefore, it will be important to clarify in pathology reports which staging system is being employed.

Reporting of NETs

According to the consensus manuscript in press in AJSP in 2010 (Klimstra et al), it is now recommended that for all NETs are graded (based on mitotic activity and/or necrosis) and staged (ENETS/WHO or AJCC/CAP systems). Reporting of Ki-67, and in doubtful cases, performance of chromogranin and synaptophysin are also encouraged.

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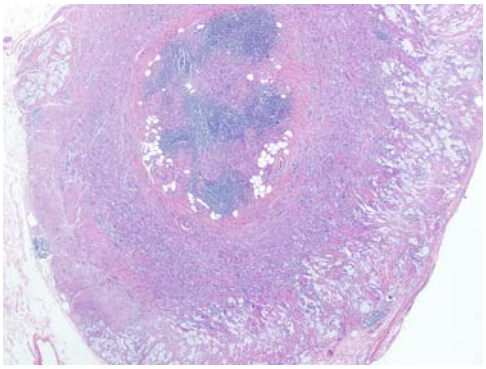

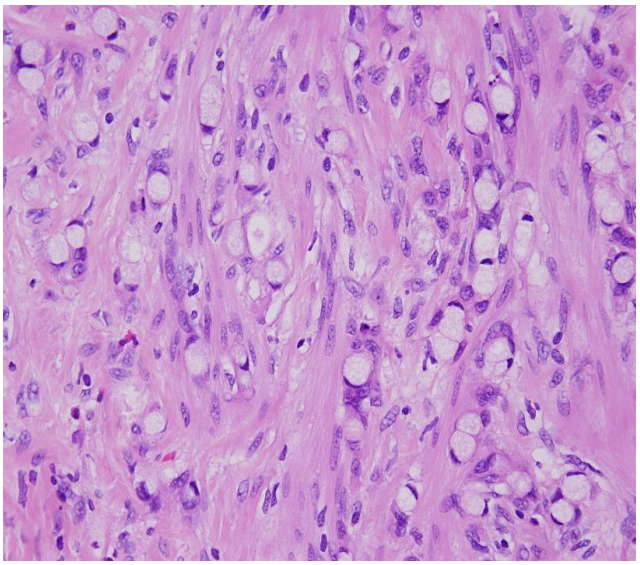

CASE 37

Ivan Damjanov

- **39- year-old woman**
- **Main complaint: Pelvic pain**

- **Surgical findings: Left adnexal mass incorporating the fallopian tube and attached laterally to the uterus but distinct from the ovary**
- **Mass weighed 690 g, and measured 17x10x10 cm , solid partially cystic**

Human Pathology (2010) 41, 716–723

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Original contribution

Morphologic, immunohistochemical, and fluorescence in situ hybridization study of ovarian embryonal carcinoma with comparison to solid variant of yolk sac tumor and immature teratoma

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Received 10 July 2009; revised 21 October 2009; accepted 23 October 2009

IMMUNOHISTOCHEMISTRY
OCT4-nuclear stainingof EC, very sensitive; does not react with YST, Chorio, teratoma; reacts with some neural tubes

CD30-cell membrane staining; negative in seminoma. Loss in metastases after treatment in 65%

SOX2-nuclear staining in EC, negative in dysgerminoma, YCT; neural tubes-positive

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Fluorescence in situ hybridization (FISH)

i(p12) overrepresentation of 12p

From L.Cheng et al Human Pathology 2010;41:716-723

FINAL DIAGNOSIS
Malignant mixed germ cell tumor of the fallopian tube (teratocarcinoma)
Patient treated with platinum based chemotherapy and re-explored 7 month thereafter

---

FINAL DIAGNOSIS
Malignant mixed germ cell tumor of the fallopian tube (teratocarcinoma)
Mature teratoma in the abdominal metastatic sites (status post chemotherapy)

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Patient alive and free of tumor 3 years after tumor resection.

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CASE 38

Ivan Damjanov

- **16-year-old woman**
- **Chief complaint: Menstrual irregularities**
- **Past history: Ovarian mass removed 18 months ago.**
- **Surgery: Paracaval abdominal lymph node enlargement treated by surgical resection**
- **The slide is from the metastasis**

FINAL DIAGNOSIS
Malignant Sertoli cell tumor of the ovary metastatic to abdominal lymph nodes.

Sertoli Cell Tumors of Ovary

Definition: Tumor composed of Sertoli cells arranged in hollow or solid tubules with rare, if any, Leydig cells (WHO 2003).
Rare - 4% of all Sertoli-stromal tumors
May occur at any age, 2-80 years but peaks in young woman (median age 30 years)
Nonfunctioning or estrogenic(40-60%)
Most benign but may be malignant
---.

Ovarian Sertoli Cell Tumors

TEILUM G. Homologous tumours of the ovary and testis. Acta Obstet Gynecol Scand 1944; 24: 480-503.

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Histology

Tubular growth pattern most common
Other patterns include cord-like, trabecullar , solid (diffuse), pseudopapillary
Some cell luteinized cells and a few Leydig cells may be present
No heterologous elements (as seen in some Sertoli-Leydig cell tumors)

Differential Diagnosis

Sertoli-Leydig cell tumor
Granulosa cell tumor
Female adnexal tumor of probable Wolffian origin (FAPTWO)
Sertoliform endometrioid carcinoma
Carcinoid
Struma ovarii (solid pseudotubular pattern)
Dysgerminoma
Metastases

Immunohistochemistry

Positive	Negative
Inhibin	EMA
Calretinin	S100
CD99	Chromogranin
WT-1	Synaptophysin
Vimentin	
Keratin (AE1/AE3 or Cam 5.2)	

Malignancy of Sertoli Tumors of Ovary

Size of the primary tumor (5 cm)
Nuclear atypia
Mitoses (>5/10HPF)
Necrosis
Caveat: Degenerative nuclear atypia like in other endocrine tumors is of no concern.

FINAL DIAGNOSIS
Malignant Sertoli cell tumor of the ovary metastatic to abdominal lymph nodes.

==========

Patient alive 3 years after diagnosis

CASE 39

Markku Miettinen M.D.

History:
44 year-old woman. A 5 cm cystic pelvic mass is excised.

Diagnosis
Multicystic peritoneal mesothelioma/multilocular mesothelial inclusion cyst

Specific notes of the case

In summary, this is a typical example of entity with some proliferative mesothelial changes, including focal hypercellularity in the cyst wall. In fact this tumor recurred 5 years after surgery, and the current material is from this recurrence. The earlier tumor had even more intracystic proliferative features. If one believes (as I do) that this entity contains a spectrum from multilocular peritoneal inclusion cysts to probable neoplasias, this case seems closer to the neoplastic end of the spectrum. However, at this point division into reactive and neoplastic examples is somewhat speculative, but presence of intracystic proliferation is a features that needs to be more systematically assesses as a potential predictor of recurrence. However, conversion into a blatant malignant mesothelioma does not seem to occur.

The present case showed immunophenotypical features of mesothelial differentiation in the cyst epithelium: Calretinin, keratins 5/6 (variable), WT1, and podoplanin expression, Note that WT1 also occurs in uterine/gynecological stromal and smooth muscle tumors, and podoplanin in lymphangiomas.

General discussion of the entity

This histologically distinctive, rare, benign mesothelial proliferation may be a heterogeneous group. It includes cases that are most likely reactive mesothelial proliferations reflecting the alternative diagnostic term. However, this entity may also include true neoplasms that have a greater proliferative capacity and may recur.

Multicystic peritoneal mesothelioma typically occurs in young females in the pelvic peritoneum adjacent to uterus and other pelvic organs, or in the upper peritoneum, including omentum. [1-5] Some patients have a history of abdominal surgery, including one patient who developed this lesion in the cesarean section scar. [6] Very few cases have occurred in men. The median age for the largest series was 38 years for women and 47 years for men. Approximately 10% of these tumors occurred in children. [4] Occurrence together with endometriosis, leiomyomatosis peritonealis disseminata, and pseudo-myxoma peritonei from an appendiceal mucinous tumor has been reported. [7-9] Lower abdominal pain is the most common presenting symptom. The prognosis has been generally excellent, especially in the cases in which complete excision of the tumor has been performed. However, local recurrences may develop in up to half of the cases. [1-5] Conservative treatment with cyst drainage has been successful in some cases. [10,11] Two fatalities reported in the largest series were one infant whose tumor also contained areas of typical epithelial mesothelioma, and a man who declined treatment. [20] A similar multicystic mesothelial lesion was reported in the pleura of a 37-year-old woman. [12]

Grossly the lesions form either multiple, variably confluent cysts. The lesions contain cysts filled with clear or blood-tinged fluid. The cysts vary from a few millimeters in diameter to several centimeters.

Histologically those examples that consist of multiple cysts lined by a single layer of mesothelial cells and spaced by loose, reactive appearing myofibroblastic proliferation and mild chronic inflammation have an appearance suggestive of a reactive condition. In some cases, a higher amount of fibrous stroma is present. However, those cases that contain multiple mesothelial cysts lined by cuboidal to focally proliferative epithelium with intracystic papillary formations, may represent neoplastic variants. Adenomatoid tumor-like features can also be present. The cyst-lining epithelial components are positive for keratins, calretinin, and podoplanin. In some cases, immunohistochemical studies reveal concomitant endometriosis elements positive for estrogen and progesterone receptors, whereas the cyst epithelium is negative.

Lymphangioma has similar multicystic quality, but the lining cells are attenuated and express endothelial markers, although some keratin expression (keratins K7 and K18) may occur. Endometriosis lesions rarely have a distinctive multicystic appearance, and are often more heterogeneous with hemorrhage, hemosiderin deposition and fibrosis.

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CASE 40

Hugo Domínguez Malagón MD

A 32 year-old white male was seen in September 2008 for a large tumor located in the upper pole of the left kidney. A radical nephrectomy was performed.
Gross findings: A well circumscribed tan yellow friable tumor measuring 7.1 x 7 x 6 cm was present in the upper pole, present largely within the medulla and focally expanding into the overlying cortex. No invasion to the capsule, renal sinus and renal vein.
Histological findings: The appearance is of a high grade well circumscribed small, round cell neoplasia with extensive geographic necrosis, arranged in solid sheaths with thin vascularized septae, occasional rosettes are identified. The cells are small to intermediate, with high nuclear-cytoplasmic ratio, scanty clear cytoplasm. The nuclei are oval hyperchromatic, with inconspicuous nuclei and abundant mitoses.
Immunohistochemistry: the tumor cells are positive for CD99, focally positive for NSE and WT1. Negative for vimentin, CD45. S100, EMA, wide spectrum cytokeratin, CK7, TdT, synaptophysin, neurofilament and CD57.
Cytogenetic studies with karyotype show trysomy 7. FISH show EWS split signals indicating translocations involving the EWS locus on chromosome 22.

DIAGNOSIS: EWING SARCOMA/PRIMITIVE NEUROECTODERMAL TUMOR OF THE KIDNEY

DISCUSSION.
Renal ES/PNET is an extraordinarily rare primary tumor, morphologically it is a small-round cell tumor and can be mistakenly diagnosed as a variety of kidney tumors with similar morphology including: monophasic synovial sarcoma, lymphoma, blastema-predominant Wilms tumor among others.
Aproximately 90% of ES/PNET have a specific t(11;22)(q24;q12) which result in chimeric EWS-FLI-1, the translocation can be detected by molecular techniques, but immunohistochemical studies for FLI-1 are sensitive and highly specific. Comparative studies have demonstrated that renal ES/PNET are positive for CD99 and FLI-1, and negative for WT-1, whereas WT rarely expresses CD99 and does not express FLI-1. Due to the different prognosis, distinction between ES/PNET and other kidney neoplasms with similar morphology is crucial and histopathologic diagnosis with extreme accuracy should be made, cytogenetic analysis and immunohistochemistry are important supporting tools. The combination of CD99 and FLI-1p is the method of choice form the diagnosis of EWS/PNET, in addition a confirmatory test EWRS1 (22q12) dual colour, break apart rearrangement probe FISH should be used.
The cases of renal ES/PNET reveal that this rare entity have variable presentation and aggressive behavior. The diagnosis of this tumor must be considered in young patients presenting with a renal mass. Standard therapy consists in combination of surgical resection, postoperative radiation and chemotherapy (regime RCT II

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SESSION IV

CASE 41

P. E. Wakely, Jr., M.D.

Clinical History:

A 53-year-old man presented with a 5-month history of sore throat and dysphagia. Examination by a head and neck surgeon revealed a 4-5 cm exophytic granular lesion involving the left tonsillar fossa and palatal region. A biopsy revealed carcinoma. Surgical resection of the mass with partial mandibulectomy and bilateral neck dissection were performed. This is the tissue you have to examine.

Gross and Microscopic Findings:

A 3.5 x 1.5 x 1.9 cm. ulcerated, rubbery lesion was present on the mucosal surface. It was approximately 10% necrotic with a tan-white, granular cut surface. Light microscopy revealed well-circumscribed as well as ill-defined islands of large malignant epithelial cells surrounded and infiltrated by a dense population of small lymphocytes and plasma cells. The lymphoplasmacytic infiltrate often obscured epithelial cell islands. There was central comedo-type necrosis and microabscesses in some epithelial islands. Malignant cells showed round-to-oval vesicular nuclei with single distinct nucleoli and indistinct cytoplasm creating a loose syncytium of epithelial cells. Mitotic figures were noticeable throughout. Immunohistology showed positive staining of the tumor with high molecular weight cytokeratin (34βE12), pan-keratin, EMA, and p53. Staining was negative with S100, chromogranin, synaptophysin, CD3, CD20, CD79a, and CD43.

Diagnosis: LYMPHOEPITHELIOMA-LIKE CARCINOMA OF LEFT TONSIL.

Discussion:

Lymphoepithelioma is an archaic term introduced by Schmincke and Regaud in separate publications of a nasopharyngeal neoplasm in the 1920s. Lymphoepithelioma is best known for its nasopharyngeal location where its nomenclature has been changed over the past few decades. It is currently classified as “nonkeratinizing carcinoma, undifferentiated subtype” in the most recent WHO classification of head and neck tumors. A neoplasm with this identical histopathology of nonkeratinizing carcinoma, undifferentiated subtype (and recognized as a subtype of squamous cell carcinoma) is presently termed lymphoepithelioma-like carcinoma (LELC) when it occurs in sites outside the nasopharynx. This is due to of its close if not identical light microscopic similarity to the original tumor described by Schminke and Regaud.

Oropharyngeal LELC is a rare neoplasm. Of all oropharyngeal locations, 90% originate from the tonsil or tongue base. In a review of almost 25 years from the Mayo Clinic

files Bansberg et al. found only 13 oropharyngeal LELC cases. LELC is reported in other upper aerodigestive tract sites including the larynx, oral cavity, hypopharynx, and salivary glands. In fact, it is difficult to find any organ where LELC does not occur since it has been reported in the breast [J Ultrasound Med 2010;29:485-8], skin [J Am Acad Dermatol. 2010;62:681-4], thyroid, kidney, pancreas, urinary bladder, colon, uterine cervix [J Cancer Res Ther 2009;5:300-1], stomach, prostate, liver, thymus, ovary [Arch Pathol Lab Med 2007;131:1715-8], renal pelvis, and lung. Tonsillar LELC shows identical histopathology as seen in its more common counterpart from the nasopharynx. The heavy infiltrate of lymphocytes and plasma cells is neither neoplastic nor integral to the malignant process, but for many years this intimate association between the two cells types was considered part of the malignancy, hence the inaccurate designation of lymphoepithelioma. Neoplastic cells are epithelial, and hence immunoreactive for pan-cytokeratin and EMA stains, but typically lack staining with the Epstein-Barr virus (EBV) (except in Chinese patients). The absence of EBV staining is completely unlike the nasopharyngeal equivalent of LELC where EBV is associated with carcinoma in practically all cases. A recent publication by Singh et al. highlighted the presence of human papilloma virus (HPV) in six patients with oropharyngeal carcinoma and the morphology of LELC. Three of these were misdiagnosed as metastatic nasopharyngeal carcinoma because of this morphology.

