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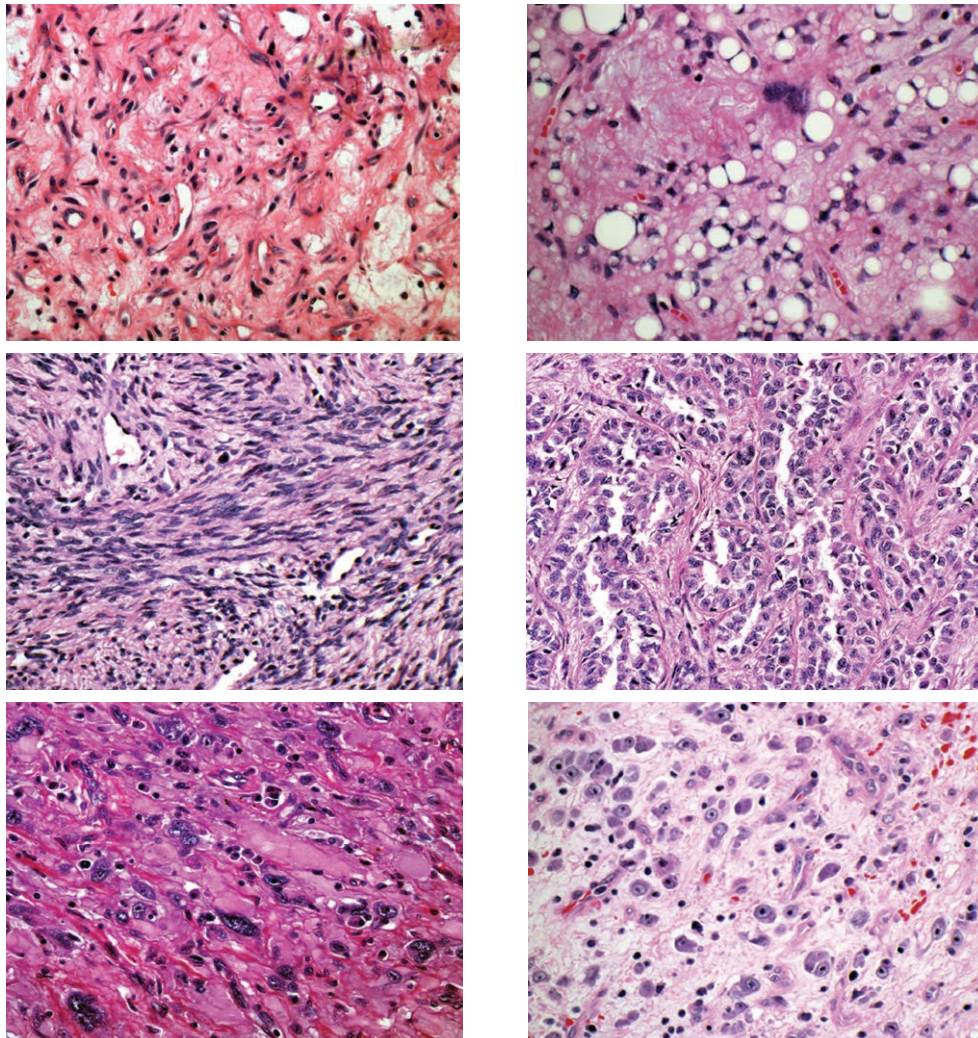
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Recently Described Soft Tissue Lesions: An Update



آغا خان یونیورسٹی ہسپتال، کراچی

The Aga Khan University Hospital, Karachi



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COLLEGE of AMERICAN PATHOLOGISTS

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Dear Readers

Hope you all had a great summer.

Year 2017's second issue of LABRAD is in your hands! This time it is non-thematic and has some useful articles like the broad clinical applications of platelet-rich plasma, clinical utility of plasma homocysteine, diagnostic tests available for Alkaptonuria and a discussion on soft tissue lesions. Another interesting article which might interest the pathologists is 'Patient Based Quality Control: Hitting a Moving Target' which discusses patient based QC procedures and how patient results can be utilized to monitor an analytical run and identify systematic errors.

Just to inform you all the 40th Annual Pakistan Association of Pathologists (PAP) Conference will be held from 14th to 16th December 2017 at Sindh Institute of Urology and Transplantation (SIUT), Karachi, Pakistan. The program will include pre-conference workshops, state of the art lectures by local and foreign faculty, plenary sessions, oral and poster presentations. The local organizers are already hard at work putting together an exciting scientific

program and we hope you will be a part of it.

