Diagnosis:
a. Chorangioma

Answers:
1. a. Beckwith-Wiedemann
2. c. Fetal thrombocytopenia and disseminated intravascular coagulation
3. a. Chorangiomas can cause arteriovenous shunting, fetal hydrops and heart failure

Discussion:
Chorangioma is a well circumscribed lesion composed of expanded stem villi with numerous endothelial lined capillaries. The villous stroma is composed of collagen, fibroblasts and scattered histiocytes. Surrounding trophoblastic tissue demonstrates minimal hyperplasia. In contrast, chorangiosis is defined by increased number of capillaries within terminal villi rather than stem villi i.e, “more than 10 capillaries in 10 scattered terminal villi.” Chorangioma is associated with a gross lesion as above while chorangiosis is a microscopic finding. Placental mesenchymal dysplasia is a diffuse process composed of expansion of stem villi with cavernous cysts as well as patchy foci of capillary proliferations that resemble chorangioma in a background of cellular villous stroma. Also the chorionic plate vessels tend to be dilated and enlarged. Placental hydrops manifests with diffuse pallor and an enlarged placenta rather than localized firm pale lesion. Microscopically, it shows villous edema with “floating Hofbauer cells.” Hypertensive decidual vasculopathy manifests with fibrinoid necrosis of basal plate vessels and acute atherosis. Villous infarcts appear shrunken rather than expansile, histologically composed of a conglomeration of villi with decreased trophoblastic basophilia, ischemic necrosis and fibrin deposition.

The placental villous capillary lesions are comprised of three broad entities: Chorangiosis, chorangioma and chorangiomatosis. Chorangiosis is seen more commonly in term placentas and is associated with decreased intervillous oxygen tension and maternal diabetes. It is characterized by increased capillary profiles within terminal villi due to excessive branching angiogenesis or folding of elongated capillaries within terminal and intermediate villi. The pathogenesis is presumed to be mediated by vascular endothelial growth factor A and insulin, induced by placental hypoxemia and maternal hyperglycemia, respectively. In contrast, a chorangioma is composed of proliferation of vessels within a stem villus, presumed to be due to a dysregulation of mesenchymal-endothelial cell interactions mediated by placental growth factors, angiopoetins (ANG-1 and 2) and their receptors (TIE-1 and 2). Chorangiomas are more frequently reported around 32 weeks of gestation and have known associations with multiple pregnancies and pre-eclampsia. Multiple chorangiomas constitute the diagnosis of chorangiomatosis which can be localized or diffuse.

The pathologic diagnosis is usually affirmed with routine light microscopy on hematoxylin and eosin stained sections with correlation of gross findings. Immunohistochemical stains CD31 and CD34 can help highlight the vasculature and are noted to be more sensitive in identifying more numerous capillaries than those identified on routine stained sections. Ogino et al have reported an interesting observation that chorangiomas and chorangiomatosis show a complete rim of MSA positive pericytes encircling the capillaries while capillaries in chorangiosis are only partially encircled by MSA positive pericytes.

The clinical significance of chorangiosis is not well established while chorangiomas and chorangiomatosis have been associated with intra-uterine growth restriction. The latter can also cause serious fetal complications such as fetal thrombocytopenia and disseminated intra-vascular coagulation as well as polyhydramnios, fetal hydrops and cardiac failure due to arteriovenous shunting.

References/Recommended Readings:
SPP Case 16-02

Diagnosis:
a. CLOVES (congenital lipomatous overgrowth, vascular malformations, epidermal nevi, skeletal abnormalities) syndrome

Answers:
1. e. Parkes Weber syndrome
2. d. Somatic mutations in AKT1
3. a. CLOVES syndrome

Discussion:
The hematoxylin-eosin-stained section shows a hamartomatous growth of vascular channels and fibroconnective tissue, which involves the superficial and deep dermis and the subcutis (Fig 3). A large number of the vascular channels have thin, delicate walls lined by one layer of attenuated cuboidal endothelium, consistent with lymphatic vessels (Fig 4). The lymphatic vessels are scattered throughout the tissue, and occasionally have a more clustered appearance. A smaller subset of the vascular channels shows a smooth muscle wall, resembling veins (Fig 5). Definitive arteries are not seen. Some of the vessels have lymphocytic aggregates associated with their walls. The background is composed of a dense proliferation of collagen; scattered smooth muscle fibers are present and seem in disarray in some areas. The subcutaneous tissue demonstrates areas of hemorrhage. There is no cytologic atypia present. The epidermis shows focal mild acanthosis.

While there is no adipose tissue present in the slide that accompanies this case, other slides (not distributed) showed the collagen bands described above infiltrating within the subcutaneous adipose tissue, without a distinct border, and the adipose tissue appeared in excess.

These histologic findings on the patient’s chest lesion, along

with the presence of an abdominal wall clinically similar lesion, overgrowth of the right hand and foot, digital anomalies, and the finding of a mutation in PIK3CA gene, are in keeping with a diagnosis of CLOVES syndrome.