The median age of head and neck non-nasopharyngeal LELC is 60 years with a broad age range. There is a definite male predominance. Most patients complain of dysphagia or pain, and a percentage will present with regional lymph node enlargement. In a series of 34 patients with non-nasopharyngeal LELC, almost one-third of patients had a neck mass as the only presenting sign, and over three-quarters of patients were found eventually with lymph node involvement. The typical clinical appearance of tonsillar LELC is an ulcerated mass involving the tonsillar pillar or fossa. Surgery and radiation therapy are the mainstays of treatment Local control is generally achieved with both modalities. About 20% of patients develop distant metastases.

At one time, pathologists were taught about the two histologic patterns of LELC, namely the Regaud pattern whereby malignant cells formed cohesive islands that were distinct from the surrounding inflammatory cells, and the Schmincke pattern where the inflammation percolated into and often obscured the epithelial nests. These patterns are of no biologic significance, and unnecessary to report. The differential diagnosis of LELC includes primarily large cell forms of malignant lymphoma, particularly ALCL, Hodgkin lymphoma, and diffuse large B-cell lymphoma. The diagnosis of UNPC or lymphoma is typically resolved through standard immunohistologic profiles: a positive cytokeratin stain favors carcinoma, and a positive CD45 stain favors lymphoma. While such an algorithm proves useful in the majority of cases, it is important to note that ALCL has been shown to be negative for CD45 in one-third of cases and, although rare, may even show focal cytokeratin positivity. We published a study a few years ago [Kneile et al] demonstrating positive CD30 staining in 11% of cases of nonkeratinizing carcinoma, undifferentiated subtype from the nasopharynx. In addition, 6 cases of LELC and 10 cases of squamous cell carcinoma from various sites were stained with CD30 and this was absent in all those cases.

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CASE 42

Göran Elmberger, M.D.

Clinical history:
67-year-old female with a one month history of rapidly growing hard irregular tumor behind left ear. The patient noted a small stable nodule in same location for many years. FNA revealed high-grade adenocarcinoma most likely of salivary gland origin. On superficial parotid resection a 20 mm sized tumor was entirely sampled. An 8 mm sized LN metastasis was found in an intraparotid LN. No formal neck dissection performed. pT1N1Mx. Post-operative radiation tx 68 Gy.

Pathological findings:
Approximately 50 % of tumor consisted of classical acinic cell carcinoma (ACC) showing a solid-lobular and microcystic arrangement. Cytologically serous as well as vacuolated and non-specific glandular cells were seen. In the central part of the tumor a small encapsulated core of very well differentiated ACC could be seen. Here dystrophic calcification was noted. The remaining 50 % of the tumor showed an abrupt but multifocal transition into a solid HG tumor with solid sheet-like growth and a highly pleomorphic cell population. Comedo-like necrosis was frequently seen. Even within the HG component serous cytoplasmic differentiation could be detected in HE and PAS. The HG component revealed aggressive infiltration of the surrounding soft tissues and benign salivary gland tissue. Abundant lymphoid stroma was present in and around LG as well as HG component. Perineural or LVI growth was not seen. An 8 mm sized LN metastasis with HG morphology was detected in an intraparotid LN. The surrounding parotid tissue showed multilocular oncocytic hyperplasia but was otherwise unremarkable.

Special studies:
IHC revealed pan CK+, CK18+, CK5-, CK7-, CK20- and HMWCK-. No myoepithelial markers positive (p63, Actin HHF35, SMA, Calponin, SMMS1). TTF1-, AR-, Chromogranin A-, Synaptophysin-, GCDFP15-, PSA-. No signs of p53 overexpression/mutation. MIB1 80 % in dedifferentiated part and < 50 % in classical component. Interestingly, some markers like CK18 showed a differential graded expression with a stronger staining within the classical component than within the HG component.

Diagnosis: Acinic cell carcinoma of parotid gland with high grade transformation (dedifferentiation) and LN metastasis.

Follow-up: Alive and without tumor at 10 months.

Discussion:
Acinic cell carcinomas constitute between 2% and 4.5% of all salivary gland neoplasms. On light microscopic examination, they display surprising heterogeneity, including not just solid "blue dot tumors," but also papillary-cystic and other patterns. Similarly, the individual cytology includes serous, vacuolated and clear cell variants. The typical clinical presentation is that of slowly growing painless mass, which often mimics a benign tumor.

Epidemiology:
Acinic cell adenocarcinoma is the second most common epithelial malignancy of salivary glands: about 17 percent of primary epithelial malignancies and about 9 percent of all epithelial neoplasms. Slightly more women than men are affected. An even age distribution ranging from young children to elderly adults is seen. Four percent of the patients are under 20 years.(1) Most cases are sporadic but a few reports on familial occurrence have been published.(2;3)

Localization:
The majority, almost 80 %, of ACC occur in the parotid gland sometimes with ductal extension.(4) 17 % involve the intraoral salivary glands in the area of buccal mucosa(5), palate(6), lip(7) and tongue base(8). Only about 4 % involve the submandibular gland(9), and less than 1 % arise in the sublingual gland(10). Within the H&N area localization in sinonasal mucosa(11;12), larynx(13), trachea(14), lacrimal gland(15) and the external auditory canal(16) also have been described. Tumor development ex Warthin's tumor(17) and ex pleomorphic adenoma(18) have been described as well as origin in a parotid lymph node(19). Central genesis within the mandibular bone is also known to occur(20).

Outside H&N ACC has been reported in many organs such as the breast(21-24) and lung(25;26). Finally intratumoral metastases of ACC has been published(27).

Histopathology:
Although serous-acinar cell differentiation is diagnostic for ACC, the spectrum of histological growth patterns and cellular features is extremely variable. Similar to normal serous acinar cells, neoplastic acinar cells are often large cells with granular, lightly basophilic cytoplasm and uniform, round, eccentric nuclei. The tumors are most readily recognized when these cells are arranged in large sheets. This archetypal pattern, however, represents only a minority of acinic cell adenocarcinomas, and it is important to recognize that the spectrum of histomorphologic and cellular features is much broader. Architectural growth patterns are categorized as solid, microcystic, papillary-cystic, and follicular. Cellular features are identified as acinar, intercalated ductal, vacuolated, clear, and non-specific glandular. These patterns are not exclusive of each other but rather describe the spectrum of histologic features of these tumors. Acinar cells are often scattered among the non-specific glandular cells, and the PAS stain helps identify them. Non-specific glandular cells are the majority of the cell population in only about 15 percent of tumors.

Variants with frequent psammoma bodies(28), neuroendocrine differentiation(29) and massive deposits of globular amyloid(30;31) have infrequently been seen.

Cytology:
Acinar differentiated cells are proved to be the diagnostic clue cells as stated by many reports. Neoplastic acinar cells are round, oval, or polygonal shaped, exhibiting abundant granular cytoplasm. Azurophilic intracytoplasmic granules can be detected if one uses a Giemsa type stain. In addition to acinar tumor cells, the cytoarchitectural pattern is another important diagnostic feature of ACC. Acinar structures are mentioned to be typical for ACC. The term acinar structure comprises both circular tumor cell formations resembling enlarged acini in sialadenosis and tumor cells in rosette-like arrangements. Acinar structures were mainly represented by rosette-like formations in about two-thirds of cases. The predominant pattern of cell arrangement, however, is represented by clusters of acinar tumor cells varying in size and partially adhering to vascular stromal fragments. Marked cell dissociation and abundance of bare nuclei are stressed as striking features. Nuclear changes suggesting malignancy are rare in aspirates from ACC. Abnormalities including marked anisonucleosis, slight-to-moderate nuclear pleomorphism, and hyperchromasia can be seen in only 20% of the cases. Multinucleation and mitoses can be noted only infrequently. Thus, in most cases the cytologic diagnosis of malignancy is only based on recognition of acinar cell differentiation. On rare occasions, sialadenosis may be confused with ACC, because hypertrophic serous acinar cells may resemble neoplastic acinar cells and enlarged acini in sialadenosis may mimic acinar structures observed in FNA's from ACCs. Moreover, cytoplasmic fragility of acinar cells originating from both lesions results in numerous naked nuclei embedded in a foamy background substance.

FNA of ACC have been reported to have rather low sensitivity and specificity with a correct diagnosis in only 68% of cases(32;33). Some authors are even more critical and report the FNA findings to be notoriously unreliable in recognising the malignant nature of parotid carcinoma preventing its precise classification and grading.(32) A malignant neoplasm that is particularly prone to diagnostic error on FNA is acinic cell carcinoma which is frequently interpreted as benign. FNA cytology is useful in diagnosing non-neoplastic inflammatory lesions for avoiding surgery or limiting surgical procedures for benign tumours. FNA can also reliably diagnose HG malignant carcinomas. For planning the extent of surgery of possibly malignant parotid tumours the clinical, radiographic, and intraoperative findings should contribute to the overall diagnostic impression.(32;32-34)

Immunohistochemical and genetical findings:
The immunoprofile of ACC is largely unspecific. However, an IHC study of CK distribution (CK7, 8, 13, 14, 18, 19) found that cytokeratin 8 was the more specific to neoplastic cells of ACC.(35) This staining may be useful in the recognition of neoplastic acinic cells IHC.(36) BMP-6 has recently been reported as a highly specific immunohistochemical marker of ACC and serous differentiation in salivary glands.(37) Infrequent expression of ER and PgR possibly of therapeutically and diagnostic interest has been reported.(38) The present commercial antibodies for alpha-amylase are often not staining ACC serous cells. Approximately 10 % of tumors are positive for S-100. Abnormal pattern of expression of Rb-pathway related proteins have been published.(39) All the ACCs exhibited substantial numbers of positive cells against Rb antibody that recognizes both unphosphorylated and phosphorylated Rb proteins. This phosphorylation appeared to be critical for inactivation of Rb-mediated growth suppression and may play an important role in the pathogenesis of ACC. On gene expression profiling of some SGT's each carcinoma entity was clustered together but MEC, SDC, and ACC were separated from each other. Significance analysis of

micro arrays identified 27 genes expressed differently between the groups. An intermediate filament protein of basal epithelial
cells, cytokeratin 14 (KRT14) was clearly differently expressed between the 3 types of carcinoma, and can be used as an aid in their differential diagnosis. The array results were validated by RT-PCR and immunohistochemistry(40)

A single case of ACC has been reported as normal after cytogenetical analysis with a combination of conventional cytogenetics via GTG-banding, molecular cytogenetics via fluorescent in situ hybridization, and chromosome morphometry(41).

A group from MDA investigated microsatellite alterations at certain chromosomal loci in ACC and reported that 84.0% of the tumors had alteration in at least one of the loci tested. In general, chromosomal regions at chromosomes 4p, 5q, 6p, and 17p were more frequently altered. Certain markers at 4p15-16, 6p25-qter, and 17p11 regions showed the highest incidence of LOH, suggesting the presence of tumor suppressor genes associated with the oncogenesis of these tumors(42)

The chromosomal locus 9p21 that contains the often deleted p16(INK4a/CDKN2/MTS1) tumor suppressor gene did not show LOH or homozygous deletions(43) absence of RAS mutations(44)low expression and mutation rate of p53(45)

Histogenesis:
Morphological similarities between secretory carcinoma of the breast and ACC has been repeatedly published and the question if ACC is an analogue tumor to secretary carcinoma of breast has been raised.(46) A recent FISH study demonstrated lack of ETV6 rearrangements in ACC support the concept that secretory carcinomas of the breast and ACCs are distinct entities.(47)

Tumor spread:
Usually, ACC initially metastasize to cervical LN's and subsequently to more distant sites, most commonly the lung. Rare patterns of spread including orbital metastases(48), hypophyseal metastasis(49) and intracranial extension(50;51) have been reported. Late manifestations including widespread cutaneous metastases(52) and widespread systemic metastases(53) are also known to occur.

Differential diagnosis:
Tumors composed of numerous acinar differentiated cells pose little diagnostic challenge except for distinguishing them from normal salivary gland. In tumors with few acinar type cells, the varied histomorphologic patterns and cell types may resemble other salivary and metastatic tumors. In all cases, recognition of acinar differentiated cells, sometimes with the help of special stains, is a key to the diagnosis.

The differential diagnosis for the papillary cystic and follicular patterns includes cystadenocarcinoma, mucoepidermoid carcinoma, metastatic thyroid carcinoma, and, in minor salivary glands, PLGA.

Although abundant clear cells are infrequent, acinic cell adenocarcinoma is included in the differential diagnosis of clear cell neoplasms, which also includes mucoepidermoid carcinoma, epithelial-myoepithelial carcinoma, clear cell adenocarcinoma, clear cell oncocytoma, and metastatic renal cell carcinoma. In contrast to the clear cells in such tumors

as epithelial-myoepithelial carcinoma, metastatic renal cell carcinoma, clear cell oncocytoma, and many clear cell adenocarcinomas, the clear cells in acinic cell adenocarcinoma are negative for glycogen. Features that distinguish acinic cell adenocarcinoma from clear cell mucoepidermoid carcinoma are the same as those already stated above. Of course, all of the above tumors, except acinic cell adenocarcinoma, lack serous acinar cell differentiation.

Cystadenocarcinomas, including papillary variants, are uncommon salivary gland tumors, but share features with papillary-cystic and follicular forms of acinic cell adenocarcinoma. Vacuolated cells and a microcystic pattern are indicative of acinic cell adenocarcinoma while mucocytes, identified with the mucicarmine stain, favour cystadenocarcinoma. The identification of serous acinar differentiated cells distinguishes acinic cell adenocarcinoma.

Although a papillary architecture is uncommon in mucoepidermoid carcinoma, epidermoid cells and mucocytes, which are strongly mucicarminophilic, and absence of serous acinar cells, distinguish it from acinic cell adenocarcinoma. Besides a search for serous acinar cells, immunohistochemical staining for thyroglobulin readily differentiates metastatic thyroid carcinoma.

In the context of neoplasia in the intraoral minor salivary glands, acinic cell adenocarcinoma is uncommon and PLGA is common. Like many acinic cell adenocarcinomas, PLGA has bland cytologic features and multiple growth patterns, such as solid, tubular, cystic, cribriform, and papillary. PLGA has an affinity for perineural growth, a homogeneous cell population, and a tendency for single-file cell infiltration at the tumor periphery.

Occasionally, the differentiation from oncocytoma and more specifically the clear cell variant cause problem. Performing the PAS+/- D and p63 IHC for detection of basal cells in oncocytomas can be helpful.(54)

Granular cell tumor(55) and ADCA NOS(56) also sometimes enter the differential diagnosis.

Prognosis and predictive features:

The National Cancer Data Base (NCDB) in USA reported that five-year survival in ACC was 83.3% (observed) and 91.4% (disease specific).(57) The biological behaviour of acinic cell carcinomas is unpredictable. Controversy and inconsistency characterize attempts at stratification of the prognosis for patients with acinic cell adenocarcinomas. Overall, most investigators agree that the majority of acinic cell adenocarcinomas are low grade. Many investigators have found that histomorphologic features do not reliably predict biologic behavior while other investigators have attempted to correlate these features with a grading system. Unfortunately, the criteria for grading have varied among investigators, and at least one scheme used clinical staging criteria, such as size and site, as part of the “grading” criteria. It would be useful if staging and grading were distinct and independent prognosticators. Some investigators provide insufficient information on their criteria for grading and use terms such as well differentiated, highly differentiated, poorly differentiated, lowly differentiated, and dedifferentiated without definition. However, the general consensus is that there are no histomorphological criteria by which locally aggressive behavior or metastatic potential could be predicted.(58-60) Neither the degree of acinar cell differentiation nor various growth patterns in ACC seem to influence prognosis. In general, the histologic features that have often been associated with tumors that recur or metastasize include frequent mitoses, focal necrosis, neural invasion, pleomorphism, infiltration (lack of circumscription), and stromal hyalinization. In one outcome based study of clinico-pathological parameters the

presence of a predominately solid architecture was strongly associated with a poor outcome(61). In this study tumour size (> 2.75 cm) was also a significant predictor of recurrent deep parotid lobe involvement. The presence of cervical nodal disease and lymphocytic infiltration were, although not significant, factors showed a tendency towards recurrence.

