ARKADI M RYWLIN INTERNATIONAL PATHOLOGY SLIDE SEMINAR CLUB

XXV CONGRESS of the INTERNATIONAL ACADEMY OF PATHOLOGY

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SUMMARY OF CASES

Case 1 (Dr. Michele Bisceglia)
Female 9, left nephrectomy for progressively enlarging multicystic kidney since birth, complicated by urinary tract infections and microhaematuria. Normal liver, pancreas and spleen. Unremarkable family history.

Case 2 (Dr John Chan)
Female 39, presented with high fever, right lower zone haziness on chest X-ray and lymphopenia. Rapid progression into respiratory failure and death within 2 days.

Case 3 (Dr Thomas Colby)
Female 49, abdominal and rectal pain, constipation and an episode of bloody diarrhea. Colonoscopy showed an area of narrowing in the sigmoid colon. Radiologically, ?Crohn's ?neoplasm. Partial sigmoidectomy showed 40 cm of non-ulcerated, mural thickening.

Case 4 (Dr Kumarasen Cooper)
Female 18, presented with an enlarging abdominal mass. A large adnexal pelvic mass was resected. The contralateral ovary and uterus appeared normal.

Case 5 (Dr Angelo P dei Tos)
Female 61, presenting with a 6.5 cm mass located in the posterior aspect of her neck.

Case 6 (Dr Giovanni Falconieri)
Female 58, with shortness of breath, bilateral patchy pulmonary infiltrates. Unremarkable past medical history. Improved with corticosteroid therapy, but relapsed after cessation of therapy. Wedge biopsy of left lower lobe of lung performed.

Case 7 (Dr Thomas Krausz)
Female 54, with "coin" lesion in the lower lobe of the right lung on chest X-ray. Wedge resection of well-circumscribed mass, 2.5 cm in diameter.

Case 8 (Dr Janez Lamovec)
Female 58, necrotic, haemorrhagic tumour of the right nipple and areola following breast trauma 6 months previously. Past history of surgery and radiotherapy for infiltrating duct carcinoma. On Tamoxifen for 5 years after surgery.

Case 9 (Dr Michal Michal)
Male 35, left orchidectomy for gradually enlarging, painless testicular mass since age of 10.

Case 10 (Dr Cesar Moran)
Male 49, with a painless testicular mass underwent right orchidectomy.

Case 11 (Dr Paul Wakely)
Male 52, nonsmoker, wedge resection of a slowly enlarging, 3 cm left lower lobe lung mass, discovered incidentally as part of a job-related screening chest X-ray. No hilar or mediastinal adenopathy.

Case 12 (Dr Noel Weidner)
Regrowth of uterine mass. The submitted specimen was a 661 gm, 15.0 x 12.5 x 8.5 cm intact uterus with cervix. The endometrial cavity was distorted by a bulging, ill-defined tumor measuring 9.9 x 9.0 x 10.0 cm.

CASE 1

CONTRIBUTOR: Michele Bisceglia, MD; IRCC "Casa Sollievo della Sofferenza" Hopsital, San Giovanni Rotondo, Italy

Clinical history. A female child of 9 years of age, complaining of abdominal and left flank pain, was admitted on a previous diagnosis of "microcystic nephropathy, NOS", which was established at birth, when a palpable abdominal mass was first discovered. At birth this mass lesion, measuring 8x5x3 cm and corresponding to a huge left kidney, was diffusely multicystic at ultrasonography and non functioning at scintilligraphy, except for a small portion located in the lower pole of the organ. No other cystic lesions were documented in the remainder of abdominal parenchymal organs, particularly the liver, pancreas and spleen. During the time the renal mass had been gradually enlarging, urinary tract infection was the most frequent complaint, and the urynalisis showed constant microscopic hematuria and frequent bacteriuria. The renal function tests and the arterial blood pressure values were always normal. On inquiry the family history of this child was unremarkable, with parents and close relatives in good health and free of disease on ultrasonography scan. A total left nephrectomy was performed.

Gross pathology. The surgical specimen was 15x8x6 cm in size and 350 gr in weight with a smooth to slightly bosselated external surface. On sectioning, myriads of roundish cysts 1 mm to 1.5 cm in size were apparent throughout *(see kodachrome)* with only a small uninvolved portion of the kidney left at the lower pole. The urinary excretory system as well as the renal vessels at the hilum were normal.

Histology. Innumerable cysts are evident which are lined by flat to cuboidal one-layer thick epithelium in absence of any multilayering or papillary tuft *(HE glass slide A)*. Cysts clearly affect any tract of the nephrons from the Bowman's space in the cortex as assessed by the presence of scattered glomerular cysts to the collecting and papillary ducts in the medullary pyramids. Several intervening thin tissue septa among the cysts containing normal renal parenchyma (glomeruli and tubules) can also be seen. On balance the exhibited histological features are identical to those seen in the autosomal dominant polycystic kidney disease of the adult type. The small portion of the kidney, which we mentioned above as seemingly normal at the lower pole of the organ *(HE glass slide B)*, actually is not histologically normal in that it also shows early formation of fewer and smaller similar cysts.

DIAGNOSIS. Based on the clinicopathologic data available, such as the uremarkable family history, the normal contralateral kidney, the absence of cysts in other visceral organs, and the absence at histology of dysgenetic/dysplastic elements, such as primitive ducts or primitive mesenchyme or metaplastic cartilage, a diagnosis of ***unilateral renal cystic disease (URCD) of nongenetic etiology*** was established.

DISCUSSION

URCD (1-10) is a poorly understood disease condition, which was first recognized and described in 1979 as *localized (segmental) cystic disease of the kidney* (LSCDK) (11), the alternative synonymic term which is still well in use (12-15). Other terms on record used both after 1979 and before that date alluding to URCD/LSCDK are *multiple unilateral renal cysts*(16), *segmental polycystic kidney disease* (17-18), and *unilateral polycystic renal disease* (19-23), which are to be discouraged as possible confusing misnomers with other renal cystic diseases. URCD/LSCDK is a rare, nonfamilial, nonprogressive renal disorder, not associated with cysts or malformations in other organs, and not related to other genetic cystic diseases. Patients affected are usually diagnosed in their adulthood (3,5,11,14,15,19-23), although young people (1,5,8) or children (1,2,11,16) and even infants and neonates (4) are on record. The most common clinical symptoms are variously represented by flank or abdominal pain, a palpable mass, gross or microscopic hematuria, and hypertension, in absence of any renal function impairment. Pathologically URCD/LSCDK involves part to most or even (more rarely) the whole of one kidney with many to innumerable, variously sized cysts separated in places by normal or compressed renal parenchyma, with an overall picture which recalls the one of autosomal

dominant polycystic kidney disease. Although some standard textbooks of systemic as well as urologic surgical pathology do include this entity among the cystic diseases of the kidney, some others do not. Thus far 51 such cases of URCD/LSCDK in total have been published in the world literature (1-23) with part of the cases having been diagnosed on the basis of the clinico-radiological features only, since many patients were not operated on and not histologically examined. In this review we have also included some cases, originally published as polycystic kidney disease (20-23), which can be nowadays recognized as most likely examples of URCD/LSCDK. The pathogenesis of the disease is obscure, although an acquired maldevelopmental origin is hypothesized. In URCD/LSCDK the contralateral unaffected kidney may occasionally show in adult patients few simple cysts as it has been documented in three of such reported cases and although it is believed that simple cysts can appear coincidentally in the contralateral kidney, since simple cysts are so common and since there is an increase in incidence of simple cysts with age in normal individuals, hypothesis has been proposed that URCD/LSCDK may represent a variation in the theme of multiple renal simple cysts. URCD/LSCDK is a condition that does not require aggressive approach and can be only periodically followed-up if asymptomatic.

The case herein presented is not an autosomal dominant polycystic kidney disease (ADPKD). ADPKD usually occurs in the context of a familial positive history and is bilateral, although approximately in 10-20% of patients the family history is lacking due to new mutations and the disease may also appear in asynchronous or asymmetrical form in around 15% of cases, mainly in children, with some extreme forms of asymmetry having been described as *unilateral ADPKD*, especially in infancy. Further, half of the patients have associated cysts in other visceral organs (mainly liver, spleen, lung) alongwith other rarer abnormalities (cerebral and coronary artery aneurysms). However, three cases have been even described of *unilateral ADPKD with contralateral renal agenesis*, which theoreticaly -in absence of a positive familial history- would not be distinguishable from a *solitary kidney affected by URCD/LSCDK*. Instead -as above said- few other cases previously described as *unilateral polycystic kidney* (20-23) can nowadays be reversed to URCD/LSCDK diagnosis.

This case is not the autosomal recessive polycystic kidney disease (ARPKD). **In ARPKD the disease condition is always bilateral and the cysts are characteristically fusiform and radiate from medulla to cortex, with the collecting ducts as the specific tubular segments of cystogenesis. The correct diagnosis rests upon the positive family history, the renal pathologic picture and liver biopsy (ductal plate malformation in the neonatal age, and liver cysts with portal hepatic fibrosis resulting in portal hypertension and hepatosplenomegaly in later ages).**

This is not a case of multiple simple cysts (MSC). *MSC are the most frequent cystic lesions of the kidney, typical of the adult and old age, located in the cortical zone and lined by a flat layer of epithelial cells. Simple cysts are rare in children and infants, usually as solitary lesions, and those prenatally detected commonly resolve during pregnancy. However, multiple simple cysts - as above stated- can occur coincidentally in the contralateral kidney in a URCD/LSCDK nor it is excluded that URCD/LSCDK represents a variation in the presentation of MSC: two cases reported in the literature (16) as multiple unilateral renal cysts do likely correspond to URCD/LSCDK. Further, simple cysts sometimes occur in multiple generations and there's accumulated some evidence that a rare autosomal dominant form of simple cyst disease may exist.*

This case is not a multicystic dysplastic kidney (MDK). MDK is usually sporadic, but rarely is familial. The clinicopathologic spectrum includes a *bilateral form* with Potter's sequence, which is inconsistent with survival, as well as a *unilateral form* which is often asymptomatic. Unilateral form of MCDK grossly exhibits a "*bunch of grapes*" appearance, loosing the normal reniform shape. The pelvicalyceal system and the ureter are usually abnormally developed (absent or atresic) as is sometimes affected also the escretory distal urinary system. Renal vessels may be abnormal or even absent on occasion. Further, other cardiovascular, digestive, and central nervous system malformations have been also found associated. The histologic landmark of MDK is represented by the dysplastic/dysgenetic elements (primitive ducts and heterologous tissue, mainly cartilage), in absence of normal renal tissue. The fate of MDK is usually

represented by stable size or by partial or complete involution, an event which is alien to the clinical behaviour of URCD/LSCDK. Again, the *aplastic dysplastic kidney*, an extreme form of the dysplasia spectrum, which is comprised of a small nub of tissue in which only primitive ducts can be seen, and the *hypoplastic dysplastic kidney*, which is an intermediate form, as well as the other distinctive entities of *kidney dysplasia associated with lower tract obstruction* and the s*egmental dysplasia*, commonly confined to the upper pole in a duplex kidney, can be excluded straight.

This case is not a pluricystic kidney of the multiple malformation syndromes (PCK-MMS). In PCK-MMS the phenotype of the PCK is different in different syndromes. Cysts may be of variable number and are usually bilateral. However a well definite phenotype is represented by the *diffuse cystic dysplasia of the kidney* (DCDK), which is seen prototypically in Meckel-Gruber's syndrome and its variants (Goldston's, Sipopoulos' and Miranda's syndromes) as well as in several others (oro-facio-digital syndrome, trisomy 9 and trisomy 13, short-rib-polydactyly syndrome, Jeune's asphyxiating thoracic dystrophy syndrome, Zellweger cerebro-hepato-renal syndrome, renal-hepatic-pancreatic dysplasia, glutaric aciduria type II, Ellis-van Creveld's, Elejalde's, and phocomelia syndrome or Robert's syndrome [pseudothalidomide syndrome]). In DCDK kidneys grossly loose their reniform shape, being characterized by numerous peripheral roundish cysts in the cortex, and microscopically exhibit a more advanced metanephric differentiation in comparison with MDK with some visible normal nephrons and primitive ducts, the marker of dysplasia, while cartilage is usually absent. The urinary excretory system is better developed than in MCDK and patent. DCDK may also occur sporadically.