CLOVES syndrome was first described by the German physician Hermann Friedberg in 1867 (1). The acronym stands for congenital lipomatous overgrowth, vascular malformations, epidermal nevi and skeletal abnormalities. The entity is fairly new and in the past, patients with manifestations of CLOVES were diagnosed as “truncal Klippel-Trenaunay”, or Proteus syndrome.

Clinically and radiologically, CLOVES is characterized by hamartomatous overgrowth of adipose and connective tissue, along with predominantly lymphatic malformations, present on the trunk and abdominal wall, and involving the intrathoracic, abdominal and retroperitoneal space. Capillary and venous malformations are also common, which can lead to the misdiagnosis of Klippel-Trenaunay. Occasionally, the patients can also have arteriovenous malformations, which are a characteristic feature of Parkes Weber and PTEN hamartoma syndrome. Patients with CLOVES often have hemifacial overgrowth accompanied by epidermal nevi. The acral deformities frequently seen in CLOVES are symmetrically large, wide feet and hands, macrodactyly and wide first web space of the hands and feet (“jar grip”, and “sandal gap”). The latter is not specific of CLOVES and it can be seen in disseminated capillary malformation with overgrowth, Klippel Trenaunay, macrocephaly-capillary malformation syndromes, as well as in normal individuals. Some degree of palmar and plantar wrinkling of the skin can be seen, but not to the degree seen in Proteus syndrome. The “S” in CLOVES was added later, when it was established that the fat overgrowth and infiltration in the paraspinous muscles and in proximity of other bones, causes scoliosis and other skeletal abnormalities.

Interestingly, patients with CLOVES may have renal size discrepancy, and can develop Wilms tumor in the larger kidney (2).

Because of the presence of dilated thoracic and central veins, these patients are at risk for thrombosis and pulmonary embolism (1). Therefore, detailed clinical follow-up is needed.

In 2013, Kurek et al. (2) described missense PIK3CA mutation in the hamartomatous lesions of six patients with CLOVES, and absence of mutation in these patients’ saliva and blood, consistent with somatic mutation. The mutations identified were c.314A>G (p.His1047Arg) in two patients, c.1624G>A (p.Glu542Lys) in two patients, and c.1258T>C(p.Cys420Arg) in the last two patients. The patient described in our case was
found to have a c314A>G(p.His1047Arg) mutation, as described in this paper.

The diagnosis of CLOVES is multidisciplinary and involves a careful integration of the clinical presentation, imaging studies and molecular findings, because there is tremendous overlap with other overgrowth syndromes. For example, the same PIK3CA mutations identified in CLOVES syndrome can also be found in a subset of cases of Klippel-Trenaunay, lymphatic malformations and fibroadipose vascular anomaly (3,4). For the purpose of providing a practical approach to the diagnosis of these complex syndromes and lesions, here is a brief description of each of them, and of the syndromes mentioned in the multiple choice questions.

Lymphatic malformations are usually present at birth, and if not, most of them appear by the age of two. They can be localized or regional. Occasionally, they can involve multiple organ systems. In the current terminology, they are referred to as microcystic or macrocystic (channels larger than 0.5 cm in diameter). They can be present in any location, but the most common are the ones in the axilla, shoulders, neck, tongue, floor of mouth, proximal limbs and perineum. Histologically they are characterized by lymph channels with lumens of variable size, lined by an attenuated layer of endothelium. The larger lymph channel walls may demonstrate a smooth muscle layer. The content of the lumens varies, and can be proteinaceous, or bloody. Thrombi can be also present. The vascular channels are intermixed with a collagenous background stroma. Immunostains like D2-40 and Lyv1 are helpful in highlighting the vascular lining, and CD34 and CD31 demonstrate only patchy staining (3).

The pathogenesis of Klippel-Trenaunay syndrome (KTS) is controversial; while some researchers postulate that it is a dominantly or paradigmatically inherited condition (5), there is not enough evidence to support this theory. The most accepted concept is that it is a sporadic condition, the result of a somatic mutation that happens during the embryonic vasculogenesis (6). The vascular anomalies in KTS consist of capillary-lymphatic-venous malformations that are present at birth, and males and females are equally affected. In the majority of cases, only the lower limb is involved and combined upper and lower limb involvement occurs in a small percentage of cases (approximately 15%). Also, the majority of the cases are unilateral, and there usually is hypertrophy without vascular anomaly in the opposite site. The head and neck are usually spared, and the thorax and abdomen is usually not involved. Multiple genetic and chromosomal abnormalities have been described in KTS, but none that define the entity (7).

Fibroadipose vascular anomaly (FAVA) was described by Alomari et al (8), as a vascular anomaly that affects the calf, forearm and thigh. It is characterized by fat infiltration of the muscle, fibroadipose tissue overgrowth, and anomalous venous channels, some with phleboliths. Occasional more delicate channels, resembling capillaries and lymphatics are seen. Because of severe infiltration of skeletal muscles, the main clinical presentations are contractures and pain. FAVA was shown to have postzygotic somatic mutations in PIK3CA (3, 8).