Only two exceptions from this rule have emerged including development of HG foci in otherwise typical ACC referred to as dedifferentiation or HG transformation of ACC and well differentiated ACC with lymphoid stroma. The well differentiated variant of ACC was reported by Michal et al(62) The findings of dense lymphoid stroma with well-developed germinal centres, surrounding a sometimes scanty epithelial component, which in each case had a microcystic growth pattern and a thin fibrous pseudocapsule surrounding the tumor were characteristic of this prognostically favourable subgroup. This type of ACC is thus mimicking an intraparotid lymph node containing a metastasis. All 12 cases showed low MIB1 proliferative activity, with a mean index of 1.7%.

Immunohistochemical staining with the MIB 1 antibody has been used to assess cell proliferation in ACC and a MIB 1 index higher than 5 per cent was seen in all patients who developed tumor recurrences(63). Most patients with MIB 1 indices higher than 10 per cent had unfavourable outcomes.. The results indicated that MIB 1 staining appears to be a significant prognostic factor in ACC.

In one recent study computer-assisted analysis of acinic cell cancer (ACC) morphological characteristics of CD34 immunoreactivity were detected. Bigger vessel size, vessel irregularity, and lower intensity of CD34-positive vessel staining may indicate unfavourable prognosis. (64-66)

In addition, incomplete resection, large size, and involvement of the deep lobe of the parotid gland have indicated a poorer prognosis. Staging is probably a better predictor of outcome than histomorphologic grading.

HG transformation (dedifferentiation):
HG transformation is defined as the histologic progression of a LG malignant neoplasm to a HG one, within which the original line of differentiation is lost. Originally, the phenomenon has been reported in some mesenchymal tumors, such as dedifferentiated chondrosarcoma and dedifferentiated liposarcoma. The phenomenon of HG transformation has been recently introduced to salivary gland pathology. HG transformation has been recognized in a variety of salivary gland carcinomas, including ACC, adenoid cystic carcinoma, polymorphous LG adenocarcinoma, mucoepidermoid carcinoma, myoepithelial carcinoma, and epithelial-myoepithelial carcinoma.

High-grade transformation in ACC:
Stanley et al first published HG transformation in ACC 1988 and subsequently a few more cases and small series has been added(42;67-74;74;74-76). Up-to-date less than 20 cases have been published and AFIP have reported another 4 cases on file. My own collection includes two cases. (one previously reported(73))

High-grade transformation of acinic cell carcinoma (ACC) is a rare phenomenon characterized by histological progression of low-grade ACC to high-grade adenocarcinoma or

undifferentiated carcinoma. These tumors have hybrid features of acinic cell adenocarcinoma, with the patterns and cell types described above, and areas of undifferentiated carcinoma

Histologically, the high-grade component is composed of polymorphic cells with high mitotic rate arranged in glandular and solid growth patterns with comedonecrosis. Spindle cell carcinoma and myoepithelial carcinoma have been described as dedifferentiated components, even though myoepithelium is not believed to play a role in acinic cell adenocarcinomas.

The MIB-1 labeling indices are elevated in the high-grade component, as compared with the low-grade conventional ACC. The high-grade component of ACC is characterized by membrane staining for CK18 and beta-catenin. In contrast, S-100 protein, a-1-antitrypsin, and lysozyme are lost only in high-grade foci of transformed ACC.

Unlike the findings in our case most cases have no history of multiple recurrences and no evidence that the differentiated acinar portion of these tumors preceded the undifferentiated carcinoma portion. It is possible that such tumors represent the proliferation of two clones of neoplastic epithelial cells that have developed in proximity to one another. In fact, heterogeneity on a molecular level has been documented analyzing the X chromosome inactivation status in cell cultures from a cytogenetically highly polyclonal acinic cell carcinoma of the parotid gland.(77)

Regardless of the histogenesis, the biologic potential and treatment of these tumors are dictated by the undifferentiated carcinoma component. Prognosis is poor in comparison to classic acinic cell carcinoma; however, the exact biologic behavior of these tumors is uncertain, as, so few cases of this entity have been published in English literature. LN metastases have been reported in 56%. Distant metastases to the lungs, pleura, brain, and peritoneum, and paraaortic, paratracheal, and mediastinal LN's were observed. 66% died from tumor dissemination, all with a median overall survival of 4.3 years. Aggressive clinical course was observed even if the HG areas represented no more than 5% of the primary lesion

FNA reports of isolated cases of dedifferentiated ACC has been published and the key for not missing the important diagnosis is recognition of both of the components—the low-grade acinic cell carcinoma and the high-grade undifferentiated carcinoma.(78;79)

Take home messages:

Dedifferentiated ACC is a highly aggressive neoplasm and failure to recognize HG transformation within ACC would result in therapeutic mismanagement of patients.

Any foci of HG morphology qualify the tumor as HG transformed ACC.

Radical surgery and adjuvant radiotherapy is recommended.

High propensity for cervical LN metastases suggests the need for neck dissection.

Resected specimens of all ACC should be thoroughly sampled to avoid a missing HG component, particularly in recurrence.

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CASE 43

Göran Elmberger, M.D.

Clinical history:

This 59-year-old man with congenital aortic insufficiency was healthy until 40 years age when he suffered from bone pain and increased inflammatory parameters. X-ray revealed lytic and sclerotic multiple lesions in long bones. Bone biopsy from femur condyle interpreted as reactive. On special clinical request to rule out Langerhans cell histiocytosis S-100 IHC was found negative. No Birbeck granulae on EM. Long term treatment with cytotoxic drugs and steroids. Otherwise, symptomatic pericarditis and radiolological signs of coated aorta and perirenal fibrosis. Recently epileptical seizures of grand mal type. CT reveals meningeoma-like tumor cerebri in fronto-temporal cortex. Brain biopsy (enclosed slide) externally interpreted as metaplastic-xanthomatous meningeoma. General increase in inflammatory parameters and scintigraphically signs of increased activity in distal long bones.

Pathological findings:

Microscopical examination showed a dural based xanthomatous histiocytic proliferation with multiple Touton-like multinucleated giant cells. The Touton-like giant cells regularly showed classical wreath-like arrangement of multiple nuclei. In some sections the histiocytic proliferation was sharply demarcated from surrounding brain tissue even if no capsule formation was seen. The histiocytes generally had abundant pale staining and foamy or finely granular cytoplasm. In minor areas a more eosinophilic granular cytoplasm was present. Focally intracytoplasmic hemosiderin pigment was noted. The nuclei were round to oval and lacked the irregular configurations typical of Langerhans' cells. Pseudocystic and microcystic degeneration was noted centrally in lesion. Parts of lesion showed birefringent collagen sclerosis. Mild lymphocyte dominated focal inflammation was seen. Minor component of plasma cells and rare eosinophilic granulocytes were also noted. Sparsely foci of empty cholesterin-like clefts with suggestive multinucleated foreign-body-type granuloma were seen. Findings not previously described were stromal and intravascular psammoma bodies, HPC-like vessels and a plexiform rich capillary network. Capillaries also focally showed sclerosis. Subdurally, small ball-like proliferates of menigothelial cells were seen. No lymphocytic emperipolesis was identified. No cellular atypia, necrosis, mitosis or granuloma were found.

Special studies:
IHC: CD45+, CD68+, Lysozyme+, MAC387+, vim+/-, CD99-, EMA-, PgR-, MNF116-, S-!00-, GFAP-, NSE-, CD1a-. Proliferation rate < 1% (MIB1).
Histochemistry: PAS +/- D -.

Review of previous femoral bone biopsies revealed irregularly thickened sclerotic bone trabeculae, bone marrow fibrosis, lymphocytic sparse inflammatory reaction, and a focal infiltrate of foamy cells consistent with late fibrotic phase of Erdheim-Chester disease (ECD). Touton giant cells could not be seen.

Diagnosis: Pseudotumoral intracranial Erdheim-Chester disease (polyostotic sclerosing histiocytosis) mimicking a primary brain-meningeal tumor as manifestation of previously undiagnosed systemic disease.

Discussion:
ECD was first described by Chester and Erdheim in 1930 under the name "lipoid granulomatose". To date approximately 200 cases has been described in the literature. ECD is a rare, idiopathic, non-Langerhans cell lipid-storing histiocytosis without detectable serum lipid abnormalities. Histologically ECD is characterized by xanthogranulomatous inflammation, with widespread systemic manifestations. The disease appears to be non-familial and mainly affects middle-aged adults. Clonality studies have yielded mixed results in ECD. A more than rare co-existence of ECD and LCH raise the possibility of a relationship between the two histiocytoses. Typical systemic features of ECD include osteosclerotic lesions of the metaphysial regions of long bones, retroperitoneal and pulmonary fibrosis, cutaneous lesions, fever, and exophthalmos. It may also affect the heart, liver, spleen, sinonasal mucosa, aorta, testis, thyroid, lymph nodes and kidneys. Neurological involvement is most commonly heralded by hypothalamic/pituitary involvement with resultant diabetes insipidus, and occasionally cerebellar symptoms. Involvement of other intracranial regions such as the cerebral cortex, spinal cord, choroid plexus and leptomeninges is uncommon. By the time the intracranial lesion is discovered, most patients demonstrate evidence of systemic disease. A rare case of isolated intracranial ECD has been described. The most common central nervous system manifestations of ECD in descending order are diabetes insipidus,

cerebellar syndromes and orbital lesions. Spinal and extra-dural masses have also been documented in the literature. In general, ECD evolves in a slowly progressive manner and may mimic multiple sclerosis because of the multifocal nature of involvement.

Both preoperative and post-operative anatomical diagnoses of the present case were initially interpreted as consistent with metaplastic-xanthomatous meningeoma. The systemic nature of the disease as well as the outcome of IHC with a CD68+, EMA- and PgR- cell population is clearly against meningeoma. In addition to ECD, other notable histiocytic disorders that involve the central nervous system and are rarely characterized as a solitary mass lesion include Langerhans cell histiocytosis (LCH) of presumed dendritic Langerhans cell origin and non-Langerhans cell histiocytoses from bone-marrow derived macrophages such as sarcoidosis, Rosai-Dorfman disease (RDD), juvenile xanthogranuloma (JXG) and hemophagic lymphohistiocytosis. In the absence of the characteristic extra-cerebral manifestations, the diagnosis of these disorders is often challenging and is one of exclusion. Awareness of the clinico-pathological spectrum of these entities can be helpful in sorting out the differential diagnosis of cerebral histiocytic lesions that may mimic neoplastic or infectious diseases. Rarely true malignant histiocytic neoplastic disorders such as histiocytic sarcoma or follicular dendritic cell (FDC) sarcoma may primarily involve the leptomeninges and brain.

The prognosis of Erdheim-Chester disease is dependent on the extent of extraosseous disease. Approximately 60% of patients die of the disease, the majority due to respiratory failure or central nervous system involvement. No effective treatment has been discovered, although chemotherapeutic agents, radiotherapy, interferon therapy, and autologous bone marrow transplantation have been employed.

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CASE 44

P. E. Wakely, Jr., M.D.

Clinical History:

A 52-year-old white male nonsmoker presented with a slowly enlarging left lower lobe (LLL) lung mass. This mass was discovered incidentally 3 weeks earlier as part of a job-related screening chest X-ray. He is a copper welder whose factory requires biennial health screenings, and therefore a chest X-ray was performed. Review of his chest X-ray from 2 years earlier showed a similar, but much smaller LLL lung mass.

CT scan confirmed a single 3 cm. LLL non-calcified mass with no hilar or mediastinal adenopathy and no other parenchymal abnormalities. His past medical history is positive for Bell's palsy a few years ago that resolved completely. He uses an inhaler for asthma and is hypertensive. Lymph node survey revealed no palpable peripheral lymphadenopathy. A left lower lobe wedge resection of the lung was performed.

Gross and Microscopic Findings:

A single 3-cm. diameter ill-defined pale tan nodular mass was surrounded by grossly unremarkable lung tissue. Light microscopy showed the mass was composed of a solid infiltrate of lymphocytes that replaced the alveolar parenchyma. At the mass periphery interstitial nodular expansion of bronchoalveolar septa by lymphocytes was present. Focally, lymphocytes infiltrated the visceral pleura. Numerous easily recognizable follicles were seen at low power. Some of these represented reactive lymphoid follicles while others showed the follicles had been colonized by neoplastic cells. Reactive follicles contained normal appearing germinal centers composed of a mixture of follicular center cells and tingible body macrophages. At the periphery of reactive follicles, primarily small-cleaved (centrocytic)

lymphocytes proliferated. Within neoplastic follicles a distinct follicular architecture mimicking follicular lymphoma at low power was created by "colonization" with these neoplastic centrocytic-type cells. In other foci a solid/diffuse sheet of lymphocytes was apparent. Centrocytic lymphocytes and small round lymphocytes commonly infiltrated the bronchiolar epithelium creating so-called “lymphoepithelial lesions”. Cytokeratin staining highlighted these lymphoepithelial lesion foci by demonstrating an intimate mixture of lymphocytes and epithelial cells. Imprint smears of the nodule showed principally small round and cleaved lymphocytes with a minority of cells being centroblastic or plasmacytoid. Mature plasma cells and monocytoid lymphocytes were distinctly infrequent. Excised hilar, interlobar, and subaortic lymph nodes were negative for lymphoma.

Special Studies:

Immunohistology showed diffuse positive cytoplasmic/membranous staining for CD20. Staining of bcl-2 was positive in all areas with sparing of reactive germinal centers. Flow cytometry performed from the tissue sample demonstrated monoclonal lambda light chain restriction with a kappa - lambda ratio of 12:48. Lymphocytes expressed CD19, CD20, but did not express CD23, CD5, or CD10.

Diagnosis: PRIMARY PULMONARY EXTRANODAL MARGINAL ZONE B-CELL LYMPHOMA OF MUCOSA-ASSOCIATED LYMPHOID TISSUE [MALT LYMPHOMA, MALToma].

Discussion:

Primary pulmonary lymphomas comprise <1% of lung tumors. Criteria for the "primary" designation should be applied only to those patients that have no history of and no evidence of an extra-pulmonary lymphoma at the time of diagnosis and for 3 months thereafter. There should be no radiologic evidence of mediastinal adenopathy at the time of diagnosis, and no evidence of peripheral blood or bone marrow disease. Some investigators also require negative CT scans of the chest, abdomen and pelvis before pronouncing the lymphoma as primary of the lung.

Extranodal Marginal Zone B-Cell Lymphoma of Mucosa-Associated Lymphoid Tissue [MALT Lymphoma] is the most common (comprising up to 75% of cases depending on the series) of primary pulmonary lymphomas. **Primary Pulmonary MALT Lymphoma** [**PPML**] typical occurs in the 6^{th}-7^{th} decade with a slight female predominance, but a wide age range from the 4^{th}-9^{th} decade exists. There is no known association with a specific environmental exposure. This patient's clinical presentation could not be more classical. The typical PPML patient is asymptomatic, and has a pulmonary nodule discovered only incidentally on chest X-ray. Most lung masses are single, < 5 cm. in diameter and unilateral. Less often patients may present with cough, dyspnea, "B" type symptoms, and chest pain; a minority have multiple lung nodules, and pleural effusion. There are no specific laboratory findings for PPML, but 43% of patients had a serum monoclonal gammopathy despite negative bone marrow exam in one series.[3] Bone marrow involvement eventually occurs in 15-20% of patients. Hilar lymph nodes are microscopically involved in less than a third of cases, although a recent series reported over 40% of patients with positive ipsilateral regional nodes. PPML has been reported in association with a "laundry list" of autoimmune diseases including Sjögren's syndrome, SLE, Hashimoto's thyroiditis, primary biliary cirrhosis, multiple sclerosis, and rheumatoid arthritis among others. The mechanism for development of PPML is thought to be secondary to chronic inflammation, and persistent nodular lymphoid hyperplasia in the lung. Because it is classified as a MALT lymphoma this implies the PPML

arises from pre-existing lymphoid tissue in the lung which has been termed "bronchus-associated lymphoid tissue'".