The Department of Pathology and Laboratory Medicine is committed to providing high quality diagnostic services for physicians across Pakistan. One of the goals of the Department is to update physicians throughout Pakistan about the advancement in laboratory sciences and the services available at the Clinical Laboratory, through Continuing Medical Education (CME) lectures across the country, laboratory updates and LABRAD. In order to keep the contents of LABRAD current and dynamic, readers are encouraged to send short articles on topics pertinent to healthcare and laboratories today. Since last year LABRAD is marked in the Institutional Repository and available on <http://ecommons.aku.edu/pakistan>. Readers from outside Aga Khan University now have the access current and previous issues of LABRAD from here and this has improved our newsletter readership. I hope you enjoy this issue. Happy reading.

Lena

Clinical Applications of Platelet-Rich Plasma

Dr Anila Rashid
Haematology & Transfusion Medicine

Platelet-rich plasma (PRP) is an autologous product derived from whole blood through the process of gradient density centrifugation. PRP functions as a fibrin tissue adhesive with hemostatic and tissue sealing properties, but it differs from fibrin glue and other platelet-poor tissue adhesives because its platelets provide a unique ability to promote wound healing and enhance osteogenesis. PRP provides an immediate surgical hemostatic agent that is biocompatible, safe, and effective. PRP accelerates endothelial, epithelial, and epidermal regeneration, stimulates angiogenesis, enhances collagen synthesis, promotes soft tissue healing, decreases dermal scarring, enhances the hemostatic response to injury, and reverses the inhibition of wound

healing caused by glucocorticoids. The high leukocyte concentration of PRP has an added antimicrobial effect. Since PRP is an autologous blood product, it carries no risk of transmitting infectious disease.

PRP has an extremely broad range of clinical healing applications in head and neck surgery, otolaryngology, cardiovascular surgery, burns and wound healing, oral and maxillofacial surgery, cosmetic surgery, and periodontics (Table 1). In addition to its effectiveness for patients with chronic non-healing wounds, it has also been used as an antiangiogenic agent and as a carrier for growth factors.

Preparation

Numerous techniques have been described for the immediate preoperative preparation of autologous PRP, but most are variations on a standard theme. Blood is drawn from the patient and fractionated using centrifugation. The platelets are concentrated in the platelet rich plasma at levels generally six to eight times the baseline levels. The resultant PRP is stored at room temperature until needed, at which time 10,000 units of powdered bovine thrombin is mixed with 10 percent calcium chloride. Next, the PRP is drawn into a 10ml syringe. The thrombin/calciumchloride mixture then is aspirated into a one ml syringe and both syringes are mounted in a mixing applicator. At the tip of the applicator, the two preparations are mixed to activate the PRP. Within five to 30 seconds, a gel is formed as the citrate is neutralized and the thrombin activates polymerization of the fibrin and degranulation of the platelets. The gel is then inserted into the surgical field as needed.

Most current methods of PRP preparation use calcium and bovine thrombin to initiate formation of PRP gel. The use of bovine thrombin has unfortunately been associated with the development of antibodies to human clotting factors V, XI, and thrombin, resulting in a risk of potentially life-threatening coagulopathies. Several commercial systems are available for preparing PRP, including the Cobe Angel Whole Blood Separation System which also can produce fibrin glue (Cobe Cardiovascular, Inc., Arvada, Colorado) and the Sequire Platelet Concentrating System (PPAI Medical, Fort Myers, Florida). Most commercial PRP preparation systems are available for offi ce use by dental practitioners, podiatrists and wound care

physicians. In comparison with previous methods that employed autotransfusion devices, current automated systems have shorter preparation times and require substantially less blood volume.

Contraindications

Treatment with autologous PRP is generally considered safe in appropriately selected patients. Potential candidates for treatment with PRP should undergo a pretreatment hematologic evaluation to rule out potential coagulopathies and disorders of platelet function. Patients who are anemic and those with thrombocytopenia may be unsuitable candidates for treatment with PRP. Other potential contraindications include hemodynamic instability, severe hypovolemia, unstable angina, sepsis, and anticoagulant or fibrinolytic drug therapy.

Conclusion

Autologous PRP is a relatively new biotechnology that has shown promise in the stimulation and acceleration of soft-tissue and bone healing. The application of PRP has been extended to many different fields, including orthopedics, sports injuries, dental and periodontal surgery, and cosmetic, plastic, cardiovascular, general and maxillofacial surgery. Few well-designed scientific studies of the clinical use of PRP are available. The exact mechanisms of action of the many components of PRP are not fully understood, and the ideal ratios of these components are unknown. In some circumstances, the cost of implementing this promising technology must be weighed against its benefits, and well-designed controlled clinical studies are needed to provide clear evidence of the capacity of PRP to improve patient outcomes.