The same way the Greek sea god, Proteus, was famous for being able to assume different forms, Proteus syndrome can have various phenotypes; hence the high frequency with which other vascular anomalies are erroneously classified as Proteus. It is caused by a somatic activating mutation in AKT1 (9). The manifestations of Proteus syndrome include cerebro-vascular connective tissue nevi (pathognomonic), epidermal nevi, lipomas, ovarian cystadenomas and salivary monomorphic adenoma. The patients also have progressive, asymmetric skeletal overgrowth, as well as ocular, renal and pulmonary abnormalities. The most important piece of information in establishing a diagnosis of Proteus is whether the overgrowth is congenital or postnatal, because the overgrowth of tissue in Proteus is considered to always be postnatal. The vascular anomalies present in Proteus are capillary, lymphatic and venous malformations. They are usually progressive, and approximately 10-20% of the patients die due to complications of their venous anomalies (emboli) (10). Evaluation for thrombopathy in consideration for anti-thrombotic prophylaxis is encouraged in patients with Proteus syndrome undergoing surgical procedures.

Parkes Weber syndrome is a fast flow, arteriovenous-capillary malformation that is present at birth, and is characterized by germline or possibly somatic mutations in RASA1 in approximately 50% of the cases. The vascular anomaly is most commonly seen in the lower limb, but upper limb involvement has also been described. The limb is symmetrically enlarged, and it is covered by skin that shows a pink, macular stain that usually involves the lower abdomen, too. Because of the arteriovenous malformations, the limb is usually warm and a bruit can be heard with auscultation. Although rarely, the syndrome can also comprise lymphatic anomalies. The overgrowth of the limb is caused by an overgrowth of the soft tissue and bone. Cardiac overload is a frequent complication of this syndrome, and can be present at birth (7).

PTEN hamartoma syndrome comprises Bannayan-Riley-Ruvalcaba and Cowden syndromes, which are both caused by germline mutations in PTEN. Clinical manifestations include macrocephaly, developmental delays, autism spectrum disorders, ileal and colonic hamartomatous polyps, penile hyperpigmented macules, skeletal abnormalities, breast tumors, lipo-
mas, angiolipomas, facial tricholemmomas, mucocutaneous neumomas. Also, there is an increased risk of malignancy, especially in breast, thyroid, kidney and endometrium (11). The associated vascular anomalies consist of fast and slow flow lesions composed of arteriovenous malformations, but also with a capillary and lymphatic component. Developmental venous anomalies of the brain are also encountered in patients with PTEN germline mutations. The lesions are most commonly located in the lower extremities, they are multiple, and present with pain and swelling (12).

In conclusion, while this case presents a patient with overlapping clinical, histological and molecular features of CLOVES and Klippel-Trenaunay, the presence of thoracic and abdominal lesions, as well as skeletal abnormalities involving the digits is most suggestive of CLOVES syndrome. Triaging the specimen appropriately, and pursuing molecular testing, was of most value in establishing the diagnosis.

References:
12. Kurek KC, Howard E, Tennant LB, Upton J, Alomari AI, et al. PTEN hamaro-

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SPP Case 16-03

Diagnosis:
E. Melanotic neuroectodermal tumor of infancy

Answers:
1. e. Odontoma
2. d. Melanotic neuroectodermal tumor of infancy
3. d. Maxilla

Discussion:
Microscopically, the tumor is composed of irregular nests and cords of small round primitive cells intermixed with a second population of large cells with associated pigment and a background of fibrous stroma. The small cells are loosely arranged and have scant cytoplasm and round nuclei with condensed chromatin. Fine fibrillar material is seen in the background. The second population of large cuboidal epithelioid cells have eosinophilic cytoplasm with dark pigmented granules and large round nuclei with vesicular chromatin. These large cells appear to line the nests of small cells, creating pseudoalveolar spaces. Tumor infiltrating bone and teeth at different stages of development may be seen in some sections. Immunohistochemical stains demonstrate patchy positivity in the tumor cells for cytokeratin, HMB45, and synaptophysin. Focal positivity is seen with myogenin. The tumor cells are negative for CD99, S100, chromogranin, smooth muscle actin, and desmin. CD45 highlights scattered lymphocytes but is negative in the tumor cells. The classic morphology and supportive immuno-
histochemical stains are consistent with the diagnosis of melanotic neuroectodermal tumor of infancy.

Melanotic neuroectodermal tumor of infancy (MNETI) is a rare tumor of neural crest origin that shows neuroblastic, melanocytic, and epithelial differentiation. The entity was first described by Krompecher in 1918 as a congenital melanocarcinoma. Since that time, it has been referred to by a variety of diagnostic terms including retinal anlage tumor, pigmented congenital epulis, melanotic progonoma, melanotic hamartoma, and melanotic adamantinoma, among others. The neoplasm occurs almost exclusively during the first year of life.
Few cases have been reported in older children and rarely in adults. Greater than 90% of the cases arise within the head and neck region with the anterior maxilla being the most common location, comprising 70% of the cases. Other less common sites include the bones of the orofacial region and the skull (16%). Rare cases have been reported in the central nervous system, soft tissues, paratesticular region, and extremities. A slight male predilection has been described.