The relatively good prognosis of PPML relates to its remaining localized to the lung for an extended period of time. About 50% of patients develop a recurrence of the tumor in 2 years. Recurrence is usually in the lung, or may occur in other extra-nodal sites such as the major salivary glands or the stomach. Survival is similar to age-matched controls with 10-year survivals reported to range from 70% to almost 90%, however, some series report 10-year survival as low as 40%. Less than 20% of PPML cases transform to a diffuse large B-cell lymphoma.

Pathology of PPML

PPML typically forms a mass that has effaced the underlying lung parenchyma. The mass consists of small and centrocytic lymphocytes as in this patient. In some examples a greater degree of plasmacytic differentiation is present with the formation of intranuclear immunoglobulin inclusions (Dutcher bodies) and monocytoid cells. Although not present in this case, amyloid deposition has been reported in 10% of cases, and seems to have a detrimental effect on survival.[3] Reactive follicles with intact germinal centers are a common feature, and occur along with follicles colonized by malignant lymphocytes. Lymphoepithelial lesions are found are almost universally. Blood vessel wall invasion was not appreciated in this case, but has been reported. This angioinvasion is not associated with necrosis or angiodestruction. The mass periphery typically shows a lymphangitic and interstitial pattern of spread into alveolar septa, the pleura, bronchioles, and blood vessels. PPML may penetrate the visceral pleura to form polypoid nodules projecting into the pleural space. A granulomatous inflammatory infiltrate including multinucleated foreign body-type giant cells can occur in up to 50% of cases.

The immunohistochemical profile in paraffin-embedded tissue shows positive staining with CD20, bcl-2, CD79a, nuclear bcl10 and ±CD43. Reactive germinal centers stain with CD21, bcl-6, and CD35. Negative staining occurs with bcl-1, CD5, and CD10. Light chain restriction is best confirmed by flow cytometry where it is present in more than 90% of tumors. Cytogenetic abnormalities can be found in almost ¾ of patients with PPML. These are heterogeneous, and include the *API2-MALT1* fusion protein due to t(11:18)(q21;21). This is the most common structural cytogenetic abnormality being reported in 30-50% of PPML. Another less common abnormality is t(14;18)qp32;q21) which juxtaposes the IGH promoter region at chromosome 14 with the MALT1 to produce the *IGH-MALT1* protein. Aneuploidy alone can be found in 40% of PPMLs.

Differential Diagnosis:

The principal entities to be distinguished from PPML are benign lymphocytic proliferations. **Lymphoid Interstitial Pneumonitis** [**LIP**] is diffuse chronic interstitial pneumonia rich in small lymphocytes and plasma cells that does not form a solid mass. It has been reported most often in AIDS-infected children. These patients progress to diffuse interstitial fibrosis in 30% of cases. Distinction of LIP from **Follicular Bronchitis/Bronchiolitis** is often arbitrary. Lymphocytes and lymphoid nodules are typically confined to peribronchial & lobular septa in the latter rather than showing a diffuse alveolar pattern of infiltration. Nonetheless, morphologic overlap occurs. LIP and follicular bronchiolitis are typically polyclonal lesions. **Nodular Lymphoid Hyperplasia [NLH]** is typically a subpleural polyclonal localized mass of reactive lymphoid tissue. Microscopically, this lymphoid collection contains normal appearing secondary follicles with germinal centers and interfollicular plasma cells. Lymphoepithelial lesions and infiltration of the visceral pleura are absent. The major histologic feature that separates PPML from a reactive

lymphocytic infiltrate is a coalescence of lymphocytes in the former into a mass(es) that destroys/effaces the underlying lung architecture. Invasion of the visceral pleura, or bronchial cartilage favor PPML, while invasion of parietal pleura, regional node involvement, and lymphangitic spread offer even greater assurance that the infiltrate is lymphomatous. Lymphoepithelial lesions and demonstration of light chain monoclonality alone without this architectural effacement are insufficient to issue a diagnosis of PPML.

Lymphomatoid Granulomatosis [**LYG**] is a heterogenous category of lymphoproliferative lesions that display an angiocentric and angiodestructive (necrosis being a common feature) population of polymorphous B-lymphocytes. These are phenotypically analogous to T cell-rich B-cell lymphoma. The grade I form of LYG most closely may resemble PPML. LYG contains a higher percentage of large lymphocytes as well as cells mimicking mononuclear R-S cells (Hodgkin's cells). The large B-cells are nearly always EBV positive in LYG unlike PPML.

Follicular Lymphoma represents about 5% of primary lung lymphomas. It has a widespread follicular histology and is morphologically similar to its nodal-based counterpart. Reactive follicles with germinal centers are typically absent. Immunohistology shows CD10, CD20, bcl-2 positive and CD43 negative follicles.

The **Large Cell Lymphomas**. Intravascular Large B-Cell Lymphoma is a variant of diffuse large B-cell lymphoma. Pulmonary vessels are filled with large lymphocytes in this condition. Diffuse large B-cell lymphoma (DLBL) and anaplastic large cell lymphoma (ALCL) may develop as primary lymphomas of the lung. Their morphology is in stark contrast to PPML in that they are composed of large centroblastic or immunoblastic lymphocytes with no germinal center formation.

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Case 45

Cesar A.Moran M.D.

Clinical History

51-year-old man presented with a history of shortness of breath and chest pain. The patient has a history of heavy tobacco use of many years. Radiographic examination revealed the presence of an intrapulmonary mass. Lobectomy was performed.

Histopathological Features

At low power, there is a cellular proliferation destroying the normal lung parenchyma. The cellular proliferation is composed of medium size cells with distinct cell borders, round nuclei, and clear cytoplasm. The cellular proliferation characteristically shows many dilated vessels giving a vague hemangiopeircytic pattern of growth. No mitotic activity and/or hemorrhage are present.

Immunohistochemical Features

A battery of immunohistochemical studies was perfomed including keratin, EMA, and CEA, all of them with negative results. In addition, HMB-45 and CD-34 show positive reaction in tumor cells.

Diagnosis

Clear Cell "sugar" tumor of the Lung

Discussion

Sugar tumor is an unusual benign neoplasm of the lung, which etiology of this tumor remains unknown. Liebow originally described the tumor in 1963 and since then less than 100 cases have been reported in the literature. The tumor can occur in any age group and it has been recorded to occur in patients from 8 to 70 years of age. The presentation is usually of an asymptomatic coin lesion of the lung. The tumors are well circumscribed that can vary in size from under 1 cm to more than 6 cm in greatest dimension.

Because of the immunohistochemical profile shown by sugar tumors of the lung, more recently it has been suggested that this tumor also belongs to the so-called PEComas, which may also involved other lung neoplasms such as angiomyolipoma and lymphangioleiomyomatosis.

Surgical resection of the tumor is the treatment of choice and the prognosis is good.

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CASE 46

Cesar A.Moran M.D.

Clinical History

11-year –old girl presented with shortness of breath. Radiographic examination revealed the presence of an intrapulmonary mass. Lobectomy was performed.

Histopathological Features

At low power view, the tumor appears well circumscribed but not encapsulated neoplasm destroying normal lung parenchyma with solid and vague papillary growth pattern. Areas of hyalinization and vascular proliferation were also present. At higher magnification, the tumor appear to be composed of two different cellular components – one composed of a monotonous cellular proliferation composed of medium size cells with lightly eosinophilic cytoplasm, round nuclei, and inconspicuous nucleoli, the other cellular component is composed of small cuboidal cells reminiscent of alveolar cell lining. Focal areas of hemorrhage were present but necrosis was not identified. In the solid component focal areas with collection of foamy macrophages was identified. Several lymph nodes show metastatic tumor.

Immunohistochemical Features

Immunohistochemical studies for keratin showed positive staining while EMA showed weak positive staining. TTF-1 shows strong positive reaction. S-100 protein, smooth muscle actin, and neuroendocrine markers were negative.

Diagnosis

Sclerosing Hemangioma (Pneumocytoma)

Discussion

Sclerosing hemangiomas are unusual benign tumors of the lung. Liebow and Hubbell described the tumor originally in 1956, which the authors interpreted as an analogous tumor to the one in the skin. The tumors appear to be more common in women between the ages of 30 and 40 years. In the majority of patients the lesion is discovered incidentally during a routine radiographic examination. Grossly, the tumors may range in size from under 1 cm to more than 5 cm in diameter, with an average of 3.5 cm.

Immunohistochemically, different studies have reported different results in term of keratin, EMA, S-100, smooth muscle actin, and Ulex europeus positivity. EM studies have suggested an origin from type II penumocytes, thus the term pneumocytoma.

The clinical behavior of SH is that of a benign tumor that can be cure by complete surgical resection. Only a few cases have been described with lymph node involvement, and in the case herein presented, it appears to be the youngest patient with such an occurrence.

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CASE 47

Cesar A.Moran M.D.

57-year –old man presented with chest pain, shortness of breath of several weeks duration. Radiographic examination revealed the presence of a large anterior mediastinal mass. Complete surgical resection was performed.

Histopathological Features

The low power is of a solid cellular proliferation with a vague storiform or HPC pattern. Higher magnification revealed the presence of a spindle cellular proliferation composed of fusiform cells with elongated nuclei and inconspicuous nucleoli. Scattered lymphocytes are also present admixed with the spindle cells. The cells do not display nuclear atypia or mitotic activity and also no hemorrhage or necrosis is also identified. Although in some part, the tumor appears to be well circumscribed, other areas show the presence of the spindle cellular proliferation penetration into adjacent adipose tissue.

Immunohistochemical studies

The tumor cells show positive staining for broad-spectrum keratin and keratin 20 while show only focal staining for EMA. Other stains including S-100 protein, chromogranin, and Bcl-2 are negative.

Diagnosis:

Invasive spindle cell thymoma

THYMIC EPITHELIAL NEOPLASMS

Primary thymic epithelial neoplasms have long been a source of controversy in pathology due to their wide spectrum of histologic appearances, biologic behavior, and clinical manifestations. In fact, such variability has been responsible for difficulties in the classification and prognostication of these tumors. This has led to a proliferation of classification systems in recent years and conflicting views on the best approach to the evaluation of these lesions by pathologists. The pathology of thymomas has thus turned into a complex and controversial issue that has generated much confusion for practicing pathologists.

Historical Considerations

The term thymoma, as currently defined, refers to a neoplastic proliferation of thymic epithelial cells. Throughout the years, numerous attempts at classification of these tumors have been presented in the literature. The most widely accepted classification scheme in the Unites States was the one proposed by Dr. Barnatz at. al. from the Mayo Clinic, which classified thymomas according to their relative proportion of lymphocytes and the shape of the neoplastic epithelial cells into predominantly lymphocytic, predominantly epithelial, mixed, and predominantly spindle cell type (Table I). Numerous clinicopathologic studies of thymoma in large series of patients utilizing this histologic approach, however, failed to find any statistically significant correlation between the morphology and the clinical behavior of these tumors.

Despite the apparent unreliability of the various morphological classifications of thymoma for predicting biologic behavior in these tumors, it was soon appreciated by several investigators that clinical staging of the lesions based on their status of capsular integrity afforded a better means for assessing their biologic behavior. For this reason, Levine and Rosai in 1978 introduced the concept of defining thymomas on the basis of their capsular status into benign and malignant, depending on whether the tumor was encapsulated or invasive (Table I). In their classification, Levine and Rosai additionally espoused the concept that invasive tumors displaying overt cytologic features of malignancy should be regarded as

equivalent with thymic carcinoma (so-called malignant thymoma type-II) (Table I).

More recently, interest in the morphological classification of thymoma was revived by the studies of Marino and Muller-Hermelink, who presented a novel histologic classification of these tumors based on histogenetic considerations. These investigators proposed that thymomas could be divided on the basis of their cytological features into those derived from the cortical or from the medullary epithelium of the thymus into "cortical" and "medullary" thymomas. Cases that contained features of both were regarded as "mixed". The authors subsequently modified their approach by adding two additional categories, the predominantly cortical or "organoid" thymoma, and a fifth category designated as "well-differentiated thymic carcinoma" (Table I).

TABLE I: Review of Major Classifications of Thymoma (1961-1989)

Barnatz et al (1961) al (1989)	***Levine & Rosai (1978)***	***Muller-Hermelink et***
Predominantly epithelial	***Benign thymoma***	***Medullary thymoma***
Predominantly lymphocytic	***- Encapsulated***	***Cortical thymoma***
Mixed	***Malignant thymoma***	***Mixed thymoma***
Spindle cell thymoma	***- Type I (invasive)***	***Predominantly cortical***
	- Type II (thymic carcinoma)	***Well-differentiated thymic carcinoma***

Many studies have been presented in the literature that would appear to validate the clinical use of the Muller-Hermelink classification. The proponents of such classification have claimed that the various morphologic subtypes directly correlate with the probability of invasiveness for these tumors, and that histologic subtyping according to this classification is predictive of clinical behavior independent of stage. Despite the undoubted wide appeal of the Muller-Hermelink classification, major objections have been voiced by experts in the field concerning its applicability for clinical practice. Most of the criticism of the studies supporting the Marino & Muller-Hermelink classification has been centered around issues of inadequate sampling, reproducibility, and reliability for accurately predicting the prognosis of these tumors, particularly when dealing with limited biopsy samples (i.e., endoscopic biopsies of large mediastinal masses).

Given this controversy, a number of new classification schemes have been proposed by different investigators for these tumors in recent years. In the new AFIP Fascicle on Tumors of the Mediastinum, Drs. Shimosato and Mukai propose a complex classification scheme that takes into consideration the extent, histology, cell type and degree of atypia of the neoplastic cells, and incorporates terminology from various other existing classifications (Table II). The most recent classification scheme presented by Dr. Kuo from Taiwan proposes that thymomas be classified according to their cytokeratin expression profiles. Based on a study of 34 immunostained thymomas, and an additional 113 thymomas without the stains, the author proposed that his approach provided a useful method for the clinical evaluation of thymomas (Table II).

Table II: Recent Additional Classifications of Thymoma

Shimosato & Mukai (1997)	***Kuo (2000)***
By extent:	***Spindle cell thymoma***
-Circumscribed	***Small polygonal***
-Invasive	***Mixed***
-With implants or metastasis	***Organoid***
By histology:	***Large polygonal cell***
-Lymphocytic, mixed, epithelial	***Squamoid thymoma***
By cell type:	
-Spindle, polygonal, polygonal-oval	
By cell atypia:	
-Absent, slight, moderate, marked	

Given the controversy and lack of consensus regarding thymoma classification, the WHO organization commissioned Dr. Juan Rosai to establish a panel for the study of this topic. After many years of deliberation, a compromise, "non-commital" formula was devised by the WHO panel for the classification of thymic epithelial neoplasms. The new WHO classification schema did not introduce a new terminology but simply assigned a combination of letters and numbers to the various histologic types in the existing classifications of thymoma (Table III). The letters A and B represent thymomas predominantly composed of either spindle or round cells, respectively. Type AB are those composed of both spindle and round cells; and type C is reserved for those showing overt features of carcinoma (i.e., thymic carcinoma). In their WHO monograph, the authors state that this schema is *not intended as a new histologic classification* of thymoma nor is it meant to replace any previous terminology, but rather should be employed as a means for facilitating comparison among the various terms

Table III: Comparison of WHO Schema With Other Classifications

WHO Schema	***Barnatz et al***	***Muller-Hermelink et al***
Type A	***Spindle cell***	***Medullary thymoma***
Type AB	***---***	***Mixed thymoma***
Type B1	***Lymphocyte rich Predominantly cortical***	
Type B2	***Mixed***	***Cortical thymoma***
Type B3	***Epithelial rich***	***Well-differentiated thymic carcinoma***
Type C	***Thymic carcinoma***	***---***

from the existing classifications.

Although the recently introduced WHO schema does not settle the issue of thymoma classification, the WHO monograph is of importance because it supported and stressed certain valuable concepts that were agreed upon by all the panel members in that publication: 1) no histogenetic basis has yet been conclusively demonstrated between the normal anatomic compartments of the thymus and any of the different histologic types for any of the existing classifications; 2) thymic epithelial neoplasms represent a spectrum of lesions that may range from histologically benign to malignant, hence the inclusion of thymic carcinoma as "thymoma type C"; and 3) the degree of invasiveness relates more closely to recurrence and outcome than the cytoarchitectural features, to the point of markedly reducing the independent prognostic value of the latter.