This is not a juvenile nephronophtisis (NPH) nor a medullary cystic disease of the kidney (MCD). NPH (*NPH1 -juvenile form; NPH2 -infantile form; NPH3 -adolescent form; NPH4*) is of autosomal recessive inheritance accounting for the 70% of all cases of the NPH/MCDK group. NPH usually presents in the I decade and is in some cases associated with retinal dysplasia and less often with skeletal and central nervous system abnormalities. Kidneys are bilaterally affected and are usually small at presentation. The main clinical features are represented by polyuria, polydipsia, functional concentration tubular defects, Fanconi syndrome, growth retardation, and renal function impairment leading to early end-stage-renal-disease (hence the alternative term of *uremic medullary sponge kidney* for alluding to the entire NPH/MCD group). MCD, of which two forms have been described, MCD1 and MCD2, the latter being often associated with hyperuricemia and gout, is an autosomal dominant disease, which usually occurs in the III-IV decades of life, sharing the same clinical renal presentation with NPH, except for growth retardation and extrarenal malformations, which are absent in MCD. The hybrid phenotype of NPH1 associated with hypeuricemia (*familial juvenile hyperuricemic nephropathy -FJHN)* is considered an allelic variant of MCD type 2. Pathologically the common distinguishing features of the NPH/MCD disease group is the presence of some conglomerates of small cysts located at the corticomedullary junction and deeper in the renal medulla, arising from the distal convoluted and collecting tubules, alongwith some tubulointerstitial damages. Of note, 15% of cases of NPH/MCD are new mutations and thus present with negative family history.

This is not a medullary sponge kidney (MSK). MSK is a non-genetically tansmitted disease -usually asymptomatic- characterized by the presence of ectasia of papillary collecting ducts in the renal medulla, congregating at the papillary tips, and involving one to all of pyramids (*cystic disease of renal pyramids*). Papillary duct ectasia is likely a congenital anomaly which is not discovered until complications supervene. It is commonly detected in adults under investigation for urolithiasis, although pediatric cases are even on record. Kidneys are bilaterally affected, with normal size and intact function, which on intravenous urography show the characteristic picture of "*flowers' bouquet"* (radial linear streakings in the renal papillae due to stasis of the contrast medium in the ectatic or cystic papillary ducts).

This is not a glomerulocystic kidney disease (GCKD) nor a glomerulocystic kidney (GCK). GCKD, which is mostly transmitted in an autosomal dominant mode of inheritance, is characterized by the presence of glomerular cysts (dilatations of Bowman's space), located in the cortex, as the predominant histological feature, occurring outside the context of a dysplastic kidney and syndromic abnormalities. The disease is bilateral and is usually discovered in infants, both within the context of a familial history of ADPKD (*familial ADPKD-GCKD*) and outside,

although presentations occurring in older children and adults are also seen (*familial GCKD in older individuals*). Grossly kidneys are usually enlarged in *familial ADPKD-GCKD,* instead -out of the remainder of the patients- some have their kidneys normal in size, and some others have theirs decreased (*familial hypoplastic GCKD*). Sporadic occurrence of GCKD, probably reflecting new mutations, is also on record (*sporadic GCKD*) mostly among adults. GCK is the term for alluding to kidneys with glomerular cysts as a major pathologic feature, which may characterize complex inheritable malformation syndromes (tuberous sclerosis, oro-facio-digital syndrome type I, brachymesomelia renal syndrome, trisomy 13, short-rib-polydactyly syndrome [Majewski type and Saldino-Noonan type], Jeune's asphyxiating thoracic dystrophy syndrome, Zellweger cerebro-hepato-renal syndrome, familial juvenile nephronophtisis). Further GCK may also be a minor finding in congenital nephrotic syndrome –Finnish type ["*microcystic disease*"], Cornelia de Lange syndrome, Down syndrome, Marden-Walker syndrome, phocomelia syndrome or Robert's syndrome [pseudothalidomide syndrome], Smith-Lemli-Opitz syndrome, trisomy 9, trisomy 18) and -occasionally- may be featured by other diseases, both congenital (hypothyroidism, retinitis pigmentosa, cerebral vascular malformation) and acquired (progressive systemic sclerosis). GCK is always bilateral, although an extraordinary case of *unilateral megalic variant associated with tuberous sclerosis* has been recently described.

This is not the classical cystic kidney (cCK) neither of tuberous sclerosis complex (TSC) nor of von Hippel Lindau syndrome (VHL). TSC is an autosomal dominantly heritable complex malformation syndrome characterized by mental retardation, epilepsy, angiofibromas (the classical triad of Bourneville's phakomatosis), ungueal fibromas, CNS lesions (i.e., tubers, subependymal astrocytic hamartoma and giant cell astrocytoma), retinal astrocytomas and retinal hamartomas, cardiac rhabdomyomas, renal and lymphnodal angiomyolipomas, lymphangioleiomyomatosis, and renal cysts and renal cell tumors (papillary carcinomas and oncocytomas). cCK is represented by either occasional or numerous up to innumerable medullary and cortical cysts, imparting a sponge-like appearance to the kidneys, and distinctively lined by granular eosinophilic cells with large nuclei, resembling proximal tubular epithelium, forming in places papillary tufts. cCK-TSC may be bilateral as well as unilateral (and -in a unique case described- even segmental), mostly occurring in the context of a full blown syndromic complex, even though occasionally cCK may be just the first manifestation of TSC. Noteworthy, sometimes cystic changes in TSC may exhibit the ADPKD phenotype (ADPKD-TSC) as the effect ("adjacent gene syndrome") of large deletions disrupting both TSC2 and PKD1 genes, the two main genes respectively responsible for TSC and ADPKD, which are closely (tail-to-tail) located on the same Chr. 16p. VHL syndrome is an autosomal dominant, multisystem neoplastic disorder, of which there are two clinicopathological types, including retinal or central nervous system hemangioblastomas, pancreatic cysts and pancreatic tumors (cystadenomas, carcinomas, islet cell tumors), adrenal pheochromocytoma (in type II syndrome), epididymal papillary cystadenoma in men, pelvic tumor of the broad ligament of Wolffian origin in women, Heffner tumor, and renal cysts and renal cell carcinomas. The cysts in cCK-VHL are characteristically lined by a variously layered epithelium with focal papillary tufts and piling which herald a tumor development or are even difficult to distinguish from an early established neoplasia. Renal cysts are mostly frequent in VHL disease, affecting two thirds of the patients, and rarely can be the initial clinical presentation in less than 10% of cases.

This is not a multilocular cyst (cystic nephroma) (MLC-CN). MLC-CN is regarded as the benign counterpart of Wilms' tumor. Usually is unilateral, of huge size and comprised of variously sized cavities up to several cm, occurring in pediatric age, with a peak incidence in late childhood. It is characterized by mature, histologically bland epithelial cysts and fibrous septa. It is now believed there is a continuous morphologic spectrum of infantile renal tumors that encompasses the histologically mature MLC-CN at one end and, at the other, the cystic partially differentiated nephroblastoma –an extremely cystic variant of Wilms' tumor-, which contain immature blastemal cells with or without embryonal stromal or epithelial elements. MLC-CN was thought to rarely affect adults, where it often exhibits cellular septal stroma that focally resembles ovarian tissue, features now recognized as being those of mixed epithelial–stromal tumor of the kidney. MLC-CN does enter the differential diagnosis versus URCD/LSCDK: however helpful distinguishing features are its encapsulation and demarcation from the normal

residual kidney, the lack of normal nephrons within the tumor fibrous septa, and the absence of any communication in MLC-CN of tumor cysts with each other or with the excretory system.
This is not the cystic variant of congenital mesoblastic nephroma (C-CMN). **CMN is a rare pediatric renal tumor that is usually diagnosed during the first 3 months of life of which two forms are known, the conventional or leiomyomatous (solid) form and the atypical or cellular form. C-CMN is usually relevant to the atypical type and always exhibits areas of the solid type with brisk mitotic activity and possible metastasizing ability.**
This is not a renal cystic lymphangioma (RCL)/peripyelic-pericalyceal lymphangectasias (PPL) nor a capsular lymphangioma of the kidney (CLK) **(hygroma renalis).** RCL and PPL, alternatively described in the literature for alluding to single or multiple, cystic lesions involving bilaterally or unilaterally the renal sinus or the renal parenchyma and variously considered to be either a hamartomatous malformation or an acquired lesion resulting from lymphatic obstruction secondary to pelvic inflammation, occurr both in adults and in children, causing kidney enlargement. The condition has a multicystic gross appearance both on external surface and on sectioning and can be confused with URCD/LSCDK, but cavities are histologically lined by flattened endothelial cells. CLK, which also occur as a bilateral or unilateral (segmental or diffuse variant) lesion, imparts a cystic appearance to the external renal surface only and is easily differentiated from URCD/LSCDK.
This case is not an acquired renal cystic disease (ARCD) of patients on long-term dialysis nor is **a *multicystic renal cell carcinoma (MCRCC)*** for obvious reasons.

Conclusions. URCD/LSCDK of non genetic origin can be reported with confidence, but awareness of the existence of this disease is essential to the correct diagnosis. This case indeed was "unilateral", "cystic", "non-familial" and "non-progressive" as well as exclusive of cysts in other abdominal organs and so a precise diagnosis of "URCD/LSCDK –diffuse variant" could well be rendered. The main differential diagnosis of URCD involves ADPKD, which usually is of later onset, bilateral, familial, progressive, and with hepatic cysts. The absence of the last four findings should always offer the correct alternative diagnosis of URCD/LSCDK versus a unilateral ADPKD diagnosis from a new mutation. Notwithstanding, there are just the following three (almost virtual) eventualities in which the diagnosis of a "diffuse form of URCD/LSCDK" cannot be made: 1. ADPKD, lacking positive familial history and affecting one kidney only (asymmetrical and new mutant ADPKD): time will resolve the enigma in that sooner or later the other kidney will manifest the cystic changes too; 2. ADPKD with contralateral renal agenesis, lacking positive familial history (new mutant ADPKD in a unikidney patient): time will not resolve the enigma until the appearance of the patient's offspring; 3. bilateral involvement by (diffuse) URCD/LSCDK: an eventuality which has never been described so far and which however -although semantically contradictory- in theory cannot be excluded. The absolutely certain diagnosis in the above three (virtual or almost virtual) events will probably lie on molecular studies as well as on fluorescence in situ hybridization analysis which allow genetic diagnostic testing for the exclusion of ADPKD.

References. **1.** J Comput Assist Tomogr 13: 273-276,1989//. **2.** J Urol 148:1921-1223,1992//. **3.** Abdom Imaging 19:366-368, 1994//. **4.** Clin Nephrol 43:204-205,1995//. **5.** Nephrol Dial Transplant 14:1999-2003,1999//. **6.** Radiat Med 17:423-426,1999//. **7.** Urology 53:1227-1228,1999//. **8.** Clin Nephrol 57:320-324,2001//. **9.** Comput Med Imaging Graph 26:247-249,2002//. **10.** Actas Urol Esp 26:50-52,2002//. **11.** AJR 132:891-895,1979//. **12.** J Urol 140:357-359,1988//. **13.** J Clin Pathol 51:703-705,1998//. **14.** AJR 176:843-849,2001//. **15.** Abdom Imaging 28:588-592,2003//. **16.** Pediatr Radiol 30:346-348,2000//. **17.** NY State J Med 72:1850-1852,1972//. **18.** J Ultrasound Med 2:425-427,1983//. **19.** NY State J Med 64:2465-2469,1964//. **20.** J Urol 107:527-529,1972//. **21.** AJR 130:1165-1167,1978//. **22.** J Urol 127:297-300,1982//. **23.** Nephrol Dial Transplant 14:2775-2776,1999.