The clinical presentation is typically that of a single rapidly growing painless, partly pigmented mass in an infant. Similar to other tumors of neural crest origin, urinary vanillylmandelic acid, serum catecholamines, and serum alpha-fetoprotein can be elevated in a small subset of patients. The levels return to baseline following excision of the tumor. Radiographic features often show an osteolytic lesion, but the lesion may also be radiopaque depending on the degree of pigmentation. MNETI may mimic odontogenic tumors when in close association with an unerupted tooth. The infiltrative borders, cortical destruction, and aggressive features make odontogenic tumors less likely. Grossly, the tumor is unencapsulated and has a solid cut surface with black, gray, or brown pigment deposition. In addition to the microscopic features of this case, mitotic activity and necrosis are absent to minimal. The immunohistochemical profile demonstrates neural, epithelial, and melanocytic differentiation. Both the large and small cells may express vimentin and neuron-specific enolase (NSE). The large cells are also positive for cytokeratins, HMB45, and dopamine-beta-hydroxylase while the small cells mainly express synaptophysin and CD56. S-100 is typically negative in both components. MNETI can also occasionally show glial and myogenic differentiation. Electron microscopy shows melanosomes at various stages of maturation and epithelial features in the large cells and neuroblastic features, including neurosecretory granules and cytoplasmic processes, in the small cells.

The differential diagnosis includes other small round blue cell tumors of childhood. The most challenging distinction is with neuroblastoma, especially on a limited biopsy. MNETI lacks rosette formation and diffuse expression of neuroendocrine markers as seen in neuroblastoma. MNETI has no known recurrent molecular alterations; however, molecular studies may help to exclude other entities with known translocations. The characteristic biphasic morphology of MNETI on larger biopsies and its immunohistochemical profile excludes most of the other entities listed. The age of presentation and location may overlap with congenital granular cell tumor of the newborn (congenital epulis) clinically, but the striking histologic features easily distinguish these lesions from one another. Odontogenic lesions in childhood are rare and usually seen after the age of 6 years during the time of secondary tooth development. The most frequently reported odontogenic tumors in the pediatric population are odontoma and ameloblastoma.

MNETI is a rapidly growing, locally aggressive tumor with an approximate 20-30% local recurrence rate. MNETI can extensively invade and infiltrate bone and soft tissue. Complete radical excision is the treatment of choice and is curative in most cases. The role of adjuvant chemotherapy and/or radiotherapy is unclear, however, and it may be indicated in cases in which surgical resection is incomplete. When arising within or extending into the central nervous system, MNETI tends to spread rapidly and have an aggressive clinical behavior despite radical surgery and chemotherapy. MNETI can undergo malignant transformation and metastasize. A literature review demonstrates a malignancy rate of approximately 6-7%. Metastasis has been reported to the regional lymph nodes, liver, bone, and soft tissue. In these cases, the metastatic tumor typically acquires a monotonous pattern of neuroblast-like cells similar to a conventional neuroblastoma. In cases of widespread metastasis, death usually ensues in months. There are currently no uniform clinical or histologic features to predict malignant transformation; some studies suggest that increased mitotic activity, which is normally minimal, and an inconspicuous large cell population may predict more aggressive behavior.

In summary, melanotic neuroectodermal tumor of infancy is a rare tumor of with unique clinical, histologic and immunohistochemical features including polyphenotypic differentiation along neural, epithelial, and melanocytic lines. Given its rapid and infiltrative growth, early diagnosis with complete excision is needed for the best clinical outcome. Close follow-up is recommended given the high recurrence rate.

References:


Carpenter BF, Jimenez C, Robb IA. Melanotic neuroectodermal tumor of in-
SPP Case 16-04

Diagnosis:

h. Subcutaneous sacrococcygeal myxopapillary ependymoma

Answers:
1. d. Scalp
2. b. Brachyury
3. d. Optical nerve pilocytic astrocytoma

Discussion:

Grossly, the specimen consisted of a 6.2 x 4.0 cm ellipse of skin and soft tissue excised to a depth of 3.0 cm. No lesions were noted on the skin surface. Upon sectioning, a 5.3 x 2.5 x 2.4 cm ovoid, well circumscribed mass was identified in the subcutaneous soft tissue (Figure 2). The cut surface was tan and hemorrhagic (Figure 3 – online only). H&E stained histological sections show a tumor with a myxopapillary appearance, with ovoid to elongated tumor cells with fine nuclear chromatin. The cells are arranged in a papillary configuration, surrounding blood vessels. There is abundant perivascular and intercellular myxoid matrix. (Figure 4(online)-6). Few enlarged, multinucleated cells with more hyperchromatic nuclei are scattered throughout the tumor (Figure 7). In some areas, the tumor is more cellular and few mitotic figures are noted (Figure 8).