It follows from review of the above that markedly different opinions continue to exist regarding the issue of thymoma classification and that a widely agreed upon classification remains to be devised. Moreover, it has become increasingly obvious to workers in this field that the complexity of thymoma classification continues to increase with every new proposal and with the introduction of new and increasingly complex (albeit colorful) terms.

New Concepts

Current opinions appear to continue to be divided between those who contend that histologic classification provides a reliable means for assessing prognosis of thymic epithelial neoplasms, and those who claim that staging represents the best if not the only valid parameter for the evaluation of thymoma. We believe the truth probably lies somewhere in-between, and that proper evaluation of these lesions would benefit from an approach that incorporates, as in other tumor systems throughout the body, a combination of histologic grading and clinical staging of the lesions.

In a recent review (Am J Clin Pathol, Vol.111:826-833,1999) we presented a novel conceptual approach to primary thymic epithelial neoplasms that is based on a combination of grading and staging of these tumors. In traditional pathology, most tumor systems follow a stepwise progression in their histologic evolution leading to progressive loss of differentiation of the tumor cells. Thus, for most epithelial tumor systems, the first step in this progression is represented by carcinoma in-situ, followed by well-differentiated (usually invasive)

Table IV: Organotypical Features of Thymic Differentiation

Normal Mature Thymus of Childhood or Adolescence	**Normal Mature Thymus of the Adult**
-Low-power lobulation	***-Bland spindle cell population with scant lymphocytes***
-Dual (epithelial/lymphoid) cell population	***-Cystic and glandular formations***
-Distended perivascular spaces	***-Rosette-like epithelial structures***
-Areas of "medullary" differentiation	
-Lack of cytological features of malignancy	

carcinoma, moderately-differentiated carcinoma, and finally poorly-differentiated carcinoma. The equivalent of such a spectrum has not been yet recognized in the thymus. One of the reasons for this is the tremendous histological variability that these tumors can display. The other reason is the traditional belief that malignancy in thymoma cannot be predicted based on features of differentiation and atypia because of the overwhelmingly "bland" appearance of the majority of such tumors. Thymic epithelial neoplasms characterized by overt cytologic evidence of malignancy (i.e., thymic carcinoma) have thus been traditionally separated from conventional thymomas and felt to represent a totally different and unrelated entity.

Careful study of our cases and review of the literature have led us to believe otherwise, and have demonstrated that the lesions which we call thymic carcinoma and thymoma are closely related entities that most likely represent opposite ends of a single spectrum of differentiation. In a recent study we were able to document the existence of tumors demonstrating direct transitions between areas showing the classical features of thymoma and areas showing unequivocal features of thymic carcinoma. Moreover, in several of these cases, we were able to identify different areas within the same tumor showing a spectrum of differentiation that ranged from classical thymic epithelial cells with round to oval, vesicular nuclei with small nucleoli and abundant rim of eosinophilic cytoplasm, to larger cells with well-demarcated cell borders and features of atypical keratinizing epithelium. These observations have led us to believe that thymic epithelial neoplasms form part of a spectrum of closely related lesions that may display varying histological appearances depending on their degrees of differentiation.

Unlike other epithelial tumor systems in which a progression from well-differentiated through poorly-differentiated carcinoma can be easily determined, thymomas have not lent themselves easily to such categorization because of their great variability in cytological composition and architectural growth patterns. Another difficulty involved in establishing the degree of differentiation in these tumors is the fact that the "normal" thymus can differ dramatically in appearance depending on the age of the individual. Thus, the normal *mature* thymus of a child will look quite different from the normal but *involuted* thymus in the adult. The mature thymus in childhood and adolescence will show the prototypical features of this organ characterized by a sharp demarcation between the cortex and the medulla and the admixture of thymic lymphocytes with large, round epithelial cells containing vesicular nuclei and indistinct cell borders with abundant amphophilic cytoplasm. The normal involuted thymus of older adults, on the other hand, will often be characterized by a paucity of lymphocytes and the epithelial cells will frequently adopt the shape of small, oval to spindle cells with scant cytoplasm and inconspicuous nucleoli. Although these contrasting appearances are an expression of the functional (i.e., active *vs.* inactive) state of the organ, they both represent the normal status of this organ at different stages in its evolution. Tumors displaying features that closely resemble these two "normal" appearances of the thymus could therefore be regarded as showing a high degree of differentiation.

Commonly accepted features of organotypical differentiation in thymomas on routine microscopy include: 1) a well-developed lobular architecture on scanning magnification; 2) dual cell population (neoplastic thymic epithelial cells and thymic lymphocytes) admixed in various proportions; 3) distended perivascular spaces; 4) areas of "medullary" differentiation characterized by discrete rounded foci predominantly composed of epithelial cells surrounded by a population of cells that resembles the normal cortex; and 5) the bland appearance of the epithelial cells, which lack overt cytological features of malignancy. To the above features we would also add the presence of a bland-appearing spindle cell proliferation with scant lymphocytes, cystic and glandular formations, and rosette-like epithelial structures, which are features that are commonly encountered in the regressed thymus of older adults (Table IV).

Applying the above parameters, tumors that exhibit most or all of the above features could be categorized as well-differentiated, whereas tumors displaying total loss of these organotypical features would be classed as poorly-differentiated neoplasms. Fortunately, the vast majority of thymic epithelial neoplasms will generally fall within the first group, i.e., that of well-differentiated tumors displaying most of the organotypical features of the normal thymus. Such tumors have been designated by convention as thymoma. Thymic epithelial neoplasms in which most or all of the organotypical features of differentiation of the normal thymus have been lost and the tumor cells already display overt cytological features of malignancy would correspond to those traditionally designated in the literature as thymic carcinoma. There exists a third, smaller group of primary thymic epithelial neoplasms that displays features intermediate between thymoma and thymic carcinoma. Such tumors are characterized by the presence of cytological atypia of the tumor cells, yet they still retain many if not most of the organotypical features of differentiation of the normal thymus. These tumors can be regarded as representing an intermediate stage in the spectrum of differentiation of thymic epithelial neoplasms (i.e., moderately-differentiated tumors). On the basis of the cytological features of atypia displayed by their tumor cells, we have proposed the designation of atypical thymoma for these tumors.

Based on the above considerations, we currently believe that thymic epithelial neoplasms can be reliably classified into three simple categories: well-differentiated thymoma, moderately-differentiated or atypical thymoma, and poorly-differentiated tumors (i.e., thymic carcinoma) (Table V). Assigning a given lesion to any of these various categories does not depend on any purported histogenetic considerations, does not require the use of special stains or advanced techniques not readily available to general pathologists in community practice, and simply requires basic familiarity with the organotypical features of differentiation of the normal thymus and attention to the degree of cytological atypia displayed by the neoplastic epithelial cells on routine microscopy.

Table V: Classification of Thymic Epithelial Neoplasms According to Grades of Differentiation

Type	Grading	Histological Criteria
Thymoma	*Well-differentiated*	*-Preservation of organotypical features of differentiation* *-No cytological evidence of atypia*
Atypical thymoma	*Moderately-differentiated*	*-Preservation of organotypical featured of differentiation* *-Mild to moderate cytological atypia*
Thymic carcinoma	*Poorly-differentiated*	*-Loss of organotypical features of thymic differentiation* *-Presence of overt cytological evidence of malignancy*

Prognostic Features

The evaluation of prognosis in thymoma remains a controversial issue. Most studies seem to indicate that staging represents the most important parameter for assessing the clinical behavior of these tumors. Many of the proponents of some of the more recent morphologic classifications, however, contend that histologic subclassification of well-differentiated thymoma represents a valuable independent prognostic criterion for determining clinical behavior and guiding therapy in these tumors. Studies using special techniques, such as determination of ploidy by flow cytometry, immunohistochemical determination of proliferative index, assessment of p53 protein expression, etc., have so far yielded conflicting and inconclusive results.

The proponents of the Muller-Hermelink classification have maintained that the different histologic types of their histogenetic classification directly correlate with invasiveness, and therefore can be reliably utilized to predict the biologic behavior of the lesions. The majority of such studies, however, have failed to demonstrate any correlation with mortality and survival on long-term follow-up. The fact that certain histologic subtypes (such the predominantly epithelial thymoma) were associated with increased invasive properties, and that others (such as spindle cell thymoma) were more often associated with indolent clinical behavior has been well-recognized in several studies predating the histogenetic classification of Muller-Hermelink and colleagues. The same studies, however, also demonstrated that when stratified according to staging, all the different histologic subtypes ultimately behaved in a similar manner.

This begs the question: is histologic subclassification of thymoma, particularly the well-differentiated variants, necessary? We believe the answer to this question is that subtyping of well-differentiated thymic epithelial neoplasms offers no distinct advantage for prognostication, and that determination of the status of capsular integrity constitutes the most important step in the evaluation of these tumors. Another factor that renders subclassification of well-differentiated thymoma nearly irrelevant is the fact that these tumors tend to display marked morphologic heterogeneity, with frequent admixtures of different histologic growth patterns and cells types often being observed within the same tumor mass. In a recent study of 630 consecutive thymomas, we compared the final histologic classification of the tumors with the number of sections examined per case. It was found that when the number of sections examined per case increased, more cases were included in the “mixed” category, and fewer cases could be assigned to either the pure “cortical” or “medullary” types. These findings suggest that histologic subclassification of thymoma, although of academic interest, may be of limited practical relevance for the assessment of prognosis, particularly in limited biopsy tissue samples.

Staging of thymic epithelial neoplasms also remains a controversial subject. The most popular staging system for these tumors was introduced in 1981 by Masaoka et al, in a study in which statistically significant differences in survival could be appreciated for the different groups of patients depending on the gross and microscopic status of the capsule, spread into adjacent structures, and presence or absence of metastases. Since then, several refinements and modifications to this staging scheme have been introduced by other investigators. A TNM staging system was also introduced by Masaoka in 1991 for thymic carcinoma, but has been felt to be impractical for the well-differentiated variants of thymoma. The most recent staging proposal was presented in the WHO monograph on the Histologic Typing of Tumors of the Thymus (Table VI). We believe until more extensive data becomes available on the discriminatory value for prognosis of these various schemas, a more simplified approach should be favored that basically addresses the distinction between encapsulated (non-invasive), locally invasive, widely invasive, and metastatic tumors.

In summary, classification of thymoma need not be a cumbersome task for general pathologists and can be readily accomplished by most experienced surgical pathologists in clinical practice. Given the relative rarity of these tumors and the questionable role that complex substratification by histologic type plays in prognostication and treatment of these tumors, we favor a simplified approach that combines histologic grading (based on organotypical features of differentiation of the thymus and cytologic atypia) with staging (status of capsular integrity/presence or absence of metastasis). It is our contention that this simplified approach affords an equal opportunity for proper management of these patients as the more sophisticated systems currently in existence, which generally will require review of the case by an expert in the field for proper typing and categorization.

Table VI: Comparison of various staging schemes for thymoma

Masaoka et al (1981)	*WHO (1999)*	*Suster & Moran (1999)*
Stage I: Encapsulated	***Encapsulated***	***Encapsulated***
Stage II: Invasion of capsule and/or perithymic fat	***Minimally invasive***	***Locally invasive***
Stage III: Gross invasion of adjacent organs	***Widely invasive***	***Widely invasive with or without implants***
Stage IVA: Implants	***With implants***	***Metastatic***
Sateg IVB: Metastases	***Lymph node mets***	
	With distant metastases	

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Case 48.

Michele Bisceglia, M.D.

History

During the course of routine work, a lung tumor, which was removed from a 7-year old boy with a previous history of nephroblastoma, was microscopically examined. The diagnosis of metastatic nephroblastoma seemed obvious on morphologic grounds and in keeping with the natural history of this tumor (1-2). However, due to unavailability of the primary tumor (which had been resected at another institution approximately five years earlier), immunohistochemical stains were performed to corroborate the diagnosis. This revealed WT1-negative and TTF-1-positive tumor cell nuclei, an unexpected and seemingly paradoxical result. Nevertheless, all of the clinical and histological parameters were consistent with a pulmonary metastasis of nephroblastoma and therefore the **diagnosis** that was issued was ***TTF-1 positive / WT1 negative Wilms' tumor (lung metastasis)***.

Concomitantly a detailed medical history of the patient was collected, and all the previous histological slides were reviewed and immunohistochemically investigated.

At the age of 2.5 years this NorthEastern African male child, with known history of celiac disease, underwent preoperative chemotherapy followed by right radical nephrectomy for a nephroblastoma (size & weight of the nephrectomy specimen: 380 g and 13x9x8 cm; tumor

size in the upper pole of the kidney: 10x7x5 cm with central necrosis). Histologically the tumor had the typical features of a triphasic nephroblastoma. Approximately 80% of the bulk was necrotic with old and recent hemorrhages, and calcifications. A prominent pseudocapsule was infiltrated, but there was no evidence of renal sinus or vein invasion. The residual viable tumor exhibited variable degrees of microcystic and glomeruloid differentiation, with easily identifiable mitoses in the blastemal compartment.
At the age of 5 (2.5 years after nephrectomy) the patient presented with acute abdominal pain resulting from the rupture of an intraabdominal tumor recurrence. There were also metastases in the anterior abdominal wall (peri-urachal region), ileo-pelvic nodes, and left spermatic cord/paratesticular region. Microscopically the intraabdominal metastases revealed triphasic nephroblastoma with brisk mitotic activity. The patient received high-dose chemo- and radiation therapy with autologous bone marrow rescue.
At the age of 7 pulmonary metastases were detected. The patient was hospitalized in our institution and operated of lung metastasectomy. The right lower pulmonary lobe was removed en bloc with an adherent portion of diaphragm. The pathologic study revealed a 6 cm sized, triphasic (predominantly blastematous), metastatic nephroblastoma, with a modest degree of stromal and epithelial differentiation, from which the histological sections circulated in this seminar were obtained.

The retrospective immunohistochemical analysis which was performed in this case yelded the following findings.

- *Primary* nephroblastoma showed nuclear TTF-1 positivity restricted to a few scattered blastemal elements. There was also strong nuclear WT1 positivity in abortive glomerular structures,
- The *abdominal recurrence* exhibited diffuse nuclear positivity for TTF-1 and WT1 restricted to the blastema.
- The *lung metastasis* - as above said - expressed diffuse strong nuclear reaction for TTF-1 in the blastema. WT1 was negative in the original block, but positive in rare primitive epithelial elements in subsequent blocks.

During the nearly 5-year course of tumor progression in this patient, there was a negative correlation between the expression of TTF-1 and WT1. While the former gradually increased with each relapse, the latter decreased to the point of being exceptionally rare.

Follow-up
Additional chemotherapy was given after pulmonary lobectomy. However, shortly liver metastases developed. The patient died following a gastrointestinal hemorrhage around 5 years after the nephrectomy. No autopsy was performed.

Comment
The reason we initially included TTF-1 in the immunohistochemistry panel we carried out on this case was for completeness sake, in view of the pulmonary location of the mass. Once TTF-1 was detected, four possibilities were considered to explain this unexpected finding: i. the lesion is not a pulmonary metastasis from the nephroblastoma, but a primary lung tumor with a nephroblastoma-like appearance. To the point, TTF-1 is expressed in the epithelial alveolar-like lining of pleuropulmonary blastoma type I (3), an early childhood embryonal neoplasm with a blastemal component that resembles that of nephroblastoma and that in about one quarter of the cases is seen in families with cystic nephroma and other renal tumors (4-5). ii. the TTF-1 positivity is in non-neoplastic lung tissue entrapped within the metastatic nephroblastoma. iii. the positivity in the metastatic nephroblastoma is due to "microenvironmental influences" exerted by the lung parenchyma. iv. nephroblastoma can exhibit TTF-1 immunoreactivity.

In order to assess the frequency of the seemingly incongruent finding of predominantly WT-1 negative / TTF-1 positive Wilms' tumor we undertook a study of the immunophenotypic expression of TTF-1 and WT1 in 47 additional renal Wilms' tumors (collected from 3 tumor pathology centers): of these 47 cases subsequently studied, 7 primary tumors expressed TTF-1 (diffuse in 2, focal in 5), while – as expected - most (i.e., 42/47 → 89.3%) showed nuclear reactivity for WT1.