CASE 2

CONTRIBUTOR: John K C Chan, MBBS; Queen Elizabeth Hospital, Hong Kong

Clinical history:
This 39-year-old lady presented with high fever. Chest X-ray showed right lower zone haziness. Peripheral blood examination revealed lymphopenia. The disease progressed rapidly, resulting in respiratory failure and death within a few days.

Histologic findings:
Histologically, the postmortem lung tissue shows prominent features of diffuse alveolar damage, with hyaline membranes identified in many areas, especially in the alveolar ducts. There are occasional exfoliated atypical pneumocytes with large vesicular nuclei and fine cytoplasmic vacuoles (it is possible that these represent virus-infected cells). Definite viral inclusions are not seen at the light microscopic level. Of significance, although there are some histiocytes in the alveolar spaces, there are few lymphocytes in the alveolar spaces or alveolar septa.

Additional studies performed to confirm diagnosis:
(1) Reverse transcription-polymerase chain reaction of the lung tissue shows positive reaction for the SARS coronavirus.
(2) Electron microscopy shows groups of coronavirus particles within vesicles in some pneumocytes.

DIAGNOSIS:
Severe acute respiratory syndrome (SARS) due to SARS coronavirus infection

DISCUSSION

The epidemic of SARS

SARS is an emerging infectious disease that has posed worldwide threat because of its high infectivity (including high risk of transmission to healthcare workers, who account for >20% of all infected individuals). The disease first started in southern China in November 2002 as a peculiar form of "atypical pneumonia", and cumulated in a major outbreak in February 2003. The epidemic in Hong Kong started in March 2003, and a significant outbreak occurred in a residential building (Amoy Garden). There were also major outbreaks in many countries, such as Singapore, Vietnam, Canada and Taiwan. The epidemic has abated, with the last cases being reported in June 2003 (except that there was subsequently a single case of laboratory transmission to a laboratory worker in Singapore). The total number of cases worldwide is 8098, and the case fatality rate is 9.6%.

In early 2004, a few sporadic cases of SARS have been reported in Southern China, but the disease appears to pursue a milder clinical course compared with the 2003 epidemic.

Clinical features

The disease can affect any age group, including previously healthy young adults. The commonest presenting symptoms are high fever and muscle ache. Some patients have dizziness or diarrhea (See appendix for W.H.O. case definition). After several days, there is onset of respiratory symptoms such as dry cough and dyspnea. Blood examination shows lymphopenia, elevated liver enzymes and elevated LDH. Chest X-ray changes (patchy consolidation, most commonly in the lower lobes) may not appear until about 5 days after onset of disease; CT scan can detect the early subpleural lesions not otherwise detectable on routine chest X-ray. The pulmonary infiltrate may progress, resulting in oxygen desaturation. The disease resolves in some patients, but progresses (and may result in death) in others.

The etiological agent: a newly identified SARS coronavirus

The etiologic agent is a newly identified coronavirus, called SARS coronavirus. It is noteworthy in human history that this etiologic agent of this new disease could be so promptly identified and characterized -- within weeks of the description of the syndrome, no doubt attributable to the advances in biological sciences.

Conventional coronavirus is the commonest cause of common cold, which is a mild and self-limiting disease. This new SARS coronavirus, on the other hand, causes quite significant pulmonary disease. Subclinical infection by SARS coronavirus, if it occurs at all, is extremely rare.

In-situ hybridization studies have shown that the virus has tropism for pneumocytes and enterocytes.

Mode of disease transmission

SARS (SARS coronavirus) is transmitted predominantly through droplets shed from respiratory secretions of infected persons, and less commonly through fomites or fecal route. As a result, close personal contact (such as household members and healthcare workers) is the important route of transmission.

The virus can be found in respiratory secretions, urine and stool, and sometimes also in the blood. The virus can survive in the environment for at least 24 hours at room temperature.

Most patients do not effectively transmit the virus to other individuals, but there are also so-called "superspreaders", who can transmit the SARS virus to a large number of individuals (such as transmission that occurred at Metropole Hotel in Hong Kong). Superspreaders and nosocomial amplification were the main factors leading to the massive outbreaks in some countries. In the Amoy Gardens outbreak in Hong Kong, the SARS coronavirus may have spread through the sewage systems of the buildings.

Methods of diagnosis confirmation

(1) Acute phase: Use of molecular techniques (such as conventional RT-PCR, or real time-PCR) to demonstrate the virus in various patient samples, such as nasopharyngeal aspirate, throat swab, stool. However, the sensitivities of these tests are limited, and it may be necessary to obtain several samples collected over several days to get a positive result. Also a negative result does not totally rule out the diagnosis.

(2) Serologic diagnosis: This is practically the gold standard for confirmation of the diagnosis (e.g. some patients might never have a positive RT-PCR test, but eventually proven to have SARS based on serologic studies). However, the rise in antibody can be slow, and can take as long as 4 weeks. Serologic studies may be done by immunofluorescence technique or ELISA.

Pulmonary pathology

The pathologic findings as listed above are fairly characteristic of this disease entity. It is not clear whether the pneumocytes found in the alveolar spaces are exfoliated due to the disease process or merely represent a postmortem artefact. Some of the pneumocytes can be multinucleated. Squamous metaplasia can be prominent in some case. In some patients, the disease does not resolve, but progresses, with organization of the hyaline membranes and exudate (similar to organizing diffuse alveolar damage), and eventual fibrosis.

Summary of major histologic findings in the acute phase:

- Diffuse alveolar damage, with hyaline membrane formation
- Airspace edema
- Bronchiolar fibrin
- Paucity of lymphocytes
- Type II pneumocyte hyperplasia
- Cytomegaly and multinucleation of pneumocytes
- Squamous metaplasia

The overall features are not distinguishable from some other viral pneumonias or acute interstitial pneumonia (Hammond-Rich syndrome). Thus confirmation of etiology has to be obtained by molecular or ultrastructural studies.

Appendix

W.H.O. Case Definitions for Surveillance of Severe Acute Respiratory Syndrome (SARS)

Suspect case

1. A person presenting after 1 November 2002[1] with history of:
- high fever (>38 °C)

AND
- cough or breathing difficulty

AND one or more of the following exposures during the 10 days prior to onset of symptoms:
- **close contact** with a person who is a suspect or probable case of SARS;
- history of travel, to an area with recent local transmission of SARS
- residing in an area with recent local transmission of SARS

2. A person with an unexplained acute respiratory illness resulting in death after 1 November 2002, but on whom no autopsy has been performed

AND one or more of the following exposures during to 10 days prior to onset of symptoms:
- **close contact,** with a person who is a suspect or probable case of SARS;
- history of travel to an area with recent local transmission of SARS
- residing in an area with recent local transmission of SARS

Probable case

1. A suspect case with radiographic evidence of infiltrates consistent with pneumonia or respiratory distress syndrome (RDS) on chest X-ray (CXR).
2. A suspect case of SARS that is positive for SARS coronavirus by one or more assays. See Use of laboratory methods for SARS diagnosis.
3. A suspect case with autopsy findings consistent with the pathology of RDS without an identifiable cause.

Exclusion criteria

A case should be excluded if an alternative diagnosis can fully explain their illness.

Reclassification of cases

As SARS is currently a diagnosis of exclusion, the status of a reported case may change over time. A patient should always be managed as clinically appropriate, regardless of their case status.
- A case initially classified as suspect or probable, for whom an alternative diagnosis can fully explain the illness, should be discarded after carefully considering the possibility of co-infection.
- A suspect case who, after investigation, fulfils the probable case definition should be reclassified as "probable".
- A suspect case with a normal CXR should be treated, as deemed appropriate, and monitored for 7 days. Those cases in whom recovery is inadequate should be re-evaluated by CXR.
- Those suspect cases in whom recovery is adequate but whose illness cannot be fully explained by an alternative diagnosis should remain as "suspect".
- A suspect case who dies, on whom no autopsy is conducted, should remain classified as "suspect". However, if this case is identified as being part of a chain transmission of SARS, the case should be reclassified as "probable".
- If an autopsy is conducted and no pathological evidence of RDS is found, the case should be "discarded".

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CASE 3

CONTRIBUTOR: Dr Thomas Colby, MD; Mayo Clinic Scottsdale, USA

Clinical History

This 49-year-old woman had abdominal and rectal pain associated with constipation. She had an episode of bloody diarrhea. Colonoscopy showed diverticulosis and an area of narrowing in the sigmoid colon. Biopsies showed focal cryptitis and architectural alterations suggestive of inflammatory bowel disease. She was treated with Mesalamine and prednisone but her symptoms progressed with increased rectal cramping and bloody rectal discharge. Radiologic studies raised the possibility of Crohn's disease and infiltrating neoplasm because of the thickened bowel wall. The patient underwent partial sigmoid colon resection. The specimen showed 40 cm of mural thickening without overlying mucosal ulceration.

DIAGNOSIS: Gastrointestinal (sigmoid colonic) Basidiobolomycosis due to *Basidibolus ranarum*.

Follow-up: Diagnosis confirmed at AFIP and Centers for Disease Control in Atlanta. Three specific antibodies to *B. ranarum* were detected in the patient's serum.

The patient had all gross disease resected. Postoperatively she received Itraconazole for several months. She remains free of disease off medication six years after surgery.

Histologic Findings

There is relative preservation of the mucosa. The muscularis propria and the submucosa are markedly infiltrated and thickened by an inflammatory infiltrate replacing musculature. There is fibrosis and eosinophils with the formation of eosinophil abscesses surrounded by granulomatous inflammation. Within the centers of these abscesses there are pale hyphal structures, many of which are surrounded by a Splendore-Hoeppli phenomenon. Special stains show these to be weakly positive with fungal stains including PAS and Methenamine silver.

DISCUSSION

Basidiobolus ranarum is a fungus belonging to the family of Entomophthoraceae of the order Entomophthorales. It is best known as a cause of subcutaneous zygomycosis, particularly in the tropical regions of Africa and Southeast Asia. *B. ranarum* was first isolated in 1955 from decaying leaves in the United States but it has been found throughout the world in soil and decaying vegetation. It has also been recovered from a number of animals in which infections have also been described, including reptiles, amphibians, horses, and dogs.

Systemic disease caused by *B. ranarum*, in particular gastrointestinal involvement, has been rare. The case presented represented the first in a series of 9 cases that have been encountered in a eight-year period in the Phoenix, Arizona area. Seven of these are included in the Lyon reference. Five of the seven were male with a mean age of 52 years. Three had a history of diabetes, two steroid use, and three Ranitidine use. Five of the seven were smokers. All were residents of Arizona. According to a case control study, no specific exposure could be implicated in the causation of this disease. All came to some sort of total or subtotal resection of the involved tissues. In addition to colonic involvement concomitant involvement of liver, retroperitoneum, kidney, stomach, and mesentery were also identified in individual cases. The organism was grown in all cases from which tissue was taken for culture.

Symptoms of gastrointestinal basidiobolomycosis have included fever, abdominal pain, diarrhea, constipation, weight loss, and rarely constitutional symptoms. Eosinophilia may or may not be present in the peripheral blood. Carcinoma was suspected in four of the seven

cases. Of the seven cases described, six of the seven were asymptomatic and off antifungal medications. The seventh was recovering at the time the paper was published.

Recently others cases of this condition have been reported. A case of fatal disseminated basidiobolomycosis was reported from Italy (Bigliazzi et al.). This patient also had gastrointestinal involvement but in addition had organisms identified in the chest, including the lung, heart, and multiple other sites. In addition, 6 cases in children were reported from Saudi Arabia, 4 from one area suggesting an environmental influence. (Al Jarie et al.)

The gross and histologic findings from the seven Arizona cases are summarized in the following table.