Table I: Clinical Applications of Platelet-Rich Plasma

Cosmetic Surgery	Otorhinolaryngology-Head and Neck Surgery
Full and split-thickness skin grafts donor sites and recipient sites Skin flaps Bone grafts Metal implants Tissue expansion Aesthetic Surgery (Face Lifts, liposuction, etc)	Radical neck dissections Pectoralis major myocutaneous flaps Facial fractures Reconstructions
Oral and Maxillofacial Surgery	Neurosurgery
Mandibular reconstruction Alveolar cleft repair Oral-nasal fistulas	Pituitary tumor removal Skull base tumor resection Intradural procedures involving tumor or release of tethered cords Dural tumors Acoustic neuroma excisions (dura tears during laminectomy)

Orthopedic/Spinal Surgery	Cardiothoracic Surgery
Total Hip Replacement Total Knee Replacement Scoliosis Repair Spinal Fusion All Open and Internal Reduction Fixation Operations Hand and Foot Surgery Bone Graft Surgery	Sternotomy Graft Conduit Sites Esophagogastrectomy
	Eye-PRP
	treatment of dormant ulcers (epithelial defects of the cornea that fail to heal) dry eye syndrome ocular surface syndrome post Laser In Situ Keratomileusis (LASIK) Surface reconstruction after corneal perforation associated with amniotic membrane transplantation.
Periodontal Surgery	General Surgery
Dental implants Guided Bone Regeneration	Recurrent Hernia Repair Anal Fistula Bariatric Surgery

Rota Virus: Diagnosis & Prevention of Infection

Ms Syeda Kiran Zaidi
Clinical Microbiology

Rotavirus is the cause of mild to severe diarrhea in more than 18 million children under five years of age and is responsible for approximately 873,000 deaths per year. Due to compromised sanitation amenities and medical facilities available in the developing world, there is increased transmission and poor access to rehydration in patients suffering from severe vomiting and diarrhea due to rotavirus. It is also an important cause of nosocomial diarrhea.

Transmission and Reservoirs

Rotavirus is transmitted primarily through the fecal-oral route, meaning an infected person must shed the virus through their stool and then a susceptible person must ingest it in order to cause an infection. Because rotaviruses grow and multiply in the intestinal villi, the gastrointestinal tract and stool serve as the reservoirs for rotavirus in infected humans.

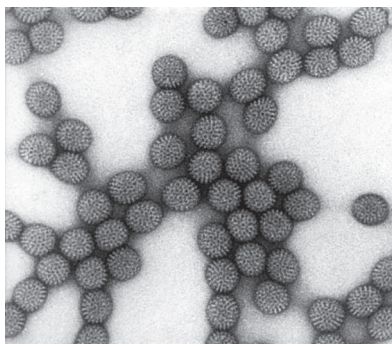
Signs and Symptoms

The most common symptoms include abdominal

pain, vomiting, fever, and diarrhea. However, in some individuals rotavirus may also be asymptomatic. Although infants under three months of age may be infected, generally the first infection after three months is the most severe.

Diagnosis

The name rotavirus is derived from the Latin word “rota”, meaning “wheel”. Rotaviruses belong to the family Reoviridae and are nonenveloped, double-shelled RNA viruses. The genome codes for six structural and six nonstructural proteins. The virus is stable in the environment. Laboratory diagnosis of rotavirus infection is usually performed by antigen detection, using enzyme immunoassay or latex agglutination techniques, which have a sensitivity and specificity above 90 per cent. Molecular techniques such as polyacrylamide gel electrophoresis (PAGE) and reverse transcription-polymerase chain reaction (RT-PCR) are used to determine the RNA migration patterns and virus genotyping, respectively. The advantage of confirming a case of rotavirus diarrhoea



Rota Virus: Electron microscopy (courtesy Google Images)

by laboratory testing is primarily epidemiological so that preventive measures may be initiated for the community at risk.

We, at Aga Khan University Hospital (AKUH) will be starting Rotavirus detection by Immuno Card STAT Rotavirus assay® (Meridian Bioscience, USA) which detects the presence of rotavirus antigen in stool through monoclonal antibodies directed against simian rotavirus strain (SA-11). It has specificity of 95.8 per cent and sensitivity of 93.1 per cent and it gives result in just 10 minutes. Limit of detection in

stool specimens is $1.8-3.7 \times 10^8$ rotavirus particles per test volume. The assay is not recommended on meconium (early neonatal) stools as their performance characteristics have not been evaluated. A positive result does not preclude the presence of other infective organisms.