Finally, both the case in point (index case) and 46 of the 47 total additional cases of Wilms' tumor were also immunostained for CD56, an immunohistochemical marker already tested in Wilms' tumors (6). All tumor tissues of the index case were positive for CD56 (focally in primary, diffusely in the abdominal recurrence and lung metastasis), and 44 of the 46 additional cases, which were so tested, were also CD56 immunopositive (diffusely in 42, focally in 2).

The immunohistochemical profile of the 8 TTF-1 positive Wilms' tumors, including the case presented herein (*Case 13 in the study*), is summarized in Table 1, of which seven primary nephroblastomas exhibited triple (TTF-1, WT1, and CD56) coexpression. Of the 8 total TTF-1 positive cases, seven expressed WT1 immunoreactivity, which was diffuse in 5 and focal in 2, respectively.

Discussion

The case presented herein was the index case leading to the investigation of TTF-1 expression in nephroblastoma, which had not been previously searched for in this tumor and was then published in the literature (7).

Thyroid transcription factor-1 (TTF-1; m.w., 38-40 kDa) belongs to the NKx2 family of homeodomain transcription factors and plays a crucial role in thyroid, lung, and ventral forebrain development. It is expressed in the early stages of thyroid, lung, and diencephalic development and it has been widely applied as a tissue marker for epithelial-derived neoplasms of the lung and thyroid, whether benign or malignant, primary or metastatic, and orthotopic or ectopic (such as thyroid tumors found in thyroglossal duct cysts and struma ovarii). This includes small cell carcinomas and well-differentiated neuroendocrine tumors from these two organs, and large cell neuroendocrine carcinoma of the lung.

Counterpoint to the claims of TTF-1 as a specific marker of lung and thyroid tissue, there is an increasing number of reports documenting its sporadic and in some circumstances frequent expression in tumors from other sites. The majority of these tumors are of neuroendocrine nature, but others belong to a wide variety of cell and tissue types, the latter totalling approximately 77, most of them (49 cases), epithelial, either primary or metastatic as per an updated review of the literature which was performed in 2008 (Table II).

In consequence of data in Table II, it would seem safe to conclude that TTF-1 expression cannot be used to distinguish pulmonary from extrapulmonary small cell carcinoma or large cell neuroendocrine carcinoma; that it can be employed to distinguish small cell carcinoma of the lung (or other extracutaneous sites) from Merkel cell tumor of skin (the latter being consistently TTF1 negative); and that it may help distinguishing pulmonary from gastrointestinal/pancreatic well differentiated neuroendocrine tumors (the latter also being consistently unreactive to TTF-1)**.**

The retrospective documentation of TTF-1 immunoreactivity in the primary tumor presented herein and the immunoreactivity found in 7 out of 47 additional nephroblastomas we subsequently studied makes it clear that, out of the four possibilities we above raised to explain the unexpected finding of TTF-1+ve/ WT1-ve Wilms' tumor, the fourth is the correct one, that is nephroblastoma may express TTF-1. Thus, nephroblastoma is to be

added to the enlarging list of extrathyroid/extrapulmonary tumors that can exhibit TTF-1 reactivity.
This constitutes a potential diagnostic pitfall, particularly when dealing with lung metastases, small biopsy samples, adult-type nephroblastomas, and/or WT1-negative tumors.

In summary, this case lead us to document for the first time the presence of TTF-1 expression in one sixth of nephroblastomas (8 of 48) , and highlight the potential source of misdiagnosis that this finding represents. Its biological significance is unclear. It may reflect the embryonal nature of this tumor, and may conceivably result - directly or indirectly - in interference with the transcriptional control of target genes and other molecular events in the pathway leading to the development of nephroblastoma.

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TABLE I

PERSONAL CASES OF TTF-1 POSITIVE NEPHROBLASTOMAS

(From Ref. 7: data reported in Table II therein)

Case No.	Specimen studied	Immunohistochemical Results		
Case 7	Primary	+	+	+
Case 9	Primary	(+)	+	+
Case 13	**Primary**	**(+)**	**(+)**	**(+)**
	Local recurrence	+	+	+
	Lung metastasis	+	(-/+)	+
Case 16	Primary	+	(+)	-
	Node metastasis	+	-	-
Case 20	Primary	(+)	(+)	(+)
Case 32	Primary	(+)	+	+
Case 43	Primary	(+)	+	+
Case 49	Primary	(+)	+	+

Legends. += diffusely positive; **(+)**= focally positive; **(-/+)**= negative in some blocks, focally positive in others; **-** = negative.

Case 13 was the "index case" of this study and is the case described herein. The histological glass slides, which have been circulated, derive from lung metastasis.

TABLE II
REPORTED TTF-1 IMMUNOSTAINING IN TUMORS OTHER THAN OF PULMONARY OR THYROID ORIGIN

(From Ref. 7: corresponding to Table III therein)

(***updated as of August 2008***)

NEUROENDOCRINE

- Small cell carcinomas of various sites: Gastrointestinal tract [88], prostate [3,16,42, 81,86], bladder [3,4,38,75], liver [18], uterine cervix [3, 12], breast [42,84,93, *****], etc: High percentage of positive cases.
- Large cell neuroendocrine carcinoma of prostate: 1 positive case [42].
- Large cell neuroendocrine carcinoma of uterine cervix: 1 positive case [57].
- Well-differentiated neuroendocrine tumor/carcinoma (carcinoid tumor) of gastrointestinal tract: 2 positive cases [11,42] out of several large series studied [3,11,42,64,72].
- Well-differentiated neuroendocrine tumor/carcinoma of pancreas: No positive cases [3].
- *Merkel cell carcinoma of skin: No positive cases.* [8,10,16,32,42,49,85]
- Paraganglioma/Pheochromocytoma: No positive cases [3,20,64].

- Well differentiated neuroendocrine tumors of thymus: No positive case [64].
- Breast carcinoma with neuroendocrine differentiation (other than small cell carcinoma): conflicting results.†

cont.

cont'd

NON-NEUROENDOCRINE

- Gastric adenocarcinoma: 2 positive cases [6,58].
- Colorectal adenocarcinoma: 10 positive cases [19,58,70].
- Primary peritoneal carcinoma: 1 positive case [5].
- Endocervical adenocarcinoma: 1 positive case [74].
- Endometrial carcinoma: 12 positive cases [6,22,74].
- Ovarian carcinoma: 4 positive cases [2,29,33,46].
- Ovarian small cell carcinoma, hypercalcemic type: No positive cases [56].
- Nasopharyngeal papillary carcinoma: 6 positive cases [14,83,91].
- Synovial sarcoma: 1 positive case (lung metastasis) [53].
- Melanoma (primary and metastatic): 6 positive cases [71].
- Brain tumors (mostly close to 3rd ventricle): 16 positive cases [27,90].
- Pituitary, parathyroid: No positive cases [64].
- Invasive ductal carcinoma of breast (both primary and metastatic): No positive cases. ‡

Legends to symbols

*. Out of around 35 fully reported cases of primary small cell carcinoma of the breast [44,55,93], plus 3 more ones just mentioned by authors reporting the results of their investigations [42,84], 6 were stained for TTF-1 [42,44,55,84,93] and 4 proved positive [42,93].

†. We have found only one study on this subject in the literature, according to which 5 of 5 breast carcinomas with neuroendocrine features proved positive for TTF1 [84]: this is in contrast with the lack of TTF-1 expression in our experience, including 5 so-called carcinoid tumors, 2 neuroendocrine carcinomas NOS, 2 invasive ductal carcinomas with neuroendocrine features, 1 invasive lobular carcinoma with neuroendocrine features, and 4 type B colloid carcinomas (M.B., unpublished data).

‡. This includes a total of more than one hundred cases studied by Kaufman and Dietel [41], Ordonez [67], Medina-Flores et al [58], and Strickland-Marmol et al [77] (59,25,20, and 10 cases, respectively, both primary and metastatic). A similar observation was also noted on 30 invasive ductal carcinomas NOS (20 primary and 10 metastatic to various sites) by one of us (M.B., unpublished data). However, for completeness sake we should mention here the questionable report of TTF-1 positivity in 4 of 5 cases of invasive ductal carcinomas [84], which has been referenced by others [46].

Note: Ref. numbers quoted herein in Table II correspond to references cited in Ref. 7 (Am J Surg Pathol. 2009;33:454-461)

CASE 49

Göran Elmberger, M.D., Ph.D.

Clinical history:

36-year-old never-smoking female with a one-year history of dyspnoea, cough, chest pain and two slowly growing CT/PET verified infiltrates in LLL. Patient was initially treated for suspected pneumonia several times. CT-guided FNA revealed atypical cells of unknown significance. In January 2009 a lobectomy was performed after frozen section reported as suspicious for bronchiolo-alveolar carcinoma (BAC). Previous history includes appendectomy at 11 years, hypothyreosis and a right-sided SOE performed in March 2005 for a 12 cm sized multicystic ovarian mucinous tumor signed out as primary mucinous adenocarcinoma with expansile invasion, stadium IA. In April 2005 a Wertheim hysterectomy including LN dissection and left SOE was performed. A well-differentiated mucinous endocervikal adenocarcinoma infiltrating in inner 1/3 of cervical stroma was found. Maximal length 13 mm and depth 6 mm. 50 LN's without metastasis. Stadium IA. After noting histological resemblance between the gynaecological tumors patient received chemotherapy.

Pathological findings:
On grossing in LLL two tumors measuring 8 and 5 cm's respectively were found.

Frozen section was reported as suggestive of BAC.

Microscopical findings in FFPE tissue from the two tumors were identical. In low-power a lepidic infiltration of a well-differentiated adenocarcinoma was seen. Remnant bronchiolar structures were intact in the inner part of the tumors. No unequivocal signs of infiltration towards parenchyma, bronchioli, vessels or pleura could be seen. In high-power view the neoplastic cells were tall, rectangular with basally localized nuclei and apical mucin vacuoles. A few goblet cells were seen. Common border of apical cytoplasm noted. Aerogenous spread with formation of satellite nodules surrounding the main mass typical of BAC's was prominent. Excessive mucin production with mucus pooling in the surrounding alveolar spaces including a prominent DIP-like macrophage reaction was seen. No metastases in hilar or mediastinal nodes (0/10).

The findings were initially thought to be consistent with a primary pulmonary mucinous BAC but the slightly aberrant cytological detail, the presence of two separate tumors, the clinical data including young age of the patient and the absence of smoking history were not typical. In looking up patient history the presence of previous mucinous gynaecological tumors further heightened the index of suspicion that the lung tumors could possibly represent secondary disease. Review of previous tumors in cervix and ovary showed identical morphology.

Special studies:
Elastin-van Giesson reveal absence of continuous elastin framework within central parts of the BAC-like ADCA.

IHC of pulmonary, endocervikal and ovarian tumors all revealed the same immunophenotype; MNF116+, CK7+, CK20-, CEA+, Vimentin-, ER-, PgR-, TTF-1-, NapsinA-, Surfactant A/B-, CDX2- & p16++. MIB-1 shows surprisingly high – 80 % proliferation rate.

RT-PCR all in 3 sites revealed presence of HPV 18 in similar titres. No attempt at type-specific sequencing was deemed necessary.

Diagnosis: **Late isolated nodular BAC-like lung metastases of a primary endocervikal mucinous adenocarcinoma initially presenting as ovarian metastasis.**

Follow-up:
After diagnosis chemotherapy regimen was changed. Alive without any signs of tumor as per March 2010.

Discussion:
The presence of nearly identical well-differentiated adenocarcinomas within a time frame of 4 years and harbouring the same HR HPV-type is highly suggestive of a primary HPV related endocervikal mucinous adenocarcinoma initially manifesting itself as an ovarian metastases and four years later as multiple lung metastases. However, other interpretations like those postulated by Li et al on the possible HPV transmission from either autoinoculation by genital-oral route or hematogenous spread of from cervix to the lung of HPV virus and the development of a secondary HPV initiated clonally unrelated lung tumor are certainly possible although in my mind less likely.(1) The issue is furthermore complicated by recent preliminary reports on incidence of HPV in primary lung carcinomas as high as 24.5 %.

Metastatic carcinoma to lung:
Pulmonary metastases are being found at necropsy in 30-50 % of patients dying from cancer. Common extrathoracic tumors can be ranked either by the propensity of these tumors to metastasize to the lung or by the likelihood of a pulmonary metastasis having originated in a particular site (Box 12.6.1; Corrin)

Box 12.6.1 Primary tumour sites

Primary tumour sites ranked in descending order of frequency according to the propensity of their tumours to metastasise to the lungs (left) and the likelihood of a pulmonary metastasis having originated in a particular site (right). The difference between the two columns is largely accounted for by differences in the frequency of cancer developing in these sites: thus tumours of the kidney frequently metastasise to the lungs but are not as common as tumours of the breast, colon, pancreas, stomach or skin. Other factors include selective attachment of tumour cells to endothelium based on particular cell surface components.

1 Kidney	1 Breast
2 Skin	2 Colon
3 Breast	3 Pancreas
4 Thyroid	4 Stomach
5 Pancreas	5 Skin
6 Prostate	6 Kidney
7 Stomach	7 Ovary
8 Uterus	8 Prostate
9 Colon	9 Uterus

Pathogenesis
Metastatic deposits in the lung can result from blood-borne tumor emboli, lymphangitic spread, intra-alveolar/endobronchial spread or by direct tumor extension. The lepidic growth pattern seen in our present case is a variant of intra-alveolar spread in which the tumor cells grow along the surfaces of alveolar walls. The tumor thus replaces the alveolar epithelium usually in a layer no more than one cell thick. Nether the alveolar spaces or walls are obliterated. This is the growth pattern seen in BAC's and AAH but is also often adopted by

other subtypes of pulmonary adenocarcinomas and many metastatic tumors either epithelial or non-epithelial.

Metastatic patterns:
Various clinico-radiological-pathological patterns of metastatic growth can be seen in the lung. (Box 12.6.2; Corrin)

Box 12.6.2 Patterns of secondary tumour growth in the lungs

- Blood-borne metastases
  - Solitary nodule
  - Multiple nodules
  - Massive tumour embolism
  - Microangiopathy
- Diffuse lymphatic permeation (lymphangitis carcinomatosa)
- Endobronchial metastases
- Intra-alveolar spread
  - Diffuse airspace
  - Lepidic growth (bronchioloalveolar cell carcinoma pattern)
- Interstitial growth

Our case showed two areas of BAC-like growth pattern thus simulating primary lung BAC.

IHC:
In daily praxis IHC is the mainstay for differentiation of primary and secondary tumors in the lung. At Karolinska we regularly perform a limited IHC panel including pan CK, CK7, CK20, TTF1, Napsin A in non-squamous - NSCLC to verify tumor origin. Panel is frequently expanded depending on sex, epidemiological situation, previous tumor history and histopathological impression. However, IHC for tumor origin analyses is not the only task nowadays. We also need IHC in poorly differentiated NSCLC to indicate squamous versus adeno-differentiation, verifying NEC's and for predictive test regarding EGFR, KRAS, EML4-ALK inv, RCC1, RMM1 & TS status.

Molecular techniques:
Molecular pathology utilizing test like cytogenetics, CGH, FISH, gene expression, SNP and microsatellite fractional analysis have all been described as useful in penetrating relations between synchronous and metachronous multiple tumors.

Viral analyses:
Analyses of HPV and EBV status has been utilized in distinguishing primarily metastases originating in UADT and GYN from primary pulmonary carcinomas. Generally we use screening with p16 IHC, ISH and RT-PCR techniques for this purpose. Obviously the type specific and quantitative reporting from both pulmonary tumor and possible extra-pulmonary primary tumor strengthens the value of the analyses.

EM:
Is useful in assigning broad categories of histogenesis such as carcinoma, mesothelioma, melanoma and sarcoma. On few occasions EM can highlight primary source of carcinoma with help of marker organelles such as surfactant lamellar bodies.