Summary of pathological findings for the 7 patients with GIB in Arizona, 1994-1999

Pathological Finding	**Number of Patients**
Gross Examination	
Anatomic site involved	
Colon	6
Stomach	1
Mucosal lining	
Intact	4
Ulcerated	3
Microscopic examination	
Granulomatous inflammation	
Present	4
Extensive	3
Tissue eosinophilia	7
Extensive necrosis	4
Fungal elements	7
Splendore-Hoeppli phenomenon	
Prominent	2
Less pronounced	2
Present	3

Histologically all the Arizona cases showed some foci identical to that in the case described. Some had large areas of necrosis with sheets of necrotic eosinophils. In addition to the distinctive eosinophilic abscess formation with central hyphal structures surrounded by a Splendore-Hoeppli phenomenon, four cases showed spherical structures the by various observers were considered to be meristospores, chlamydospores, zygospores, chlamydioconidia, and jemma. *B. ranarum* is known to produce zygospores with a beak-like protuberance but none of the structures in this case had that feature.

The histologic features in all the cases observed in Arizona are sufficiently distinctive in characteristic to allow a presumptive diagnosis based solely on the morphology. The marked infiltration and destruction of tissue by an infiltrate rich in eosinophils in which eosinophilic microabscesses form and are surrounded by granulomatous inflammation are very distinctive. In addition, the pale hyphal structures with their associated Splendore-Hoeppli phenomenon are quite characteristic. The diagnosis can be confirmed by culture in routine fungal media. The Centers for Disease Control in Atlanta has developed a number of serologic tests to detect specific antibodies to *B. ranarum*. These are not commercially available but these can be performed at the CDC by special request.

Histologic Differential Diagnosis

Fungal organisms that resemble basidiobolus in tissue sections include aspergillus and aspergillus look a likes but careful examination should allow ready separation from *B. ranarum*. The fungi in the order Mucorales have hyphae similar to basidiobolus but they are typically associated with vascular invasion and thrombi and found in a background of suppuration and infarctive necrosis with poorly formed granulomas rather than the distinctive eosinophilic infiltrate and well formed granulomas of basidiobolomycosis. *Conidiobolous coronatus* is a cause of nasofacial zygomycosis and is histologically indistinguishable from basidiobolos. Gastrointestinal conidiobolomycosis has not been reported.

This case is presented to increase the awareness of pathologists for this very unusual infection which, if the cases from Phoenix are any indication, may be increasing in frequency.

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CASE 4

CONTRIBUTOR: Dr Kumarasen Cooper, MB,ChB; University of Vermont, USA

Clinical History

An 18-year-old female presented with a history of an enlarging abdominal mass. At surgery, a large adnexal pelvic mass was resected. The contralateral ovary and uterus appeared normal.

Gross Examination

A large intact multiloculated red-brown tumor with cystic and solid areas was received. The tumor measures 39.0 x 34.5 x 20.0 cm and weighed 20 pounds. The surface was bosselated and comprised a white-gray fibrous capsule. The multilocular cystic areas ranged in size from less than 1.0 cm to 12 cm in diameter. Some cysts contained yellow-amber serous fluid, whilst others were filled with bloody fluid. The solid areas comprised approximately 50% of the total tumor mass with a yellow-tan, lobular, fleshy cut surface. Foci of necrosis and hemorrhage were also noted.

Microscopic Examination

The tumor is characterized by solid nests and sheets of cellular neoplasm with focal follicle formation. The solid nodules comprise proliferating granulosa cells and are arranged in sheets and surround the follicles. The nodularity of the solid areas is accentuated by fibrothecomatous septa. The neoplastic granulosa cells are rounded and large with an abundant eosinophilic cytoplasm. Nuclear grooving and Call-Exner bodies are not present. Focal larger vacuolated cells indicating an increase in lipid content (luteinization) are also present. Mild to moderate nuclear atypia with an increased mitotic activity (up to 2/high power field) is noted. The follicles vary in size and shape with some containing pale eosinophilic secretions. Granulosa cells of varying thickness line the follicles with a layer of theca cells separating the surrounding stroma. These follicles are smaller than those seen in adult macrofollicular granulosa cell tumors.

Immunohistology

The diagnosis of granulosa cell tumor is confirmed with the following immunopositive markers: inhibin (R1, Serotec), CD99 (HBA71, 013, Signet) and Calretinin (polyclonal, Zymed). EMA (E29, Dako), keratin (AE1/AE3, Chemicon International) and MART-1 (Melan-A, A103, Biogenex) were negative. In addition, PLAP (polyclonal, Dako), beta-hCG (polyclonal, Dako), alpha-fetoprotein (A-013-01, Biogenex), synaptophysin (Snp88, Biogenex) and C-kit (polyclonal, Dako) were also negative.

DIAGNOSIS: Juvenile Granulosa Cell Tumor

DISCUSSION

Juvenile granulosa cell tumors (JGCT) occur in children and young adults, usually before age 30. The majority of prepubertal girls with JGCT come to attention because of isosexual pseudoprecocity (precocity without ovulation or progesterone production). Adolescents commonly present with menstrual irregularities, amenorrhea, abdominal swelling/pain (as in the present case) or virilization. Postpubertal women usually present with abdominal pain or menstrual irregularities. As with ovarian tumors, ascites, tumor rupture, torsion and/or infarction may be seen at surgery.

The differential diagnosis in this age group includes germ cell tumors, small cell carcinoma and stromal tumors. The germ cell tumors of this younger age group include yolk sac tumor, dysgerminoma and embryonal carcinoma. These tumors lack the typical combination of solid cellular nests interspersed with follicle formation seen in JGCT. Further, these germ cell tumors are variably immunopositive for AFP (YST and some embryonal carcinomas), β-hCG (syncytiotrophoblasts of dysgerminoma and embryonal

carcinoma), PLAP (dysgerminoma and other germ cell tumors) and CD117 (c-kit) (YST, mixed germ cell tumors and some embryonal carcinomas).

An important differential diagnosis to consider is the small cell carcinoma of the hypercalcemic type. This uncommon aggressive tumor presents in young women in their twenties. The nuclei are undifferentiated, uniform, small and hyperchromatic (may occasionally be large with open chromatin) with brisk mitoses. Notably, this tumor may form follicular structures with eosinophilic material. Small cell carcinoma, however, is cytokeratin and synaptophysin immunopositive and negative for inhibin.

The stromal tumors in the differential diagnosis include tumors of the fibroma-thecoma continuum and sclerosing stromal tumor. The fibrothecomas are solid, white to yellow, and present in older women. They may develop extensive sclerosis and calcification. The sclerosing stromal tumor occurs in young women as a large, solid and cystic edematous white tumor with cellular islands alternating with a myxoid, edematous stroma and with a hemangiopericytomatous vascular pattern.

The vast majority of patients with stage 1 tumors are cured by salpingo-oophorectomy alone. Tumor rupture or dissemination at the time of diagnosis is a poor prognostic indicator, with most patients dying within a few years. Serum inhibin levels are useful in the follow-up of these patients. Recurrences are treated with cisplatin-based chemotherapy with some success. Surgical cytoreduction with salvage chemotherapy (including bleomycin and taxol) has met with some success.

In recent years, several immunohistochemical markers identifying ovarian sex cord stromal tumors have become commercially available. These have been found to be of value in the distinction between sex cord-stromal neoplasms and their histological mimics. These antibodies include α-inhibin, Calretinin, Melan-A and CD99. Of these, α-inhibin appears to be the most useful, being the most sensitive and specific immunohistochemical marker of ovarian sex cord-stromal tumors. Inhibin is a peptide hormone produced by ovarian granulosa cells, which selectively inhibits the release of follicle stimulating hormone (FSH) from the pituitary gland, acting as a modulator of folliculogenesis. It is produced and overexpressed by granulosa cell tumors (GCT), being therefore useful both as a marker for early tumor growth and tumor recurrence before clinical manifestation. It is particularly useful in identifying granulosa cell tumors (both adult and juvenile) with an unusual growth pattern, e.g. distinguishing the sarcomatoid growth pattern of GCT from soft tissue tumors. Inhibin is a sensitive immunohistochemical marker of a wide range of gonadal stromal tumors, including Sertoli-Leydig cell tumors, steroid cell tumors and thecomas. A note of caution: α-inhibin is not entirely specific, with immunoexpression being demonstrated in a small proportion of ovarian adenocarcinomas; emphasizing the need for a panel of antibodies when investigating a differential diagnosis.

Calretinin is a calcium-binding protein that is expressed in neurons and mesothelial cells (and their neoplastic counterparts). Many ovarian sex cord-stromal neoplasms are immunopositive with Calretinin: Sertoli-Leydig cell tumors and granulosa cell tumors, being both nuclear and cytoplasmic positive. However, fibrothecomas are negative. Calretinin has also been found to be immunopositive in the majority of female adnexal tumors of probable Wolffian origin (FATWO). Whilst the sensitivity of Calretinin has been compared to α -inhibin, positive staining of Calretinin in several tumors that may morphologically mimic sex cord-stromal tumors highlights the need for a panel of antibodies when investigating ovarian sex cord-stromal neoplasms.

CD99 (MIC2 antibody/HBA71/013) was first demonstrated as a marker for Ewing sarcoma/peripheral primitive neuroectodermal tumors. Subsequently, CD99 was identified in a range of other tumors, including hematopoietic neoplasms (lymphoblastic and myeloid), solitary fibrous tumors, mesenchymal chondrosarcomas, small cell carcinomas, and sex cord-stromal tumors. The latter involved immunoreactivity with granulosa and Sertoli cells. Interestingly, the degree of differentiation in Sertoli-Leydig cell tumors correlated with the degree of CD99 immunoreactivity, with well-differentiated tumors reacting more strongly. CD99 immunopositivity has also been demonstrated in sclerosing stromal tumors and sex cord tumor with annular tubules (SCTAT). These findings suggest a diagnostic role for CD99

as part of a panel in the investigation of tumors which include GCT and Sertoli-Leydig tumors, especially poorly differentiated variants.

The Melan-A gene was cloned from a human melanoma cell line. The same gene was found by another group using a different cell line and named MART-1. These two independent antibody clones recognize the same antigen and have proven to be beneficial in the diagnosis of melanocytic lesions. The antibody A103 has been reported to highlight adrenocortical tumors and ovarian tumors such as Sertoli-Leydig cell tumors, sex cord-stromal tumors (including adult and juvenile GCT), thecomas and FATWO. Although moderately sensitive and specific, A103 immunoreactivity appears to be of some value in the assessment of sex cord-stromal tumors, being positive in approximately 50% of tumors examined.

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CASE 5

CONTRIBUTOR: Angelo P Dei Tos, MD; Departments of Pathology and Oncology, Treviso, Italy

Case history

Female of 61 yrs old presenting with a 6.5 cm mass located in the posterior aspect of her neck. Microscopically the lesion appeared well demarcated, composed of a patternless spindle cell proliferation, associated with an hemangiopericytoma-like vascular network. Variation in cellularity was present. Additional important features of the neoplasm were the presence of multifocal nuclear pleomorphism accompanied by a mitotic activity ranging between 5 and 7 mitoses/10 high power fields. Focally the spindle cell proliferation presented a fascicular "fibrosarcoma-like" growth pattern. Immunohistochemical analysis revealed positivity for CD34, Cd99 and bcl-2.

DIAGNOSIS: Atypical/Malignant Solitary fibrous tumor (SFT)

DISCUSSION

Solitary fibrous tumor (SFT) represents an ubiquitous mesenchymal neoplasm first reported as a pleural based lesion (so called localized mesothelioma)[1]. With time it has become evident that SFT can arise anywhere including the peritoneal surface[2], mediastinum[3], retroperitoneum[2], upper respiratory tract[4], orbit[5] and urogenital tract[6]. The occurrence of systemic signs such as hypoglycemia and digital hyppocratism as been rarely reported which is related to the production of insulin-like growth factors by neoplastic cells. Age range is very broad (20-70) and there is no sex predilection.

Grossly SFT is most often represented by a relatively well circumscribed mass, with a white-gray cut surface, sometimes featuring myxoid degeneration, hemorrhage and, very rarely, necrotic foci. Microscopically, SFT is characterized by a variably cellular spindle cell proliferation organized in a short storiform pattern (so called "patternless" pattern), set in a collagenous stroma sometimes showing cheloid-like features. A characteristic hemangiopericytoma-like vascular pattern is almost invariably present. Immunohistochemically SFT typically exhibits positivity for the ubiquitously expressed antigen CD34[7]. CD99 as well as bcl2[8] are invariably expressed but again their specificity is very limited. Despite the fact that CD34 is expressed in a broad range of mesenchymal neoplasms, it has to be underlined that in context with morphology it still retains great diagnostic value. Rarely SFT may present with extensive myxoid degeneration of the stroma to the extent that the most typical morphologic picture is obscured[9]. In the recent years SFT has become a very trendy lesion[10,11], and several reports have described its occurrence as a primary soft tissue lesion arising at different anatomic sites[12-14].