Ependymomas usually arise within the spinal cord or in the brain. They account for 60% of tumors of glial origin in the spinal cord and comprise 90% of primary tumors in the filum terminale and cauda equina. Sacral extraspinal ependymomas, which are also known as soft tissue ependymomas, account for less than 5% of all primary sacrococcygeal malignancies [2-3]. As primary tumors, they are found in subcutaneous tissues of the sacrococcygeal region and presacral space and the majority are myxopapillary ependymomas [4].

Myxopapillary ependymomas are typically primary, intradural tumors arising in the filum terminale [1-4]. They can occur in the soft tissue in four general situations: from metastases or direct extension of a primary tumor of the CNS, seen after surgical excision; from direct extension to the soft tissue of the sacrococcygeal area from a primary ependymoma of the lower spinal cord, cauda equina, or filum terminale; from a primary presacral, pelvic, or abdominal tumor and from a primary tumor of the skin and subcutaneous tissue of the sacrococcygeal area without demonstrable connection to the spinal cord or filum [3-4]. Primary subcutaneous sacrococcygeal myxopapillary ependymomas (SSMPE) are believed to arise from the coccygeal medullary vestige or subcutaneous ependymal rests, which are located in the caudal portion of the neural tube. Their site is often marked by a dimple on the skin surface [3]. Sacrococcygeal soft tissue myxopapillary ependymomas are often slow growing masses and may be large at presentation.

The morphology is easily recognizable and the tumor cells stain positively for GFAP and S-100 protein. Cytokeratin positivity has also been described in SSMPE. Hussein and Sur (2008) published two cases of SSMPE which showed positivity for CAM5.2, AE1AE3, and CK7. In our case, the tumor is positive for CAM5.2 and AE1/AE3 in addition to the glial markers. CK20 is negative. This is important in differentiating this tumor from a metastatic carcinoma. Given the similarities in morphology between metastatic carcinomas, chordoma and SSMPE, the clinical history, tumor location, immunohistochemical profile and radiological findings should all be considered when a diagnosis of SSMPE is being entertained[5]. These tumors may behave aggressively with metastasis to regional lymph nodes, lung, bone and liver in up to 20% of the cases after a prolonged disease-free interval, sometimes of up to 10 years [6]. Adequate treatment of SSMPE consists of wide resection, with long-term disease free survival without additional treatment [6].

The differential diagnosis for masses in the sacrococcygeal region in children includes sacrococcygeal teratoma and pilonidal cyst or sinus. Sacrococcygeal teratomas are either cystic and solid or predominantly cystic; they are rarely solid. Over 50% have calcification or ossification and most sacrococcygeal teratomas are discovered in the newborn period. Imaging studies help in the diagnosis, especially if fat is present in the lesion. Pilonidal cysts or sinuses are benign lesions usually present at


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the base of the spine or in the intergluteal cleft. Histologically, a dense inflammatory infiltrate is present in the dermis and there is abscess formation with granulation tissue as well as a foreign body reaction around/to hair shafts.

Syringocystadenoma papilliferum occurs more frequently in the head and neck region and is characterized by papillary structures with a prominent plasma cell infiltrate in the stroma. Hidradenoma papilliferum occurs in the vulvar or perianal area and is a dermal based tumor with a surrounding fibrous capsule. It is characterized by proliferation of papillary, cystic, apocrine glandular structures. Tubular apocrine adenoma is composed of tubular structures. It has no predilection for a specific location. Extraaxial soft tissue chordomas are rare and most cases occur in the acral sites and proximal extremities or trunk. It is usually a multilobated mass comprised of fibrous septa surrounding cords or nests of epithelioid or spindle cells in a myxoid background. Physaliferous cells are characteristic of this tumor, although not required for diagnosis. Positivity for brachyury immunohistochemical stain is seen in almost 100% of the cases.

Neurofibromatosis type 1 is associated with the development of optical nerve pilocytic astrocytoma. Sebaceous carcinoma has a strong association to Muir-Torre syndrome. Pediatric basal cell carcinoma is seen in basal cell nevus syndrome also known as Gorlin Syndrome. The cribriform morular variant of papillary thyroid carcinoma is associated with familial adenomatous polyposis syndrome while adrenal cortical carcinomas are seen in a number of syndromes including, Beckwith-Wiedemann, Li-Fraumeni, multiple endocrine neoplasia 1, Carney complex and congenital adrenal hyperplasia.

References/Recommended Readings:

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SPP Case 16-05  BIOPSY CASE

Diagnosis:
d. Loeys-Dietz syndrome

Answers:
1. e. Vascular Ehlers-Danlos syndrome
2. d. TGFBR2
3. c. Shprintzen-Goldberg syndrome

Discussion:
Heritable connective tissue disorders (HCTD) are multisystem diseases. Many HCTDs have overlapping clinical features affecting the organization and integrity of the supporting connective tissues, such as bone, skin and blood vessels. Manifestations of the disorders, namely vascular compromise in the form of arterial dissection and/or aneurysmal rupture, cause significant morbidity and mortality. Herein we discuss the clinicoradiologic and pathologic findings in our proband’s genetically confirmed Loeys-Dietz syndrome (LDS) with review of the literature to include other HCTDs.