Lung metastasis from cervical carcinoma:

Endocervikal adenocarcinoma accounts for up to 25% of cervical cancer and its incidence rate has steadily increased over the last few decades. Patients with adenocarcinoma of the cervix generally have a worse prognosis than those with squamous cell carcinoma of similar stage and tumour size. Thoracic metastases from squamous carcinoma and adenocarcinoma of the cervix were studied and the incidence of chest metastases was higher for adenocarcinoma (20%) than for squamous carcinoma (4%).(2) Few patients diagnosed in the early stages of squamous carcinoma developed metastases; for adenocarcinoma, chest metastases occurred regardless of the stage at diagnosis. For both histologies, parenchymal (often cavitary) nodules only were seen; lymphangitic pattern was not observed. Both adenopathy and malignant effusion are common (44% of metastases) in thoracic metastasis from squamous carcinoma. Adenopathy but not effusion was common in adenocarcinoma. The lung may be the only site of metastatic cervical carcinoma in 12 %(3;4) to 25 %.(5) Multiple nodules are usual, but in 30 % of the cases they may be solitary. Other patterns of metastasis such as "cannonball", cavitation, endobronchial and lymphangitic spread have all been described. At times hilar and mediastinal LN's or malignant pleural effusion are the only evidence of metastasis.

Lung metastases, with or without other sites of recurrence, from uterine neoplasms after a long disease-free interval are rarely reported.(6) There is a case report of clear cell adenocarcinoma of the cervix that recurred in the lung and cerebellum after a prolonged disease-free interval (17 years) in a woman with a known maternal history of exposure to diethylstilbestrol in uteri.(7) To distinguish metastatic endocervikal adenocarcinoma in the lung from metastatic adenocarcinoma of endocervikal, endometrial or other origin and lung primary adenocarcinoma, immunohistochemical and molecular studies are useful. Endocervikal adenocarcinomas are typically characterised by positivity for carcinoembryonic antigen (often with diffuse membranous and cytoplasmic staining), CK7, EMA, and p16, and by negativity for ER (focally positive), vimentin, CK20, CD10 (except mesonephric subtype), and p63.(8-14) Human papillomavirus (HPV), which is believed to be a necessary cause of cervical cancer, especially types 16 and 18 for endocervikal adenocarcinoma, can be specifically detected by PCR amplification in both primary(15;16), and metastatic(17) tumours. These HPV infections lead to increased p16 expression. Our Case represents a metastatic endocervical adenocarcinoma to the lung with long interval (4 years after hysterectomy). Repeated pulmonary resections have been reported leading to disease-free conditions in long periods.(6)

Lung cancer and HPV:

The presence of human papilloma virus (HPV) in lung carcinoma has been recently reported and reviewed.(18) The reports highlight HPV presence in lung cancer samples and indicate its potential role in lung carcinogenesis. The overall incidence of HPV in lung cancer has been reported to be as high as 24.5%, which essentially renders HPV the second commonest risk factor for lung cancer if the cause–effect link is indeed valid. Interestingly, there is geographic variation with higher mean incidence rates reported in Asia (35.7%) compared to Europe (17%) and America (15%). This variation has been attributed to the different detection methods used and possibly to the epidemiology of the HPV itself, although information on HPV prevalence worldwide is absent. The main questions therefore that need to be answered are: what is the route of transmission of HPV to lung tissue and how
can it be potentially tumorigenic? It is known that HPV normally invades healthy tissue by direct mucosal contact and it is postulated that it reaches the lung site via blood circulation, while the possibility of transmission from the cervix to the oral cavity and then to the larynx and lung is also plausible. Once it presents to the host cells it is believed that it attaches to

those cells expressing heparin sulphates, that act as primary receptors for HPV and it is internalised to interfere with p53 and Rb proteins. This is achieved by E6 proteins, encoded by HPV, binding to the host cellular tumour suppressor proteins and triggering its degradation through the ubiquitin pathway. Contrary to this theory is evidence of HPV in normal lung tissue of patients with HPV positive lung cancer and the question that arises is whether HPV is easily integrated to tumour genome than healthy cells and therefore is an epiphenomenon rather than the cause of the tumour. This remains to be answered. However, if the cause–effect link is true then molecular HPV typing could potentially be used as a marker of lung cancer as well as to discriminate primary from metastatic squamous cell carcinoma. Kountouri et al stated that it could be safely concluded that evidence for a causative link between HPV and lung cancer is mounting but the jury is still out.(19) Conclusions from a recent met analysis on HPV and lung cancer were that further studies are needed to elucidate the role of HPV in lung carcinogenesis with careful thought given to study design and laboratory detection methods for a more accurate assessment of HPV status in lung tumors.(20)

In another study the molecular subtyping of HPV was performed in squamous cell carcinomas of the lung (n=26) as well as putative primaries of head and neck (n=21) and female genital tract (n=5) of the same patients, to test whether additional information to discriminate lung primaries from metastases can be gained by a direct comparison of the HPV status in both tumors. In 3 (14.2%) patients with head and neck as well as lung squamous cell carcinoma, an identical HPV subtype could be detected in both tumors suggesting metastatic disease. In 9 (42.9%) cases, discordant HPV status strongly suggested secondary primaries of the lung. In the remaining 9 (42.9%) patients, no HPV was evident in either tumor. In all patients with carcinomas of the cervix uteri an identical HPV subtype was detected in the cervical and in the lung tumor. In conclusion, the results suggest HPV typing, a method routinely used in cervical biopsies for years, as a very useful diagnostic tool to discriminate primary from metastatic squamous cell carcinoma of the lung, which in our cohort in 57.1% of cases allowed for almost definite classification.(21)

Human papilloma virus (HPV) typing and Comparative Genomic Hybridisation (CGH) analysis has also been reported useful in the classification of multiple tumours of the upper aerodigestive tract for the differentiation between secondary malignancy versus metastasis. (22) These techniques probably could be equally adapted to use in multiple cervical – pulmonary tumors.

Challenges in recognizing pulmonary metastasis:
Recognizing pulmonary metastasis is of great clinical importance but can sometimes be exceedingly difficult. The fact that pulmonary metastases are much more common than pulmonary primary tumors should lead to a high level of suspicion in signing out cases with new pulmonary tumors. Besides doing screening IHC panels on all new cases of NSCLC it is of paramount importance to check patient medical records directly. Too often the clinicians forget to mention important history in their requisition sheet. Fortunately at the Karolinska we see all new cases in multidisciplinary tumor boards before clinical decisions regarding therapy are taken. Our present case is a good example of factors 1,2,4 and 5 in table below. Obviously our routine IHC would register negative findings in lung specific IHC but that occurs in 20-30 % of primary lung ADCA and even more so in primary lung mucinous neoplasms. Absence of ER staining and CEA positivity would not be unusual for lung cancer. Only high index of suspicion and knowledge of the patients previous GYN history were helpful in correctly diagnosing present case.

Challenges in Recognizing Pulmonary Metastasis
1. Unusual clinical presentation
2. Long interval between occurrence of primary and metastasis
3. No known primary neoplasm
4. Misdiagnosis on previous surgical specimen
5. Resemblance to lung primary
6. Change in phenotype from primary due to progression or therapy

Metastatic mucinous carcinomas to the ovary:
The distinction between primary and metastatic carcinomas of the ovary is a common and difficult topic. Tumors that have a mucinous cell type offer a particular challenge, and because of this descriptions of "primary" mucinous ovarian carcinomas in the earlier literature are likely to have contained some tumors that were actually metastatic. Mucinous metastases in the ovary usually derives from colo-rectum, pancreatico-biliary tract, appendix or stomach. Uncommon sources of mucinous metastases to the ovary are breast, lung, endocervix, salivary gland and skin. In a recent report 77 % of mucinous ovarian carcinomas were metastatic.(23) Gross and histopathological findings that supports metastasis are: 1) bilaterality, 2) microscopic surface involvement by epithelial cells (surface implants), and 3) an infiltrative pattern of stromal invasion. Findings that were less frequent but present exclusively or almost exclusively in metastatic carcinomas were: 1) a nodular invasive pattern, 2) ovarian hilar involvement, 3) single cell invasion, 4) signet-ring cells, 5) vascular invasion, and 6) microscopic surface mucin.(24)

TABLE 2. *Summary of helpful comparative gross and routine microscopic features of primary and metastatic mucinous cystic ovarian tumors (caveats are listed at bottom)*

Features	Primary	Metastatic
History of extra-ovarian primary mucinous adenocarcinoma (e.g., colon, pancreas, gallbladder, stomach, appendix, uterine cervix, urachus, esophagus)	Rare	Usual*
Deposits of mucinous adenocarcinoma outside the ovary such that ovarian neoplasia, if primary would be stage II or higher	Uncommon	Usual
Bilateral tumor	Uncommon	Common
Size over 15 cm	Common	Uncommon
Gross surface deposits of tumor	Rare	Occasional
Associated with teratoma, Brenner tumor, or adenofibroma	Occasional	Rare†
Associated with endometriotic cyst or endometriosis	Occasional‡	Rare†
Multiple discrete nodules on gross evaluation§	Rare	Occasional
So-called mural nodules with unusual and particular microscopic appearances‖	Uncommon	Rare (not seen to date)
Surface implants on microscopic examination	Rare	Common
Surface mucin	Rare	Occasional
Nodular growth with nodules often differing in pattern	Rare	Common
Hilar involvement	Rare	Occasional
Benign or borderline-appearing areas	Common	Occasional
Lymphatic or blood-vascular invasion	Rare	Occasional
Single cell growth or signet-ring cells	Rare	Occasional
Resemblance to mullerian (endocervical-like) carcinoma	Occasional	Not seen to date (see text)

* Extra-ovarian primary tumors may be discovered intraoperatively or in a minority of the cases postoperatively.
† Metastatic tumors associated with teratoma, Brenner tumor, adenofibroma, or endometriosis could occur as a chance collision phenomenon.
‡ Primary tumors associated with endometriosis are usually of mullerian (endocervical) cell type and unilocular.
§ Nodules in these instances are composed of conventional adenocarcinoma.
‖ Nodules in these instances are composed of reactive cells (predominantly), anaplastic carcinoma cells (predominantly), or sarcoma.

Endocervical adenocarcinomas occasionally metastasize to the where they can mimic a primary mucinous or endometrioid ovarian carcinoma.(25;26) Positive staining for EA and positive strong nuclear and cytoplasmic staining of p16 in > 75%of the tumor cells is compatible with metastasis from cervix.(27) CEA can be weak or absent in mucinous endocervical carcinoma. P16 has been reported to stain a substantial fraction of primary mucinous and endometrioid ovarian carcinomas but in theses cases HPV DNA could not be found.(28)

Take home messages

When pulmonary nodules are found in female patients who have undergone a hysterectomy, even many years ago, obtaining a detailed history of gynecological diseases and considering late recurrence as one of the differential diagnoses is important.

Although unusual radiographic features of pulmonary metastases, such as BAC-like growth, solitary mass, cavitation, calcification, haemorrhage around the tumour and pneumothorax, are often encountered, radiologists could play a significant role in separating metastatic tumours from primary tumours or benign conditions.

Pathologists also need to carefully examine biopsied or resected specimens with a high index of suspicion.

Immunohistochemical analyses are the mainstay in our approach and use of a standard panel is recommended. However in some occasions as our present case IHC is not very helpful.

Viral and molecular analysis can offer help in more complicated situations.

Comparison with previous tumor material should always be a high priority.

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CASE 50

Hugo Dominguez-Malagon, M.D.

An 18 year-old female was seen in another hospital for a nodule in her buccal mucosa, the tumor was excised but the slides and report are not available. One year later the tumor recurred and was re-excised with margins.
Pathological findings. The neoplasia has a complex appearance, There are zones of hyaline fibrosis forming bands or nodules, cystic ductal structures lined by polygonal cells with ample eosinophylic cytoplasm and decapitation secretion of apocrine type, and some are arranged in cribriform structures, there are acinar structures lined by polygonal cells with ample cytoplasm containing large brightly eosinophylic granules. In some places there are ductal and lobular structures with atypical epithelial cells forming papillary or cribriform structures. In few of these ducts the cells are arranged in solid structures with comedo-type necrosis.

Immunohistochemistry: The luminal cells are positive for CK7, and CK34βE12. Basal cells positive for CK14 and S100, focally for Calponin
Electron Microscopy: The acinar cells are polygonal, showing parallel membranes with junctions, many of them contain many large electron-dense granules with homogeneous appearance. Nuclei are ovoid and have fine chromatin and inconspicuous nucleolus.

DIAGNOSIS: SCLEROSING POLYCYSTIC ADENOSIS OF MINOR SALIVARY GLANDS, WITH HIGH GRADE INTRADUCTAL CARCINOMA.

Discussion: Sclerosing Polycystic Adenosis (SPA) is a recently characterized entity described by Smith in 1996, and its nature is still controversial, it resembles fibrocystic disease and sclerosing adenosis of the breast. It is a rare entity with about 45 cases described to date, predominantly affects major salivary glands, and occasionally the minor glands of the oral mucosa.
The age of presentation ranges from 9 to 80 years, It is more frequent in female patients but it varies in different series. Patients manifest a slow-growing tumor, with occasional tenderness or pain. Grossly it is a well delimited, non encapsulated firm nodule with a lobulated surface. The stroma is abundant with areas of fibrosis and chronic inflammation. There are dilated ductal structures lined by polygonal or flattened cells frequently arranged in a cribriform pattern. Occasionally there are closely packed ductal structures resembling sclerosing adenosis, hyperplasia of ductal and acinar structures with apocrine metaplasia are commonly seen. Brightly intracytoplasmic eosinophylic zymogen granules are seen in tubular and acinar cells.
In some cases there is ductal and acinar atypia of moderate degree, but the lobular architecture is preserved. Local recurrences occur in 19% of cases, and in some of these, low-grade in-situ carcinoma has been diagnosed. In recent publications, using the polymorphism of the human androgen receptor (HUMARA) a clonal nature of the lesion has been demonstrated. However, the monoclonality of a lesion not necessarily proves it to be neoplastic. Up to date, none of the cases, including the recurring ones have produced metastases.
The present case illustrates a case of a recurring SPA with intraductal lesion with histological characteristics of a high-grade intraductal carcinoma with comedo-type necrosis. The study of large number of cases with long follow-up is necessary to assess the malignant potential of the lesion.

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CASE 51

Saul Suster, M.D.

Clinical History:
A 53 year old man was seen for progressive chest pain and dyspnea. A chest X-ray showed widening of the mediastinum. A CT scan showed a well-circumscribed mass localized in the anterior mediastinum that measured 10x8x6 cm. There was no evidence of infiltration into the surrounding structures. A complete surgical excision was done.

Pathologic Findings:
The tumor was grossly well-circumscribed and completely encapsulated. On cut surface it showed a fleshy appearance with large lobules separated by thin bands of fibrous tissue. On histologic examination, the tumor had a biphasic appearance with islands of large epithelioid, cohesive tumor cells separated by broad expanses of cellular stroma containing a dense fascicular proliferation of spindle cells with focal storiform pattern. The spindle cells in the intervening stroma showed features of fibroblasts and were devoid of cytologic atypia or mitotic activity. The islands of epithelial cells showed foci displaying marked nuclear pleomorphism with bizarre nuclei, prominent nucleoli, and rare mitotic figures. There was no evidence of necrosis or vascular invasion.

Special studies:
Immunohistochemical studies showed strong positivity in the islands of epithelioid cells for cytokeratin AE1/AE3, CK19 and p63. The spindle cells in the stroma stained positive for vimentin and focally for SMA. Some of the spindle cells also showed weak membranous positivity for EMA. Stains for CD3, CD1a and CD99 highlighted a few scattered immature small lymphocytes admixed with the epithelial cells.
Electron microscopy from the islands of epithelioid cells showed round to polygonal cells with abundant cytoplasm, intercellular junctions, and abundant intracytoplasmic tonofilaments. The spindle cells in the stroma showed elongated nuclei with scant cytoplasmic organelles and absence of intercellular junctions.

Diagnosis: THYMOMA WITH PSEUDOSARCOMATOUS STROMA ("METAPLASTIC" THYMOMA).