The morphological prediction of the clinical behavior of SFT seems to be a real challenge. Approximately 10-15% of cases behave aggressively. Metastases are most frequently observed in lungs, bone and liver. Malignant behavior seems to be more associated with the presence of cytologic atypia, increased cellularity, mitotic activity greater than 4 mitoses/10HPF and necrosis, however their absence does not exclude "per se" the possibility of aggressive clinical course [15,16]. In general high mitotic activity seems to correlate with hypercellularity and loss of alternation of hypocellular and hypercellular areas. Lesions deeply located (retroperitoneum, mediastinum and pelvis) appears to behave more aggressively than those arising in the limbs. Large size seems also to correlate with higher risk of aggressive behavior[17].

There exists general agreement on the fact that since recent times most SFT has been lumped within the category of hemangiopericytoma[18]. It has to be underlined that hemangiopericytoma is a term which has been used to describe a wide variety of lesions sharing the presence of thin-walled blood vessels often exhibiting a "staghorn" configuration. The entity known as lipomatous hemangiopericytoma may represent part of a morphologic continuum with SFT[19]. Same concept may apply to giant cell angiofibroma, however both

morphological and clinical differences exist that permit to keep these two entities separated[20,21].

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CASE 6

CONTRIBUTOR: Giovanni Falconieri, M.D; General Hospital "Santa Maria della Misericordia," Udine, Italy

Clinical history: A 58-year-old woman with an unremarkable past medical history complains of shortness of breath, nonproductive cough, and fever responsive to steroid therapy. After suspension of therapy, she experiences a rapidly progressive and dramatic recurrence of symptoms, requiring prompt hospitalization. When the patient is admitted, a chest X-ray film reveals diffuse and bilateral pulmonary opacities. A wedge-shaped biopsy on the left lung is performed.

Gross and microscopic description: The specimen is received in formalin and consists of a fragment of peripheral lung with glistening pleura and a patchy airless cut surface. Hematoxylin-eosin-stained sections show clumpy, granular pink aggregates within the distal airways and cuboidal changes of the alveolar cells. Eosinophils, either scattered or in discrete clusters, are recognized in both the alveoli and the interstitium. The airway lumina are often plugged by organized masson-type bodies, sloughed epithelia, and macrophages. No significant pathologic changes are noted over the pleural surface. Modest fibrotic changes are present. Special stains are negative for microorganisms, including acid fast for mycobacteria and Gomori methenamine for fungi.

DIAGNOSIS: Eosinophilic pneumonia/eosinophilic lung disease

DISCUSSION

Acute interstitial lung diseases represent a heterogeneous group of conditions sharing a dramatic clinical presentation (rapidly progressive shortness of breath, pleuritic pain, fever, and other systemic symptoms), a restrictive pattern on pulmonary tests, airspace consolidation on X-ray films and CT scans, and tissue evidence of acute lung injury (alveolar exudates, increased alveolar type II cells, airspace organization, widened alveolar septa).

On the basis of clinical presentation, imaging studies, and the recognition of additional histologic "modifiers," acute interstitial pneumonias further segregate into distinct clinicopathologic entities which include (a) adult respiratory distress syndrome (ARDS) the prototypical acute interstitial pneumonia associated with so called "diffuse alveolar damage" (hyaline membranes and extensive cuboidal changes of alveolar epithelia); (b) pulmonary infections, showing generally prominent necrotic changes, as frequently observed in viral (especially herpes or adenovirus), bacterial (legionella pneumophila), or fungal infections; (c) pulmonary hemorrhage syndromes, in which collections of siderophages are present in addition to the elements of the acute pattern; (d) acute fibrinous organizing pneumonia, characterized by alveolar fibrin and exudates organization; and (e) acute eosinophilic pneumonia (AEP), in which a preponderant eosinophil-rich exudate is present within the distal airways.

AEP belongs to a larger group of eosinophilic lung diseases.[1,2] Clinically, patients with AEP typically have a short history of febrile illness, restrictive hypoxemic respiratory insufficiency, and diffuse/mixed alveolar interstitial radiographic changes. Most patients are adults; withoutsex predilection. Peripheral ground-glass opacities (often migratory) and a "photographic negative" pattern of perihilar infiltrates on high-resolution CT scans have been described.[3] An excess (>25%) of eosinophils may be also detected in bronchoalveolar lavage fluids. As outlined earlier, AEP microscopically features increased fibrin, pink macrophages, and reactive-appearing enlarged alveolar type II cells. The key feature is the presence of eosinophils within the airspace: *interstitial eosinophilia is not a major diagnostic criterion!* A subsidiary alveolar organization that is similar if not identical to that of bronchiolitis obliterans organizing pneumonia is often seen.[3,4] A prompt response to corticosteroid therapy is characteristic.[2] A chronic variant of eosinophilic pneumonia has been described featuring

slow-onset, less pronounced symptoms and a microscopic picture of patchy lymphoplasmacytic interstitial infiltrates, airway consolidation, and alveolar filling by eosinophils and histiocytes. [2,5] Patients with chronic eosinophilic pneumonia also respond promptly to steroid therapy but they experience symptom relapses more frequently once the treatment is stopped, thus requiring additional treatments. Although some distinctive features have been delineated, a significant overlap exists between acute and chronic eosinophilic pneumonia, making the distinction impractical: hence, the unifying concept of "eosinophilic pneumonia". The cause of eosinophilic pneumonia often remains obscure, although in several cases, history of asthma, atopic dermatitis, and drug or chemical action may be elicited. Eosinophilic pneumonia has been recently described in the new cigarette smoker (>1 pack/day). [6]

Differential diagnosis: Because of its excellent response to steroid administration and natural history, eosinophilic pneumonia should be distinguished from other acute interstitial lung diseases entailing a more serious prognosis. Significant numbers of eosinophils are not expected in any acute interstitial lung disorders other that AEP; therefore, their finding should alert the pathologist to this possibility, especially if present within the airspaces and if the microscopic feature is complemented by significant eosinophilia (>25%) in the bronchoalveolar lavage fluid or peripheral blood. A careful search for eosinophils is required, however, especially in patients who received steroids relatively close to the lung biopsy procedure, in which case both acute injury changes and eosinophil exudation may be attenuated or even eliminated. Mild nonnecrotizing vasculitis as well as poorly formed granulomas may be observed in eosinophilic pneumonia, significantly overlapping with features of Churg-Strauss syndrome. Peripheral blood eosinophilia—usually a limited and early finding in AEP—history of asthma, and evidence of extrapulmonary vasculitis are more consistently seen in Churg-Strauss syndrome. [7] Certain parasitic infections (especially *Strongyloides stercoralis*, *Toxocara canis*, *Aspergillus*, and *Coccidioides immitis*) may also be associated with prominent eosinophilia, vasculitis, and granuloma formation. [5] Chronic desquamative interstitial pneumonia generally has a more chronic clinical presentation, "ground-glass" radiographic infiltrate, few alveolar eosinophils, and limited acute injury changes. [3] Langerhans cell histiocytosis (not typically in the clinical differential for acute lung disease) usually occurs in cigarette smokers, has ill-defined reticulo-nodular opacities or honeycombing on X-ray films, and shows irregular scarring (stellate "medusa-like") changes of the lung parenchyma with cystlike space formation adjacent to the lesion, which features, in addition to eosinophils, the diagnostic Langerhans cells and their folded nuclei.[8] Positive immunostaining of Langerhans cells for S100 and CD1a may further assist in the differential diagnosis. In late resolved lesions, however, these may be sparse or absent altogether.

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CASE 7

CONTRIBUTOR: Dr Thomas Krausz, MD; University of Chicago, USA

Clinical history
54-year-old female with a 50 pack/year history of smoking, recurrent pneumonia and emphysema. A routine chest X-ray revealed a 2.6 cm nodule located in the right posterior mid-lung field. The presence of the lesion was confirmed on CT and the patient underwent a wedge resection of the tumor of right lower lobe.

Gross findings
The wedge resection specimen, with overlying pleura on one surface, contained a moderately firm nodule, 2.6 cm in diameter. Sectioning revealed a well circumscribed, round tumor with a tan, homogenous cut surface.

Microscopic findings
The neoplasm has a biphasic appearance as a result of an intimate admixture of epithelial and spindle cell elements. The structural organization of the epithelial component is also varied: a predominantly tubular/glandular architecture is alternating with compressed tubular, focally nested, trabecular and solid areas. Mucin production is not a feature of the tumor, however, the glands in many fields are filled by dense, eosinophilic, colloid-like secretion. Most of the glandular structures are double layered, with the inner layer composed of low columnar to cuboidal epithelial cells. The epithelial cells lining tubules are rather monotonous, but there is focal, mild nuclear pleomorphism and intranuclear cytoplasmic inclusions are prominent in several fields. In addition to the inner epithelial layer, there is also an incomplete outer layer of cuboidal to attenuated cells, consistent with myoepithelial differentiation (confirmed immunohistochemically and ultrastructurally). These myoepithelial cells lack the typical clear cell phenotype and instead, have eosinophilic cytoplasm similar to the cells of the inner layer. In the solid/nested areas, the epithelial cells are mostly polygonal and some of them exhibit an oncocytic appearance with abundant, granular, eosinophilic cytoplasm. In contrast, some of the adjacent epithelial cells have a clear or a microvacuolated appearance. Mitotic figures are less than 1/50 HPFs in the tubular areas and 1/10 in the solid regions.

Approximately 30% of the tumor is composed of short fascicles of slender to plump, eosinophilic spindle cells. The cytoplasm of rare spindle cells has a granular, oncocytic appearance, and in places, these cells merge gradually with the more solid epithelial component. The tumor focally extends to the submucosa of a small tertiary bronchus but there is no intrabronchial growth. The absence of intratumoral elastic tissue excludes entrapment of both alveoli and bronchi.

Immunohistochemistry
The epithelial cells lining the tubules are strongly immunoreactive for EMA, Cytokeratins (Cam 5.2, CK7), surfactant apoprotein and TTF1 but negative for CK20, high molecular weight keratin and myoepithelial markers. In contrast, the spindle cells are positive for CK7 and myoepithelial markers (high molecular weight keratin, SMA, S-100, calponin and p63), but negative for desmin. They are also negative for EMA, Cam 5.2, CK20, TTF-1 and surfactant apoprotein. The immunoprofile of the outer/basal cell layer of the tubules also supports myoepithelial differentiation. In addition they are also weakly positive for CAM.5.2 and TTF-1. Neuroendocrine markers (NSE, Synaptophysin, Chromogranin) were negative, as was thyroglobulin. Cell proliferation marker Ki-67 (MIB-1)was positive in less than 5% of nuclei of the tubular epithelial cells but was increased (20%) in the solid/nested areas and in the spindle cells. Clearly, the immunohistochemical study confirmed a biphasic tumor with both epithelial and myoepithelial differentiation.

Electron microscopy
Ultrastructural study also demonstrated both epithelial and myoepithelial differentiation. The cells lining the glandular structures contained not only epithelial characteristics, but also type 2 pneumocytic features with numerous lamellar bodies corresponding to surfactant and short microvilli on their luminal surface. Surfactant material was also present in the lumen of the glandular structures.
The cytoplasm of the spindle cells contained thin filaments with focal densities, confirming myoepithelial differentiation. Basal lamina was also present, incompletely encompassing the spindle cells.