First described by Bart Loeps and Henry Dietz in 2005, the syndrome that bears their names is characterized by the triad of arterial tortuosity with aortic aneurysm and dissection, hypertelorism and bifid uvula/cleft palate. This autosomal dominant connective tissue disease is associated with mutations in either of the two genes encoding the transforming growth factor-ß (TGF-ß) receptors, TGFBR1 and TGFBR2, causing LDS types 1 and 2 respectively. Further analysis has demonstrated mutations in the genes encoding the TGF-ß Signaling Effector Mothers Against Decapentaplegic homolog 3 (SMAD3) and/or the TGF-ß ligand, TGFß2, cause LDS types 3 and 4 respectively. All four gene mutations result in altered TGF-ß signaling resulting in similar phenotypic features in all groups of patients. These manifestations show pervasive systemic involvement with significant overlap with other HCTDs, particularly Marfan (MFS) and vascular Ehlers-Danlos (vEDS) syndromes.

The cardiovascular components of LDS prove the most significant: affected patients display diffuse arterial involvement comprised of vascular tortuosity with an increased risk of aneurysms and dissections throughout the arterial system, associated with an aggressive vascular course. Indeed, initial LDS reports detailed a younger mean age of death (26.1 years) differing from treated patients with MFS (70 years) and vEDS (48 years). Further, up to 98% of patients with LDS harbor an aortic root aneurysm with thoracic aortic dissection resulting in the leading cause of death (67%) followed by abdominal
aortic dissection (22%) and cerebral hemorrhage. Not only do LDS patients have a higher risk of arterial dissection with rupture, but also these pathologies present at both a younger age and with smaller aortic diameters when compared to MFS, prompting the necessity for earlier detection, treatment, monitoring and surgical intervention. Other cardiovascular defects that have been associated with LDS include patent ductus arteriosus (PDA) with or without aneurysm, bicuspid aortic valve, atrial septal defects, coronary and pulmonary artery aneurysms, mitral valve prolapse with insufficiency, left ventricular hypertrophy and atrial fibrillation. In contrast to both MFS and LDS, there is no predisposition for involvement of the aortic root in vEDS.

Additional defining components of LDS include hypertelorism and bifid uvula/cleft palate; however, other craniofacial, skeletal and cutaneous manifestations associated with LDS are comparable with findings in other HCTDs. Craniofacial abnormalities include malar hypoplasia, craniosynostosis, Chiari-I malformation and congenital cervical spine defects including dysplasia of the odontoid process/dens of C2, C1 arch deformity and rotary subluxations. Of note, the severity of craniofacial abnormalities has been correlated with poor cardiovascular outcome. Common skeletal stigmata are similar to those in MFS. Examples include pectus deformity, scoliosis, flat feet, dural ectasia and arachnodactyly. In contrast to MFS, patients with LDS typically show normal height and proportional body size and lack ectopia lentis. Other skeletal findings in LDS include camptodactyly, talipes equinovarus, hindfoot valgus and developmental dysplasia of the hip. Osteoarthritis is a key feature in LDS type 3, caused by mutations in the SMAD3 gene, and affects the distal extremities, knees, hips and spine. Cutaneous findings in LDS show a striking resemblance to those present in vEDS patients consisting of thin, velvety and translucent skin with easy bruising. Another feature of vEDS also seen in LDS type 2 patients is spontaneous organ rupture. Thus, pregnancies should be considered high risk. There are reports detailing complications arising either during pregnancy or postpartum in which cases of aortic dissections, uterine hemorrhage/rupture and arterial rupture are described.

Analogous to the clinical phenotypes, histological findings show significant overlap between LDS and MFS. In both syndromes, there is loss or fragmentation and disarray of the elastic fiber content of the arteries with concomitant increase in collagen deposition. Interestingly, both show medial degeneration of the cystic (CMD) and diffuse types (DMD). In a study by Malezewski et al, it was reported that an increased amount of collagen coupled with DMD and a lack of CMD could help distinguish LDS from MFS; however, the authors note that a definitive diagnosis cannot be made from light microscopy alone due to histological overlap between the two. The histological architecture in our case supported their findings: absence of normal parallel arrangement of elastic fibers and diffuse medial degeneration with intralamellar loss and fragmentation of elastic fibers with increased deposition of ground substance. Although no unique microscopic findings exist, certain salient features are helpful in differentiating LDS from other aortic root diseases. Aortitis with inflammation and giant cells is not present in LDS. In addition, LDS lacks lamellar medial necrosis, which is identified in several acquired aortic root disorders. Special stains recommended for the visualization of these pathological changes include Masson trichrome, elastic van Gieson and/or Movat pentachrome. At present there are no immunohistochemical protocols of proven diagnostic utility.