Comment:
Thymoma with pseudosarcomatous stroma represents a rare entity described by Suster et al in 1997. Out of the 6 cases reported, four had been initially misinterpreted as thymic carcinosarcoma. The tumors were all discovered incidentally on chest X-rays and ranged in size from 6-14 cm. in greatest diameter. All the tumors were well-circumscribed and encapsulated, without evidence of infiltration through the capsule. All patients were treated by simple surgical excision. Clinical follow-up from 5-20 years (average FU= 10 years) showed that all the patients were alive and well without evidence of recurrence or metastases. The tumor was interpreted as a rare morphologic variant of thymoma with an unusually cellular spindle cell stroma that simulated a carcinosarcoma.

Five similar cases were subsequently published by Yoneda et al 2 years later. The tumors were equally well-circumscribed and encapsulated and on limited follow-up did not display any features of malignancy or aggressive behavior. The authors also noted focal EMA positivity in the stromal spindle cells, and on the basis of that finding postulated that the

spindle cell component was derived from the epithelial component by a mechanism of mesenchymal metaplasia. They also believed that the tumors were malignant and designated them "metaplastic thymic carcinoma".

More recently, the WHO fascicle on Tumors of the Lung, Pleura, Thymus and Heart have acknowledged that these tumors are, for the most part, very low-grade lesions that in the majority of instances behave in a benign fashion and are adequately cured by simple surgical excision. They have proposed the designation of "metaplastic thymoma" for these lesions and abandoned the claim that they represent a form of thymic carcinoma. Two case reports have appeared in the recent literature, however, that document examples of this tumor that developed metastases or underwent malignant transformation.

The tumor is quite distinctive and easy to diagnose on H&E once one is familiar with its features. The differential diagnosis involves thymic carcinosarcoma or a metastasis from a biphasic malignant neoplasm from another organ, such as a pulmonary blastoma or pulmonary carcinosarcoma. The key to the correct diagnosis is the bland appearance of the spindle cells which are completely devoid of cytologic atypia. The cytologic atypia often observed in the epithelial elements appears to be degenerative and does not seem to influence the prognosis in these tumors.

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CASE 52

Eduardo Zambrano, M.D.

Clinical history: 15 year-old male with left-sided submandibular mass of approximately 3 months duration. The patient reported waxing and waning pain, and intermittent swelling and regression of the mass, but denied fever, otalgia, numbness, or change in voice. No evidence of cystic degeneration, hemorrhage or invasion was found on ultrasound. The tumor was removed and left neck dissection was subsequently performed. Postoperatively, the patient received radiation therapy to 66 Gy in 33 fractions, and remained free of disease at last follow up, 14 months after his initial diagnosis.

Pathologic findings: The specimen measured 4.5 x 2.7 x 2.4 cm. On cut section, a tan-white, rubbery, well-circumscribed lesion (2.5 x 2.3 x 1.8 cm) was observed. Histologically, the tumor exhibited areas of solid, trabecular and cord-like growth patterns with infiltrative margins. Perineural and perivascular invasion were noted. Tumors cells were predominantly undifferentiated and featured enlarged nuclei with vesicular chromatin and prominent nucleoli. Increased mitotic activity with atypical mitoses and areas of necrosis were present. Foci of abrupt keratinization and squamous differentiation including intercellular bridges, individual cell keratinization and squamous eddies were noted. Areas of cystic change,

cholesterol granulomas and psammomatoid concretions were also focally identified. Left neck lymph nodes dissection revealed one out of 33 lymph nodes being positive for metastatic tumor. Variable reactivity with cytokeratin AE1/AE3, CAM 5.2, p63, and p16, and focal positivity for S-100 and CD117 were noted. Histochemical stains for mucicarmine and DPAS, and immunohistochemical stains for calponin, smooth muscle actin, chromogranin, synaptophysin, CD34 and CD56 were negative. EBER in situ hybridization was negative. Ki67 staining showed a proliferation rate of over 50%. Fluorescent in situ hybridization revealed all tumor cells harboring rearrangement of *NUT* as evidenced by split apart of one allele, without rearrangement of *BRD3* or *BRD4*, consistent with a *NUT*-variant carcinoma.

Diagnosis: *NUT*-ASSOCIATED POORLY-DIFFERENTIATED CARCINOMA OF THE SUBMANDIBULAR GLAND.

Discussion: Pediatric salivary gland tumors are exceedingly rare, with mucoepidermoid carcinomas comprising approximately 80% of salivary gland malignancies in children and adolescents. Acinic cell carcinoma, adenocarcinoma, adenoid cystic carcinoma, metastases and poorly-differentiated carcinoma NOS comprise the remaining minority of malignant tumors in the pediatric age group. Poorly-differentiated carcinomas of the salivary gland present diagnostic challenges, frequently difficult to resolve by immunohistochemistry, and usually portend poor prognosis. Although mucoepidermoid and lymphoepithelial carcinomas were initially considered in the differential diagnosis of this case, the absence of mucin and of a dense lymphocytic infiltrate with negative EBER in situ hybridization, excluded both possibilities. Features in this case including high-grade anaplasia with high mitotic rate, necrosis and focal squamoid differentiation suggest a poorly-differentiated squamous cell carcinoma. Subsequent molecular studies for *NUT* and *BRD3/BRD4* gene rearrangements revealed a positive signal involving the *NUT* gene with negative *BRD3/BRD4* signals, classifying this salivary gland tumor as a ‘‘*NUT*-variant’’ carcinoma.

NUT-associated midline carcinomas (NMC) are rare, poorly-differentiated, highly-aggressive tumors that uniformly involve midline structures, typically of the head and neck as well as mediastinum. Although the exact frequency is unknown, such tumors have been reported in all age groups with most studies identifying them predominantly in adolescents and young adults, with an average age of 25 years at presentation (age range: 3 to 78 years). A recent study, however, showed NMC made up 18% of poorly differentiated carcinomas of the upper aerodigestive tract in all age groups with a median patient age of 47 years, suggesting a more uniform age distribution for NMC. Males and females are approximately equally affected (M:F, 1:1.3). This group of tumors is defined by rearrangements of the nuclear protein in testis (*NUT*) gene on chromosome 15q14. The majority of these cancers harbor the *BRD4-NUT* fusion oncogene resulting from a t(15;19) translocation, and the remaining cases harbor *NUT*-variant fusions. While these tumors affect the head and neck region in 50% of cases, only two cases have been previously reported involving a salivary gland.

The *BRD4-NUT* rearrangement defines a distinct class of tumors with characteristic highly aggressive behavior with median survival time of 28 weeks. *NUT*-variant carcinomas demonstrate a longer median survival time of 96 weeks but still carry poor prognosis. The causes of the translocation remain unknown and the functions of BRD4 and NUT proteins remain only partially understood; however, the *BRD4-NUT* rearrangement is thought to represent a tumor-initiating event since all tumor karyotypes to date have been simple. The BRD4 protein has been found to bind chromatin during mitosis, and is thought to serve as a marker of active transcription, binding genes undergoing transcription immediately prior to mitosis to mark them for resumption following completion of cell division. The normal function of NUT protein is poorly understood; however, it is hypothesized that the BRD

moiety tethers NUT to chromatin affecting transcription. siRNA knockout studies against the BRD4-NUT fusion protein have shown induction of dramatic and irreversible squamous differentiation and arrested growth suggesting BRD4-NUT fusion protein blocks differentiation by binding to chromatin.

Since poorly differentiated tumors, including *BRD4-NUT* positive and *NUT*-variant carcinomas, lack distinguishing histological or immunohistochemical features, any poorly differentiated midline neoplasm or head and neck tumor that does not exhibit lineage specific differentiation markers (except squamous) should be considered for *NUT* rearrangement testing in order to provide accurate diagnosis, prognosis and treatment regimens.

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CASE 53

Saul Suster, M.D.

Clinical History:

A 28 year old woman was seen for shortness of breath and chest pain. A CT scan showed a large anterior mediastinal mass. At surgery, the mass was 12 x 10 x 8 cm, well-circumscribed and encapsulated, and on cut surface showed areas of hemorrhage and necrosis. There was no history or evidence of tumor elsewhere.

Pathologic Findings:

The tumor was grossly well-circumscribed and encapsulated and showed a homogeneous, bosselated yellow-gray cut surface. Histologic examination showed sheets of large, polygonal tumor cells with round, vesicular nuclei surrounded by abundant eosinophilic cytoplasm. In areas the tumor showed a trabecular arrangement that simulated a sinusoidal pattern. Other areas has scattered empty spaces containing mature adipocytes that closely resembled liver parenchyma. Towards the periphery of the tumor, focal areas displaying small spindle cells with hyperchromatic nuclei could also be identified. Discrete areas containing sheets of adipocytes with occasional scattered signet-ring lipoblasts could be identified throughout the tumor. No mitotic activity, invasion or foci of necrosis were found.

Special Studies:

Immunohistochemical stains showed strong positivity of the tumor cells for vimentin, but negative reaction with cytokeratins (AE1/AE3, CK7, CK20, CAM 5.2), EMA, CEA, AFP, PLAP, CD10, inhibin, renal cell carcinoma antigen, D-2-40, C-Kit, desmin, SMA, Myo-D1, Myogenin, chromogranin, CD31, CD34, S-100, HMB45 and Hepar-1 antibodies. A Ki-67 proliferation marker showed ~10% of nuclear staining in the tumor cells. An immunoperoxidase stain for MDM2 showed nuclear positivity in many of the tumor cells. Fluorescent in-situ hybridization was carried out for MDM2 amplification at 12q15. The tumor cells showed high-level amplification of the MDM2 locus.

Diagnosis: EPITHELIOID PLEOMORPHIC LIPOSARCOMA OF THE MEDIASTINUM.

Comment:
The epithelioid variant of liposarcoma is a rare tumor that was first described by Drs. Miettinen and Enzinger in 1999 from the AFIP. The authors reported 12 patients with a distinctive variant of liposarcoma that showed epithelioid features and focally resembled a solid carcinoma. The patients were all adults with a female predilection, and the tumors were located in the axilla, back, chest wall, thigh, groin, retroperitoneum, chest wall and pleura. They ranged in size from 3.5 to 17 cm. in greatest diameter and were composed of firm, multinodular masses with areas of hemorrhage and necrosis. On clinical follow-up, 5/10 patients died of tumor within 1 year, 2/10 patients died of unrelated cause, and 1 patient was alive and well after 24 years following a leg amputation.

Epithelioid liposarcoma is characterized immunohistochemically by uniform vimentin positivity. About 50% of cases may show focal S-100 protein and cytokeratin positivity. All other specific differentiation markers are usually negative. Liposarcomas of the mediastinum are relatively rare tumors that are most commonly observed in adults involving the posterior mediastinum. The tumors are often large and show frequent recurrences; the most frequent histologic type is well-differentiated liposarcoma (atypical lipomatous tumor). Pleomorphic and dedifferentiated liposarcomas have also been described in the anterior mediastinum, but there is only one case reported previously in the literature of an epithelioid liposarcoma by Huang and Antonescu from Sloan-Kettering Memorial Hospital in New York.

The differential diagnosis for this tumor in the anterior mediastinal location involves mainly metastases from other tumors that can adopt similar histologic features, including hepatocellular carcinoma, renal cell carcinoma, adrenal carcinoma, epithelioid sarcoma, epithelioid hemangioendothelioma, GIST, paraganglioma, melanoma, and the hepatoid variant of yolk sac tumor. Immunohistochemical stain will suffice in most instances to rule out the latter possibilities. Confusion may occur in cases in which S-100 protein or cytokeratin are positive in the tumor cells of epithelioid liposarcoma. A high index of suspicion must be held in such cases and a search for areas suggestive of well-differentiated or pleomorphic liposarcoma should be undertaken through extensive sampling.

Recent studies have demonstrated that certain types of liposarcoma are characterized at the genetic level by a specific translocation involving chromosomes 12;15 due to amplification of the MDM2 and CDK4 genes. Amplification of these genes by FISH or detection by immunohistochemical techniques with monoclonal antibodies can serve to identify the translocation and serve as an aid in the diagnosis. It should be kept in mind however, that immunohistochemical staining for MDM2 is not exclusive for liposarcoma and has been observed in a variety of other types of sarcomas. Attention to the histologic features and clinicopathologic context of the lesion is therefore important for a definitive diagnosis.

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CASE 54

Saul Suster, M.D.

Clinical History:
A 48 year old man was seen for a large anterior mediastinal mass. No tumor could be identified elsewhere on thorough clinical and radiographic exams. The resected tumor measured 15x12x10 cm and as focally attached to the pleura and pericardium, but unassociated with the bronchus or other structures in the vicinity. A complete surgical excision was carried out.

Pathologic Findings:
The cut surface of the specimen showed a glistening, mucinous appearance with a few focal areas of hemorrhage and necrosis. Histologic examination showed a proliferation of glandular structures that were lined by atypical epithelial cells. The cells contained enlarged nuclei with prominent nucleoli and scattered mitotic figures. Many of the cells showed distention of the cytoplasm by intracellular mucin, and some displayed a signet-ring cell configuration with mucin vacuoles displacing the nuclei toward the periphery of the cell. In some areas, the tumor exhibited large pools and lakes of mucin containing a few individual cells or isolated small glands floating in the mucin.

Special Studies:
Immunohistochemical stains confirmed the epithelial nature of the tumor cells and showed strong positivity of the tumor cells for cytokeratins (AE1/AE3, CK19, CK20) and for MOC31. A stain for MDX2 showed strong nuclear positivity in the majority of the tumor cells. Stains for AFP, PLAP, CD117, CK7, TTF1, chromogranin, synaptophysin, ER and PR were negative. Mucicarmine and PAS stains showed extensive positivity both intracytoplasmic and in the extracellular mucin.

Diagnosis: PRIMARY MUCNOUS ADENOCARCINOMA OF THE THYMUS.

Comment:
The initial diagnosis rendered in this case was that of “Mucinous adenocarcinoma; must rule out metastasis from the gastrointestinal tract or other source”. Following the operation, the

patient was extensively investigated with chest and abdomen CT scans, barium swallow, barium enema, multiple endoscopies, liver and abdominal ultrasound, PET scan and cystoscopy, all of which were normal. The diagnosis was then revised to primary mucinous adenocarcinoma of the thymus.

Thymic carcinoma is a rare entity with less than 200 cases reported to date. The tumor is defined as a primary malignant thymic epithelial neoplasm displaying obvious histologic features of malignancy with total absence of any of the organotypical features of thymic differentiation. A large number of histologic variants have been described, including squamous, clear cell, papillary, basaloid, lymphoepithelioma-like, mucoepidermoid, small cell neuroendocrine and anaplastic carcinoma. Primary adenocarcinoma of the thymus, however, is extremely rare and the recent literature documents approximately 14 cases, mostly as case reports.

Primary adenocarcinoma of the thymus comes in two types: conventional and mucinous (colloid carcinoma). The tumors are characterized by an atypical glandular proliferation of intestinal or biliary type. The non-mucinous types behave as low-grade carcinomas and are often associated with cystic changes in the thymus. The mucinous types are more aggressive tumors associated with earlier dissemination and distant metastases. The main differential diagnosis is with metastatic adenocarcinoma, embryonal carcinoma and yolk sac tumor of the mediastinum, and a primary mucinous neuroendocrine carcinoma of the thymus. The latter entity, in particular, can look virtually indistinguishable from the present case and the only way to separate them is by demonstrating immunoreactivity for neuroendocrine markers in the tumor cells.

The most important tool for the pathologist in the diagnosis of mucinous carcinoma of the thymus is the clinical history. Thymic carcinoma, in general, represents a diagnosis of exclusion because there are presently no specific or pathognomonic markers or cytogenetic abnormalities that will permit separating these tumors from metastases. The use of CD5 and C-kit stains can be helpful in some instances, but even these stains are not exclusive or distinctive for thymic carcinoma and can be positive in lung cancers and other conditions capable of producing metastases to the mediastinum. The pathologist should therefore be very careful not to issue a definitive diagnosis of thymic carcinoma unless supportive clinical information and evidence from radiologic studies conclusively demonstrate that there is no tumor elsewhere.

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