DIAGNOSIS: Pneumocytic adenomyoepithelioma

DISCUSSION

This is a morphologically rather unusual pulmonary neoplasm. The differential diagnostic challenge is considerable. However, the histologic features in conjunction with the immunohistochemical and ultrastructural data convincingly show a tumor with both epithelial and myoepithelial differentiation. Such differentiation leads to diagnostic consideration of pulmonary salivary gland-type of tumors. These are very rare in the lung and include both benign tumors (mucus cell adenoma, oncocytic adenoma, pleomorphic adenoma, myoepithelioma, acinic cell tumor) and malignant ones (adenoid cystic carcinoma, mucoepidermoid carcinoma, malignant mixed tumor) as well as low grade neoplasms (epithelial-myoepithelial carcinoma)[1-3].
All the benign tumors, with the exception of a cellular variant of pleomorphic adenoma, can easily be excluded on basic morphologic ground. Obviously, this is not a conventional pleomorphic adenoma (mixed tumor) because myxoid or cartilagenous differentiation is absent on thorough sampling[4]. Even in a cellular variant of pleomorphic adenoma, one would expect to identify a small amount of myxoid or chondroid matrix[5]. Lack of this feature makes this diagnosis doubtful.

From the malignant group, adenoid cystic carcinoma and epithelial myoepithelial carcinoma can be considered. Adenoid cystic carcinoma, similarly to the current case, would exhibit both epithelial and myoepithelial differentiation, but in contrast, would show focal cribriform epithelial structures together with focal deposition of basement membrane material in a cellular milieu of less phenotypic variability[1-2]. In the case presented, the cell population is strikingly heterogenous with the presence of cuboidal cells, low columnar cells, polygonal cells, oncocytic cells, clear cells and spindle cells.

Epithelial-myoepithelial carcinoma is a well-recognized entity in salivary glands and is considered to have a low malignant potential. In the lung, only nine cases have been described, none of which have reported metastases[6-14]. Therefore, some authors recommended the term pulmonary epithelial-myoepithelial tumor of unproven malignant potential[13]. In this type of tumor, the outer myoepithelial cell layer of the tubules typically has a clear cell phenotype, which was not present in our case. However, some epithelial clear cells were observed within the solid/nested areas.

This tumor does show histologic similarity to the case described by Tsuji et al (1995) under the title adenomyoepithelioma of the lung, in which a 16x15x13 cm tumor was found in the right middle/upper lobe of a 66-year-old man[15]. It was located within the lung parenchyma and had some endobronchial extension. Histologically, it showed both solid and glandular areas with biphasic epithelial and myoepithelial differentiation. However, in contrast to our case, it did not exhibit type II pneumocytic differentiation. The authors considered it to be a tumor of low-grade malignant potential[15].

The term adenomyoepithelioma (as first defined by Hamperl, 1970 and most commonly applied in breast tumor pathology) is a more appealing designation in cases like ours over the more restricted morphologic spectrum of epithelial-myoepithelial carcinoma (as defined in salivary glands)[16]. One could argue that in view of the presence of seromucinous glands in bronchial walls, a terminology used for salivary gland neoplasms rather than that of breast is more appropriate. However, our tumor was not endobronchial and was not attached to large airways but located in the peripheral lung parenchyma. Also, and most importantly, the epithelial cells lining the tubules showed type II pneumocytic differentiation, proven both immunohistochemically and ultrastructurally.
Pneumocytic differentiation, with the exception of one case, has not been documented in salivary gland type pulmonary tumors. Interestingly, the one published case was a salivary gland-type mixed tumor (pleomorphic adenoma) and in comparison to our case, was also not located in the bronchus, but in the lung parenchyma[4]. The pneumocytic differentiation in a pulmonary mixed tumor (pleomorphic adenoma) and in an adenomyoepithelioma is an intriguing phenomenon and favors a differentiation pathway from a pulmonary stem cell over origin from bronchial seromucinous glands.

In view of the type II pneumocytic differentiation and biphasic morphology, pneumocytoma (previously sclerosing hemangioma) and alveolar adenoma can also be considered in the differential diagnosis. Pneumocytomas most commonly have a papillary growth pattern but solid, sclerosing and hemorrhagic variants have also been described[17-18]. In contrast to adenomyoepitheliomas, myoepithelial differentiation has not been documented in pneumocytomas. The exact differentiation lineage of the interstitial cell component of pneumocytomas has still not been characterized[17]. It would be interesting to study, whether in some cases, the interstitial cells of pneumocytomas show myoepithelial differentiation. Alveolar adenomas may have a spindle cell component, however no myoepithelial differentiation has been demonstrated by immunohistochemical and ultrastructural study.[19]

On the basis of biphasic glandular epithelial and myoepithelial differentiation combined with broad phenotypic heterogeneity of the myoepithelial component and type II pneumocytic differentiation of the epithelial cells we propose the term pneumocytic adenomyoepithelioma. Pneumocytic adenomyoepithelioma expands the previously recognized morphologic spectrum of salivary gland type tumors of the lung. In the absence of an infiltrative margin, lack of necrosis and marked cytologic atypia, as well as low proliferative activity, a benign behavior is the most likely. At twelve months follow up, the patient is well and without recurrence of the tumor.

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CASE 8

CONTRIBUTOR: Dr Janez Lamovec, MD; Institute of Oncology, Ljubljana, Slovenia

Clinical history: A 58-year-old woman presented with a necrotic hemorrhagic tumor of the right nipple and areola that appeared after breast trauma 6 months previously.
She had a history of upper outer quadrantectomy of the right breast for infiltrating duct carcinoma followed by radiotherapy 8 years before present tumor developed. She has been taking Tamoxifen for 5 years after surgery. A simple mastectomy was performed.

Pathology: Gross specimen was the resected right breast. In the central part, in the region of the nipple and areola, there was a protruding, oval tumor, covered with dark-brown eschar. It measured 3.5 x 3.5 cm. On cut surface, it was of spongy appearance, red-brown and dark red in color. The tumor measured 2 cm in thickness and was clearly delineated from the subcutaneous adipose tissue, except for a single tongue of a red-brownish tissue at its base. The rest of the breast tissue was mostly adipose with little grossly recognizable parenchyma. In the skin of the breast, several gray-bluish patches and mottled areas were seen that on cut surface involved dermis to the uppermost subcutis.
Microscopically, this is for the most part necrotic and hemorrhagic tumor involving skin, subcutaneous tissue and the deep structure of the nipple and areola, focally extending into the submamillary breast tissue. In contrast to gross circumscription, innumerable and not clearly delineated extensions of tumor tissue at the periphery of the main tumor mass are seen. Centrally, but also peripherally, scattered discontinuous solid areas and foci of tumor cells are found, most peripherally, however, tumor cells form trabeculae and barely to clearly luminized vascular formations. The lumina of the latter are mostly empty, some filled with blood. Tumor cells in the solid areas are mostly epithelioid, with large oval to spindle nuclei, finely threaded chromatin and distinct large nucleoli. Nuclear cytoplasmic ratio is markedly increased, the cytoplasm pale amphophilic or indistinct, cell borders unclear. In some cells, intracytoplasmic lumina are evident, some containing red cells. Mitoses are numerous, many atypical. In foci with trabeculae, and vascular formations, tumor cells are more flattened and spindly, with smaller nuclei and only occasional mitotic figures. In several foci, anastomosing channels show papillary protrusions or endothelial tufts. The channels dissect through collagenous matrix. Several lactiferous duct and large central ducts are surrounded by more solid tumor tissue. In the central parenchyma of the breast, the tissue is focally fibrosed, heavily infiltrated by lymphocytes and plasma cells with more numerous capillary vessels.
The skin lesions away from the tumor (not submitted) were represented by numerous anastomosing vascular structures with jagged outlines and dense inflammatory infiltrate; very poorly delineated from surrounding dermis. Cells lining these anastomosing spaces were flat, with rare hyperchromatic nuclei. Such spaces were empty.
Immunohistochemically, tumor cells were strongly positive for Factor VIII and CD31 but negative for CD34, EMA and keratins. Positive reaction for the former two markers was also seen in skin lesions described above.

Diagnosis: Angiosarcoma, poorly differentiated (grade III), nipple and areola of the breast, after quadrantectomy and radiation therapy for invasive duct carcinoma. Benign vascular proliferations of the skin of the breast.

Follow up: One year and a half after mastectomy, the patient shows no evidence of disease. Beside surgery, she received no other treatment.

DISCUSSION

In the recent classification of breast tumors by WHO, angiosarcoma is defined as malignant tumor composed of neoplastic elements with the morphological properties of endothelial cells (1). It is a type of the neoplasm that arises in the breast more frequently than in any other

organ of the body (2). In a large series of all nonphyllodes sarcoma of the breast, angiosarcoma was most commonly seen (3). Epidemiologically, it is divided into primary (de novo) tumors and secondary tumors such as angiosarcoma following irradiation of the breast after breast conserving surgery or radical mastectomy. Angiosarcoma of the skin and soft tissue of the extremity with chronic lymphedema following radical mastectomy (Stewart-Treves syndrome), although not a breast angiosarcoma proper, has also been epidemiologically included into the category of mammary angiosarcoma. (1).

Angiosarcoma arising in the irradiated breast after breast conserving surgery is being reported with increasing frequency but the magnitude of risk is small (4). However, since more and more women undergo breast-conserving treatment, the incidence can be expected to increase (5). Angiosarcoma following radiation therapy for breast cancer may also appear as a cutaneous lesion of the breast that is limited to dermis and subcutis and sometimes extends to the underlying breast parenchyma (6).

One of the earliest reports on a series of breast angiosarcoma cases came from AFIP, with a complete clinico-pathological data for 10 patients. From illustrations and description of these 10 cases, it is evident that histological appearance of breast angiosarcoma varies from a very benign looking, hemangioma-like tumor to high grade sarcoma with less obvious vasoformative tendency (7).

Grossly, angiosarcoma may appear as a hemorrhagic, friable, spongy tumor of different sizes and variable circumscription its gross appearance may suggest the nature of the tumor. In some cases, tumor is not hemorrhagic and may not appear much differently from carcinoma of the breast.

Microscopically, angiosarcoma shows quite heterogeneous morphological aspects and may be schematically separated into three different categories bearing in mind that overlapping is common (8,9). The first category belongs to well differentiated, low grade (type I) tumors that exhibit open anastomosing vascular channels infiltrating breast parenchyma. The channels are generally lined by one layer of endothelial cells that are flat with predominantly inconspicuous nuclei. Some nuclei may be hyperchormatic, some of them protruding into the lumen. Mitoses are extremely rare. In some foci, endothelial tufting may be present. No necroses, hemorrhages or anaplasia is evident. In rare case, tumor may simulate capillary hemangioma but shows hypechromatic nuclei.

Moderatelly differentiated, intermediate grade (type II) tumors also exhibit anastomosing channels and in larger part resembles low grade tumors but shows scattered cellular foci. In addition, more nuclei are hyperchromatic, endothelial tufting is more common and papillary formations are focally present. Mitoses are still rare but may be found in papillary foci.

Type III, high grade, poorly differentiated angiosarcoma is predominantly solid but in few areas vascular spaces can be identified. It shows extensive areas of hemorrhages, necrosis, and marked cellular pleomorphism with numerous mitoses. In several areas, tumor may appear indistinguishable from MFH or any type of spindle cell sarcoma. In most tumors of higher grades, there is at least some admixture of better differentiated tumor, sometimes at the periphery of the bulk of the tumor.

Post-radiation angiosarcomas of the breast and breast skin are usually high grade tumors, with solid areas, exhibiting epithelioid or spindle sarcoma cells. Low grade foci may be seen.

Another post-irradiation lesion that may appear in the skin of the breast and rarely in the parenchyma proper is the so-called atypical vascular lesion. Its relationship to angiosarcoma is as yet undetermined. The lesion is composed of anastomosing vascular channels lined by one layer of relatively uniform endothelial cells with, with rare hypechromatic nuclei, with little dissection of collagen fibres. The channels are empty, the lesion is relatively circumscribed, with marked chronic inflammation. The lesion is located in the upper or mid-dermal area and do not extends into subcutis (6). Recently, the spectrum of benign changes of the skin following irradiation, predominantly because of breast carcinoma, has been extended to include benign lymphomangiomatous papules and plaques (10).