Approximately 19% of patients with aortic dissections do not have the systemic manifestations of HCTDs and are classified as familial thoracic aortic aneurysm and dissection (FTAAD) caused by mutations in the ACTA2, MYH11 and MYLCK genes. Genetic analysis is necessary for definitive diagnosis. Given the clinical, radiological and pathological overlap of HCTDs, MacCarrick et al suggest that a diagnosis be rendered only after molecular characterization in conjunction with clinically documented aneurysm/dissection. In our case, the patient was tested for several of the HCTD mutations and found to have a heterozygous mutation in the TGFβRI gene. In regard to earlier detection, Wellobh et al studied outgrowth endothelial cells in LDS patients and normal controls and in the former demonstrated significant up-regulation of Gremlin-1, a bone morphogenetic protein antagonist, which appears to contribute to the vascular pathology of LDS. This finding prompted the study of plasma levels of Gremlin-1, which were markedly elevated in the LDS cohort, and which may serve as a serological marker for early detection and guidance for further targeted testing. Due to the aggressive vascular course of LDS, frequent imaging analysis consisting of echocardiography (ECHO) and either computed tomographic or magnetic resonance angiography (CTA/MRA) of the head, neck, chest, abdomen and pelvis is mandatory following baseline testing. The advantage of MRA lies in the absence of radioactive exposure, a feature that is important in the pediatric population requiring multiple exams; however, CTA possesses finer spatial resolution than MRA and better delineates aneurysms in medium to small caliber vessels. Recommended surveillance should consist of ECG every 6-12 months and CTA/MRA once every two years or earlier based on prior image analysis. Surgical treatment for LDS patients should be considered when aortic root diameters approach 4 cm or when the diameter of the aortic root increases >0.5 cm/year. Due to the increased risk of endoleaks, disease progression and need for surgical reintervention, endovascular stent-grafts are not generally recommended for aortic aneurysms in patients with HCTDs, although aortic stent-grafts may be used as bridges before definitive
Cervical spine abnormalities and scoliosis/kyphosis are common issues with LDS and should be treated per typical protocols. The current standard medical therapy for both MFS and LDS patients targets the dysregulated TGF-β signaling pathway where treatment involves the use of β-blockers or angiotensin-II receptor blockers, such as atenolol and losartan, respectively. These agents decrease TGF-β signaling and have been shown to slow the rate of progression of aortic root dilatation. Celiprolol, a β-blocker, was recently reported to reduce cardiovascular events in patients with vEDS but larger trials are necessary to define its efficacy. In addition to medical therapy, exercise restrictions are imposed to decrease stress on the vascular tree; these include the avoidance of contact sports, exercising to the point of exhaustion and isometrics.

Although there are many overlapping features among HCTDs, the more common syndromes possess discriminating clinical and radiological manifestations. Knowledge of these key findings allows the multidisciplinary team to narrow the differential diagnosis, drive molecular testing for genetic confirmation of the disease state, assess the level of cardiovascular disease and formulate a patient management plan prior to mortal complications.

References:


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SPP Case 16-06 CYTOLOGY CASE

Diagnosis:

a. Fibrolamellar Hepatocellular Carcinoma

Answers:

1. d. Rarely, it may be associated with liver cell adenomas.
2. b. Focal nodular hyperplasia is the only liver tumor that is most commonly seen in the left lobe of the liver.
3. a. DNAJB1-PRKACA

Discussion:
The touch preparation shows loosely cohesive aggregates of large polygonal to rounded cells with large round nuclei and copious dense, finely granular eosinophilic cytoplasm. The nuclei show granular chromatin with prominent macronucleoli. Focal intracytoplasmic bile production, vacuoles, and binucleation are present. Mitoses are inconspicuous. Focal fibrous connective tissue surrounded by malignant tumor cells is seen. Sheets of benign-appearing much smaller polygonal cells adjacent to malignant tumor cells are appreciated, consistent with normal hepatocytes. The resection specimen contains a light brown and green tumor with a central fibrous scar, 10.4 cm in greatest dimension. Microscopic examination of the tumor section reveals cords and trabeculae of large polygonal to rounded eosinophilic malignant cells surrounded by broad lamellar fibrosis. The non-neoplastic liver parenchyma is unremarkable. Immunohistochemically, the tumor cells are positive for beta-catenin (membranous staining) and AE1/AE3 (patchy), but negative for AFP and glypican 3. A final diagnosis of fibrolamellar hepatocellular carcinoma (FL-HCC) was rendered.
FL-HCC is a rare primary malignant liver tumor, accounting for 0.85% of all primary hepatic malignancies in the United States. However, FL-HCC is important in pediatric pathology, because it represents almost 1/3 of all pediatric HCCs. It typically affects adolescents and young adults with no history of primary liver disease or cirrhosis. The median age at presentation is 21 years. Patients are usually asymptomatic until tumors become large like our patient. Occasionally, patients may present with gynecomastia and Budd-Chiari syndrome. FL-HCC is the only liver tumor that is most commonly seen in the left lobe of the liver. The level of alpha-fetoprotein (AFP) is usually normal, but around 10% of these tumors have elevated AFP. CT scan typically demonstrates a large, solitary mass which enhances strongly in arterial and portal venous phases. A poorly enhancing central scar with calcification may be seen. FL-HCC has an unusual propensity to metastasize, particularly to regional lymph nodes. Peritoneal and pulmonary metastatic foci have also been reported. Surgical resection remains the cornerstone of therapy. However, local recurrences are frequent. Currently, there are no good chemotherapy regiments. Selected agents such as 5-fluorouracil with interferon-α and gemcitabine with oxaliplatin showed varying degrees of success. The 5-year survival rate has been reported ranging from 37 to 76% in patients treated with resection or transplantation; in non-resectable cases, the median survival is 12 to 14 months.