Immunohistochemically, tumor cells variably express endothelial antigens, such as Factor VIII, CD31, CD34. Well and intermediately differentiated tumors practically always exhibit positive reaction while it may not be so in poorly differentiated angiosarcoma. The new

endothelial marker, FLI 1 appears to decorate a whole spectrum of endothelia-derived tumors (11).

In the differential diagnosis, angiosarcoma may be confounded with several other lesions. Low grade angiosarcoma should be differentiated from hamangioma. The latter us usually small, it rarely reaches 2 cm in diameter. It is well circumscribed grossly and microscopically. It may have anastomosing channels but these are rare. Pseudoangiomatous stromal hyperplasia (PASH) may superficially resemble angiosarcoma; the use of immunohistochemistry resolves the problem easily - by negative reaction of cells surrounding stromal slits for endothelial markers(12). Recently described papillary endothelial hyperplasia of the breast may mimic angiosarcoma, papillary projections covered by cells without significant atypia, the lack of solid foci, and location in the vessel or association with thrombus are distinguishing features (13). Rare acantholytic squamous cell carcinoma of the breast may to some extent on H&E slides simulate angiosarcoma; the latter is keratin positive and negative for endothelial antigens (14).

Prognosis of breast is mostly dependent on the grade of the tumor. Survival varies from 91% at five and 81% at 10 years for grade I tumors to 31% at 2 and 14% at 5 and 10 years for grade III tumors. (9). Metastases are most common in the lungs, skin, bone and liver. Axillary lymph nodes are only exceptionally involved. The treatment of choice is simple mastectomy. The role of chemotherapy remains uncertain (2). In rapidly growing angiosarcomas, a hyperfractionated radiotherapy followed by surgery has been recommended (15).

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Case 9

CONTRIBUTOR: Dr Michal Michal, MD; Medical Faculty of the Charles University in Pilsen, Czech Republic

Case history: Patient was 35-year-old, phenotypically normal male who had gradually enlarging left testicular mass. The tumor grew for 25 years. The testis was elastic and painless. Contralateral testicle had normal size and consistency. No enlarged lymph nodes or metastases were revealed by ultrasonography and computed tomography. Serum levels of α-fetoprotein, beta-HCG and CEA were within the normal range. Left orchiectomy was performed. No adjuvant chemotherapy and radiotherapy was indicated. Patient is 9 years after the surgical excision free of disease.

Grossly, the testis was replaced by uniform whitish soft tissue. No infiltration of testicular adnexa was noted. It was round-shaped and well circumscribed and it had white color 4 x 5 x 5 cm in size. Unaffected testicular tissue was compressed by the tumor. No infiltration of testicular adnexa was noted.

Pathological findings: Histologically, the tumor showed groups or single germ cells surrounded by dense sex cord stroma. There were areas, where spindle cell stromal component predominated (Figure 1), however, in most areas the dominant cells of the microscopical fields were blastic germ cells (Figure 2). Germ cells were characterized by abundant clear cytoplasm and blastic nucleus with fine chromatin having one to several inconspicuous nucleoli. Germ cells were localized on cellular background of diffusely growing spindle shaped stromal component which, in rare places formed thin trabeculae with peripheral palisading. Germ cells often varied in size ranging from small deeply blue staining to large blastic germ cells. They revealed frequent mitoses including atypical ones. No entrapped germ cells within preexisting canals of the testis were seen in any of the reviewed slides. On immunohistochemistry many germ cells reacted positively with antibody to AE1-AE3 by paranuclear dot-like or rod-like positivity (Figure 3). The germ cells were negative for placental alkaline phosphatase and inhibin antibodies. Spindle cell stromal component was positive for inhibin. All tumor cells were immunohistochemically negative with antibodies to CD 117, EMA, CAM 5.2, S-100 protein and α-smooth muscle actin. MIB 1 antibody stained almost exclusively the germ cell (20%).

Ultrastructurally, the germ cells had round to oval nuclei with finely and evenly dispersed nucleoplasm and one to three nucleoli. The cytoplasm was abundant with small amount of common organelles including mitochondrias, some annulate lamellae and stacks of endoplasmic reticulum. Glycogen particles were not recognized. Sex cord-stromal cells were smaller with irregular shapes endowed with basal lamina. They were interconnected by desmosomes. The nuclei had deep indentations, small nucleoli, which were much less apparent than in the germ cells. The cytoplasm of these stromal cells contained moderate numbers of mitochondrias and stacks of endoplasmic reticulum and intermediate filaments. No structures similar to Charcot-Böttcher inclusions were found.

Diagnosis: Unclassified mixed germ cell sex cord-stromal tumor) of the testis (Talerman's tumor)

DISCUSSION

Talerman's tumors occur in the gonads of phenotypically and genetically normal subjects. They were first described in the ovaries by Talerman (6,8,10,12). Later there were published several cases of this tumors in the testis (1,1,2,3). Existence of these tumors in the testis was, however, questioned recently by a group of authors. These pathologists reviewed nine testicular sex-stromal tumors containing germ cells, which closely mimicked Talerman's tumor but differed from the latter, in that the germ cells were entrapped and nonneoplastic rather than neoplastic (11). The nonneoplastic nature of the germ cells in their cases was supported by their peripheral distribution and association with entrapped seminiferous

tubules. The germ cells tended to occur in clusters having vaguely tubular shape, consistent with occurrence within preexisting seminiferous tubules. In one case inhibin stain showed association of germ cells with nonneoplastic Sertoli cell. The germ cells in their cases lacked large, vesicular nuclei, prominent nucleoli, closely resembling type A spermatogonia (11).
Our case differed from any of the cases of Ulbright et al (11). The tumor was composed of large germ cells with vesicular nuclei and spindle sex cord-stromal cells. The germ cells wee not seen in any of the 20 sampled blocks to be associated with preexisting tubules and in most of the slides the germ cells formed predominant part of the microscopic fields. In addition, the size of the germ cell varied ranging from small cells with small hyperchromatic nuclei to large blastic cells with vesicular nuclei. In that respect, the nuclear variations in the germ cells in our case had some similarities to nuclear variations seen in the spermatocytic seminomas (4). Atypical mitoses in many germ cells present in our case are another proof of the neoplastic nature rather than nonneoplastic nature of these cells. Lack of immunoreactivity with antibody to placental alkaline phosphatase in our case as well as in the case of Matoska and Talerman (1) does not necessarily mean nonneoplastic nature of the cells, since germ cells in spermatocytic seminomas are consistently negative with this antibody as well. Positivity with antibody to cytokeratin AE1-AE3 is an interesting and hitherto unreported feature of the tumor.

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CASE 10

CONTRIBUTOR: Cesar Moran, MD; M D Anderson Cancer Center Houston, TX, USA

Clinical History
A 48-year-old man presented with tender swelling of the right testicle of several weeks duration. Physical evaluation did not disclose any other important pathology. A right orchiectomy was performed.

Gross Features
The tumor was described as solid, light brown to tan, well circumscribed but not encapsulated, measuring approximately 3 cm in greatest dimension. The cut surface did not show evidence of necrosis or hemorrhage.

Histopathologic features
The low power magnification showed a well-defined tumor obliterating normal testicular parenchyma. The neoplastic cells were arranged in a prominent nesting pattern separated by thin fibroconnective tissue. At higher magnification, the tumor cells showed pale eosinophilic cytoplasm with round to oval nuclei and inconspicuous nucleoli. Rosettes were commonly seen. In focal areas, there was the presence of a pseudoglandular pattern composed of ribbons of cells forming gland-like structures. The higher power magnification showed similar characteristics, namely cells with pale eosinophilic cytoplasm, round to oval nuclei, and inconspicuous nucleoli. Mitotic activity, areas of necrosis and/or hemorrhage were absent. Adjacent testicular parenchyma was within normal parameters.

Immunohistochemical features
A panels of antibodies on formalin-fixed paraffin-embedded tissue were performed, including broad-spectrum keratin cocktail, chromogranin, synaptophysin, P53, EGFR, and CD117 (C-kit). Keratin cocktail, chromogranin, and synaptophysin were positive while P53, EGFR, and CD 117 (C-kit) were negative.

DIAGNOSIS: Well Differentiated Neuroendocrine Carcinoma (Carcinoid Tumor) of the testis

DISCUSSION

Neuroendocrine carcinomas of the testis are unusual tumors that have been calculated to represent no more than 1% of all testicular tumors. As has been the case with other anatomical areas in which these tumors may occur, the terminology and criteria for diagnosis used has been that of similar tumors occurring in the lung. However, the diagnostic criteria to designate these neoplasms have been changed over the last few years.

Obendorfer introduced the term carcinoid in 1907 to separate a group of tumors in the small intestine that behave less aggressively than conventional carcinomas. Years later, Gosset and Masson suggested that these tumors derived from Kultchisky cells by demonstrating argentaffin granules in the cells. Williams and Sanders presented their classification based on embryologic divisions of the gut and divided them into foregut, midgut, and hindgut with their specific hormonal association. However, controversy has always existed regarding the best way to predict prognosis. In 1972, Arrigoni et al introduced the term “Atypical Carcinoid” to designate a group of tumors characterized by increased

mitotic activity in the presence of recognizable carcinoid pattern (1 mitoses x 1-2 hpf = 5-10 mitosis x 10hpf), pleomorphism, and irregularity of nuclei with prominent nucleoli, hyperchromatism, increased cellularity with disorganization of the architecture, and tumor necrosis. Despite these criteria for "Atypical Carcinoid," numerous publications on the subject have used different histopathologic criteria, which have confused the proper classification of these neoplasms. More recently, the histopathologic criteria for the diagnosis of "Atypical Carcinoid," has changed, namely in the mitotic count, which currently has been lowered to > 2 x 10hpf.

Neuroendocrine carcinomas (Carcinoid) in the testicular region have been recognized for some time in the literature; however, for the most part, it has been in the form of single case reports. Thus, it is very difficult to draw from the literature whether the tumors described can be categorized today as well or moderately differentiated tumors. Clinically, the age group expands from fairly young men to adult individuals (average age: 32 years). The patients may present with a painless tumor mass, tender swelling of the testicle, or with other symptoms including carcinoid syndrome. None of the patients has had an associated carcinoid syndrome. All the patients have invariably undergone orchiectomy. In about 10% of the cases, metastatic disease has been described. It has been stated that testicular neuroendocrine tumors may be encountered in three different clinical settings: 1) as a component of a teratomatous tumor; 2) as a metastatic lesion; and 3) as a de novo neuroendocrine neoplasm. The first two clinical settings are relatively easy to explain; however, the third one becomes a challenge since the presence of neuroendocrine cells or Kultchisky cells has not been described in the testicle. Berdjis and Mostofi in 1977 presented a series of ten cases of what the authors called "Carcinoid tumors of the testis," two of the patients died in a period of 4 to 6 years. Although one of them clinically had metastatic disease to the posterior abdominal wall, no autopsy was performed on either patient. On the other hand, in at least two of the cases described, the gross description casts some doubt as to whether these tumors were pure neuroendocrine carcinomas. One tumor is described as semi-necrotic mass and the other as hard cartilaginous mass (cases 1 and 2). It is possible that both of these cases may have been part of a teratomatous tumor. Both patients received radiation therapy. One had a survival of 16 years, while no follow-up was recorded in the second case. Nevertheless, it is apparent that at least 20% of the tumors in this series of cases followed an aggressive behavior. Unfortunately, the microscopic description of all the cases is not in enough detail to possibly divide the cases into low or intermediate grade category. Zavala-Pompa, et al, described three cases, including a case previously recorded in the literature. Two of the cases described can be categorized as low-grade (well- differentiated) tumors, while one case very likely corresponds to an intermediate grade (moderately-differentiated) tumor. Unfortunately, it is in the latter case in which no meaningful follow-up was obtained to assess behavior.