Grossly, FL-HCCs usually show a single well-circumscribed mass. Tumors are bulging, hard, with a central stellate scar, and the cut surface is white-brown with focal greenish color like our case. The characteristic histological features of FL-HCC are the presence of large polygonal cells with a deeply eosinophilic cytoplasm and macronucleoli arranged in trabecular-type cords or nests within a lamellar network or surrounded by a mesh of thick fibrous stroma or connective tissue. The oncocytic appearance is secondary to mitochondrial accumulation. The cytoplasm sometimes demonstrates bile production and Mallory-Denk bodies as well as hyaline globular cytoplasmic inclusions. Vascular invasion and necrosis may be seen. Notably, conventional hepatocellular carcinoma (HCC) may sometimes be found combined with FL-HCC.

Touch preparation and fine needle aspiration (FNA) cytology is characterized by loosely cohesive aggregates of large plump polygonal cells several times larger than non-neoplastic hepatocytes. The cells have abundant oncocytic granular cytoplasm, large nucleus and nucleolus and low nuclear:cytoplasmic ratio. Some characteristic intracytoplasmic hyaline and pale inclusions, bile production as well as intranuclear inclusions can be appreciated. By immunohistochemistry, FL-HCCs are positive for CK7, EMA, HepPar-1 and CD68, variably positive for AE1/AE3, negative for AFP, CK19, synaptophysin and chromogranin. Our previous study showed that FL-HCC was negative for glypican 3 and had weak membranous staining for beta-catenin.

The etiology and pathogenesis of FL-HCC remains poorly understood. FL-HCC has much fewer gene mutations compared to conventional HCC. There is also less frequent methylation of the promoters of tumor suppressor genes. Next generation sequencing revealed a heterozygous deletion of approximately 400kb on chromosome 19. A functional chimeric transcript incorporating DNAJB1 and PRKACA (DNAJB1-PRKACA chimera) has recently been reported in more than 80% of patients. Detection of DNAJB1-PRKACA seems to be a very sensitive and specific finding. Molecular profiling of FL-HCC shows three robust molecular classes of tumors: the proliferation class with altered expression of genes regulating proliferation and mammalian target of rapamycin signaling activation; the inflammation class with altered expression of genes regulating inflammation and cytokine enriched production; and the unannotated class with a gene expression signature that was not associated previously with liver tumors. Interestingly, the mutational landscape of FLC did not match any of the prevalent somatic single nucleotide variants (SNVs) found in other liver cancers, which suggests that FL-HCC is a distinct type of liver tumor.

Sclerotic variant of hepatocellular carcinoma (SV-HCC) is probably the most frequent differential diagnosis. SV-HCC has fibrous septa separating trabecular cell plates. However, SV-HCC shows thin collagen fibers instead of the thick fibrous collagenous bands seen in FL-HCC. Interestingly, SV-HCC is associated with hypercalcemia and hypophosphatemia, but not with bone metastases. A central scar on imaging may alert the radiologist to another condition in the differential diagnosis, so-called focal nodular hyperplasia (FNH). FNH is a benign entity without malignant potential. A central stellate scar is seen in 60–70% of cases. An important hint is that the FL-HCC scar is often calcified, which is uncommonly observed with FNH. FNH may be multiple. Rarely, these lesions may be associated with hemangiomas, epithelioid hemangioendotheliomas, liver cell adenomas, and FL-HCCs. Liver cell adenoma is another differential diagnosis. Clinically, liver cell adenomas can occur at any age but usually are diagnosed in the patient’s 20s or 30s. Liver cell adenomas have a strong association with long-term use of contraceptive steroids (birth control pills) and often have a partial capsule without central scar. Liver cell adenomatosis, characterized by multiple (arbitrarily defined as more than ten) hepatic adenomas arising in an otherwise normal liver, does not have a strong association with estrogen or anabolic steroids. Hepatoblastoma also has no relationship to cirrhosis. However, hepatoblastoma usually affects children less than 5 years old and have marked elevations of AFP.
In summary, FL-HCC is a rare malignant liver tumor with distinct clinicopathologic features. The majority of tumors harbor the DNAJB1-PRKACA chimeric transcript. Touch preparation is very impressive and useful for diagnosis.

**References Readings:**


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