When we used current criteria to separate these tumors into low and intermediate grade of malignancy, we noted an important difference. In our experience, in one case labeled of intermediate grade, the patient died within one year after surgery, while in five additional patients in whom the tumor was categorized as low grade, the behavior was that of a low-grade neoplasm (alive and well 2-4 years later). We can safely assume that current criteria to separate neuroendocrine tumors appear to correlate with clinical behavior. On the other hand, immunohistochemical studies correlate with the neuroendocrine nature of the neoplasm by showing strong diffuse positive reaction for neuroendocrine markers such as chromogranin and synaptophysin. In regard to the treatment of these tumors, it appears that for the low-grade tumors, surgical resection in the form of orchiectomy with close follow up is the treatment of choice. Whether higher-grade tumors should get additional therapy remains a choice based on an individual clinical analysis, which should also include clinical staging.

The differential diagnosis of testicular neuroendocrine carcinomas will include Sertoli cell tumors, which at low power magnification may give a neuroendocrine-like pattern; however, these tumors would not show positive staining for neuroendocrine markers.

Another tumor that may pose a problem is granulosa cell tumor. In this setting, the presence of Call-Exner bodies may be confused with rosette formation.

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CASE 11

CONTRIBUTOR: Dr Paul E Wakely, Jr., MD; The Ohio State University College of Medicine, Columbus, Ohio, USA

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 employed as a copper welder whose factory requires biennial health screenings. Review of a chest X-ray from 2 years earlier showed a 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. Microscopically, the mass consisted of a solid infiltrate of lymphocytes that replaced the alveolar parenchyma. At the periphery of the mass 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 follicles while in others the follicles had been colonzied 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. In the 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. These were best appreciated using a cytokeratin stain that demonstrated 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: **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 a 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 12

CONTRIBUTOR: Dr Noel Weidner, MD; University of California, San Diego, CA, USA

Case history: Regrowth of uterine mass. The submitted specimen was a 661 gm, 15.0 x 12.5 x 8.5 cm intact uterus with cervix. The endometrial cavity was distorted by a bulging, ill-defined tumor measuring 9.9 x 9.0 x 10.0 cm.

Determination of Malignancy for Uterine Smooth-Muscle Tumors

The vast majority of smooth-muscle tumors can be quickly assigned to a benign group (i.e., low cellularity, no atypia, & no mitotic figures) or a malignant group (i.e., frankly invasive, significant cellular atypia, coagulative necrosis, & high mitotic count)[1-5]. However, occasional exceptions occur, and it can be difficult to predict behavior from histologic appearances alone – that is, some with bizarre cells can be benign and some cytologically bland tumors can metastasize (see note below).

(Note: For example, bizarre [a.k.a., atypical, or symplastic] leiomyoma mimics leiomyosarcoma. Symplastic leiomyoma can be distinguished from leiomyosarcoma, since symplastic leiomyomas have highly atypical nuclei with a smudgy, dark chromatin pattern ["degenerative"], and these atypical cells are interspersed among non-atypical cells. Moreover, mitotic figures are rare, tumor borders are non-infiltrative, and there is no coagulative tumor-cell necrosis. So-called "benign metastasizing" leiomyoma is the occurrence of benign-looking, smooth-muscle tumors in lung, lymph node, and/or other sites and associated with typical uterine leiomyomas – the latter may have been previously resected.[115-122] This is a diagnosis of exclusion after the uterine tumor has been thoroughly sampled to rule-out leiomyosarcoma and after documenting the absence of smooth-muscle tumors elsewhere [e.g., GI tract] that might serve as an alternative primary site. These "metastatic" nodules show circumscribed growth of bland-looking smooth-muscle cells growing in fascicles. In lung there may be entrapped, cleft-like, epithelium-lined spaces. Patients with "benign metastasizing" leiomyoma have long-term survival. The proposed pathogeneses includes multifocal primary tumor, vascular dislodgement from uterine leiomyoma, intravenous leiomyomatosis, and delayed metastasis from an inadequately sampled low-grade uterine leiomyosarcoma.)

For uterine smooth-muscle tumors, mitotic frequency is the most important predictor of outcome, although other histologic parameters contribute. Unfortunately, use of two categories (benign vs. malignant) places some tumors with very low risk of malignancy into the malignant category in order to guarantee a favorable outcome in the former.[6-18] Hence, the moniker "smooth-muscle tumor of uncertain malignant potential" (STUMP) is applied to those tumors possessing features that have been associated with occasional aggressive behavior.[6-18] STUMP designates a group of smooth-muscle tumors with a definite, yet very low, risk of recurrence and metastasis. (Actually STUMP designates smooth-muscle tumors of low malignant potential [STLMP] – but, STLMP is an acronym difficult to pronounce.) Hysterectomy should constitute adequate treatment for such tumors, but if only myomectomy is performed, follow-up without hysterectomy may be justified for those opting to preserve fertility. In contrast, leiomyosarcomas (i.e., when diagnosed using strict morphologic criteria) are highly aggressive neoplasms associated with survivals of 15% to 25%.[6-18] Bona fide leiomyosarcomas show a high frequency of local recurrence and distant metastasis, especially intra-abdominally and to lung. Unfortunately, histologic grading has not been found useful in stratifying groups into varying degrees of malignancy.

Assessment of cytologic atypia, infiltrative growth, coagulative tumor necrosis, and mitotic figure content are currently used in determining malignancy in smooth-muscle tumors of the uterus – the assessment of each integrated with or influencing the relative value of the other (see Table below). More specifically, the common group of normocellular tumors (i.e., without cytologic atypia, coagulative tumor necrosis, or infiltrative growth) are considered

benign, although those having mitotic counts beyond 15 per 10 HPFs (400x) are classified as STUMP.[6-18] Some have suggested calling tumors showing mitotic counts between 5 to 15 per 10 HPFs leiomyomas with increased mitosis or mitotically active leiomyomas; otherwise they look like the usual benign leiomyomas.[6-18] Such tumors occur almost exclusively in the reproductive years and are associated with pregnancy, progestogen usage, or secretory phase of the menstrual cycle.

In the next group, smooth-muscle tumors having cellularity greater than that of the normal myometrium, mild cellular atypia, infiltrative growth, abnormal mitotic figures, and mitotic counts from 5 to 10 per 10 HPF are classified as STUMP - those exceeding 10 per 10 HPFs are leiomyosarcomas, while those with mitotic counts below 5 per 10 HPFs are benign leiomyomas.[6-18] However, there is an important exception – myxoid smooth-muscle tumors with infiltrative growth and/or vascular invasion are myxoid leiomyosarcomas, even when they have low mitotic counts (the abundant myxoid stroma dilutes the mitotic figure counts). Myxoid smooth-muscle tumors therefore require thorough sampling of the tumor borders as well as the non-myxoid areas. A myxoid smooth muscle tumor can be safely labeled benign only if the entire growth is well circumscribed with no mitotic figures. However, the tumor should be labeled STUMP, if rare mitotic figures are present, or leiomyosarcoma, if it has invasive borders or contains more than 1 mitosis per 10 HPFs. True myxomas, identical to those occurring in the somatic tissues, have rarely been reported in patients with the Carney's complex - cardiac myxoma, spotty pigmentation, and endocrine hyperactivity.[19] True uterine myxomas are often small (< 2 cm), have a uniform appearance, and show no smooth-muscle differentiation.

Assessment of Malignant Potential of the Smooth-Muscle Tumors of the Uterine Corpus

<table>
<tr><td></td><td colspan="5">Mitotic Count/10 HPF</td></tr>
<tr><td></td><td>0</td><td>2</td><td>5</td><td>10</td><td>15</td></tr>
<tr><td>Normocellular, no nuclear atypia</td><td colspan="4">BENIGN</td><td>UNCERTAIN MALIGNANT POTENTIAL</td></tr>
<tr><td>Cellular tumor, minimal nuclear atypia or invasive margins, or abnormal mitotic figures</td><td colspan="2">BENIGN</td><td>UNCERTAIN MALIGNANT POTENTIAL</td><td colspan="2">LEIOMYOSARCOMA</td></tr>
<tr><td>Moderate to marked atypia or epithelioid morphology or intravascular growth or coagulative necrosis</td><td>BENIGN</td><td>UNCERTAIN MALIGNANT POTENTIAL</td><td colspan="3">LEIOMYOSARCOMA</td></tr>
</table>

In the third group of smooth-muscle tumors have moderate or marked cytologic atypia, epithelioid cellular morphology, intravascular growth, or true coagulative tumor-cell necrosis. Those in the third group, having mitotic counts between 2 to 5 per 10 HPFs, are considered STUMP. Tumors of the third group with less than 2 mitotic figures per 10 HPFs are benign leiomyomas; those with more than 5 mitotic figures are leiomyosarcomas. This third group also includes serosal smooth-muscle tumors that have detached from the uterus and implanted on the omentum, peritoneum, or pelvic wall (i.e., so-called "parasitic" smooth-muscle

tumors).[6-18] In one study, coagulative tumor-cell necrosis was found to be a powerful predictor of poor clinical outcome; thus, smooth-muscle tumors showing this feature are placed in the third group.[19-23]

Coagulative tumor-cell necrosis is characterized by abrupt transition between necrotic cells and preserved cells. Coagulative tumor-cell necrosis should be distinguished from hyalinizing necrosis that occurs in benign smooth muscle neoplasms. Hyalinizing necrosis is characterized by a zone of collagen interposed between the dead cells and the preserved cells, reminiscent of organization of an infarct. An alternative approach for determining malignancy of smooth-muscle tumors, based on presence or absence of coagulative tumor-cell necrosis, is depicted in Table below.[19-23]

Alternative Approach for Evaluation of the Malignant Potential of Uterine Smooth Muscle Tumors (Excluding Epithelioid and Myxoid Types)

		Coagulative Tumor Necrosis	
		Absent	Present
Diffuse Significant Cellular Atypia	Absent	LEIOMYOMA ("Leiomyoma with increased mitoses" if MI ≥ 5)	MI ≥ 10: LEIOMYOSARCOMA MI < 10: SMOOTH MUSCLE TUMOR OF LOW MALIGNANT POTENTIAL (limited follow-up experience)
	Present	MI ≥ 10: LEIOMYOSARCOMA MI < 10: ATYPICAL LEIOMYOMA WITH LOW RISK OF RECURRENCE	LEIOMYOSARCOMA

Note: MI, mitotic index (number of mitotic figures per 10 high power fields). Diffuse significant cellular atypia = Generalized moderate or severe cytologic atypia, which can usually be appreciated on low-magnification examination. Tumors with focal significant cellular atypia, no coagulative tumor necrosis, and MI <20 are designated "atypical leiomyoma (yet with limited follow-up experience)."

How to Perform the Mitotic Count: Counting mitotic figures helps distinguish benign from malignant mesenchymal or epithelial-mesenchymal neoplasms of the uterus. These counts are usually expressed as the number of mitotic figures per 10 high-power fields (HPFs). But, some pathologists doubt the reproducibility and reliability of making such mitotic counts (i.e., the so-called "no-count" pathologists).24-32 They object because of the variabilities in field size between microscopes, problems in distinguishing mitotic figures from apoptotic nuclei or lymphocytes, variable histology, variably delayed fixation, and variable training and meticulous application in counting.[24-32] Using standardized field area or application of special stains in assessing DNA synthesis (e.g., Ki-67) may help solve these issues. We adhere to the guidelines proposed by Kempson and Hendrickson[5] **1)** First, specimens should be promptly and thoroughly fixed followed by proper (first-rate) processing, sectioning, and staining. **2)** Adequate sampling for atypical tumors (other than the typical leiomyoma) is at least one block per centimeter of tumor diameter. **3)** Histologic sections should be no thicker than 5 microns. **4)** Only clear-cut and convincing mitotic figures should be counted, that is, in which at least some chromosome twigs or arms are identifiable and in which the cell does not appear to be shrunken, apoptotic, or lymphocytic. (A stain such as periodic acid-Schiff (PAS) may facilitate the distinction from pyknotic nuclei.) **5)** Use only a 40x objective with 10x or 15x eyepiece. **6)** Perform counts in areas with highest mitotic activity, starting from a field with a mitotic figure(s) and then moving at random to nine other consecutive fields to give the total

mitotic figure count per 10 HPF. **7)** Finally, repeating three other sets of 10 HPFs and taking the highest count in any single set of 10 HPFs, that is, don't use the average.

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