Prevention/Vaccines

Rotavirus is susceptible to strong disinfectants such as formalin and 95 per cent ethanol. Washing hands is not enough in preventing a rotavirus infection, as the viruses are relatively resistant to soap and water. Currently there are two FDA approved vaccinations for rotavirus. RotaTeq was approved in 2006, and two years later in April of 2008 Rotarix was approved. Both vaccines are live attenuated viral vaccines taken orally in a scheduled series. RotaTeq is given in a three dose series at two, four, and six months of age, whereas Rotarix is given in a two dose series at four and six months. Studies report an 85-98 per cent efficacy rate against severe rotavirus infection, and a 74-87 per cent efficacy rate against rotavirus infection of any severity.

Anti-Musk Antibodies for Seronegative Myasthenia Gravis

Dr Syed Bilal Hashmi
Chemical Pathology

Myasthenia gravis is the most common disorder of neuromuscular transmission. It can present at any age, but there tends to be a bimodal distribution to the age of onset with an early peak in the second and third decades with female predominance and a late peak in the sixth to eighth decade with male predominance. This autoimmune disease is caused by autoantibodies directed against muscle's nicotinic acetylcholine receptor (AChRs) or muscle-specific receptor tyrosine kinase (musk). There are two clinical forms of myasthenia gravis, ocular and generalized. In neonates, a transient form of myasthenia, called neonatal myasthenia gravis, can occur as a result of the trans placental passage of maternal antibodies that interfere with function of the neuromuscular junction but symptoms disappear after few weeks.

The cardinal feature of myasthenia gravis is

fluctuating skeletal muscle weakness, in any skeletal muscle group. The weakness may fluctuate throughout the day, but it is most commonly worse later in the day or evening. More than 50 per cent of patients present with ocular symptoms of ptosis or diplopia while 15 per cent of patients present with bulbar symptoms like dysarthria, dysphagia and fatigable chewing. Less common presentations include isolated neck weakness, isolated respiratory muscle weakness, and distal limb weakness.

The diagnostic approach to myasthenia is focused on confirming the clinical diagnosis established by the history and typical examination findings. More reliable laboratory methods that aid in the confirmation are serologic tests for autoantibodies and electrophysiologic studies.

Anti- acetylcholine antibodies are used as a first

line test to diagnosis myasthenia gravis and for distinguishing acquired disease (90 per cent positive) from congenital disease (negative). They are also used for monitoring disease progression or response to immunotherapy. The antibodies are present in 80-90 per cent of patients with myasthenia gravis.

Anti- musk antibodies are used for diagnosis of autoimmune myasthenia gravis in patients with new onset acquired myasthenia gravis evident clinically and electrophysiological but negative first-line serological tests, i.e. autoantibodies against AChRs. These autoantibodies are present in half of seronegative myasthenia gravis patients. It also helps in distinguishing autoimmune from congenital

myasthenia gravis, diagnosing ocular myasthenia gravis and monitoring clinical course and response to immunomodulatory treatment of a myasthenia gravis patient.

The diagnosis of myasthenia gravis in immunosuppressed or patients on immunosuppressive drugs should be made with caution because these autoantibodies may be falsely low in such cases. Also 10 per cent of patients with acquired, presumably immune-mediated myasthenia gravis do not have detectable serum autoantibodies to AChR or MuSK. In these seronegative patients, the diagnosis is based on the clinical presentation, the response to cholinesterase inhibitors and electrophysiological findings.

Clinical Utility of Plasma Homocysteine

Salima Ratani and Hafsa Majid
Microbiology and Chemical Pathology

Role of Homocysteine in Atherosclerosis

Atherosclerosis is a pathologic process caused by the buildup of plaque/ fatty material on the inside of blood vessels leading to decreased blood flow commonly affecting the coronary, cerebral and peripheral arteries. Recently, there is increased evidence that raised homocysteine levels in blood are an independent cause of atherosclerotic vascular disease and recurrent venous thromboembolism.

Homocysteine is an intermediary amino acid produced during the conversion of methionine to cysteine (Figure 1). Homocysteine has primary

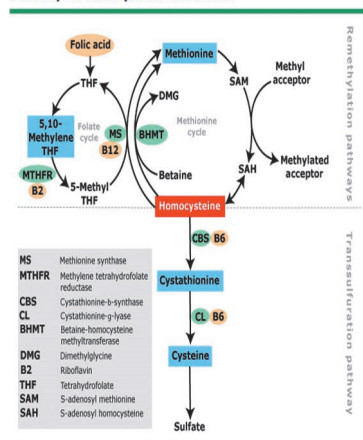
atherogenic and prothrombotic properties. Homocysteine-induced vascular injury include intimal thickening, elastic lamina disruption, smooth muscle hypertrophy, marked platelet accumulation, and the formation of platelet-enriched occlusive thrombi. Homocysteine causes vascular injury by following mechanisms:

- Increased leukocyte recruitment
- increased smooth muscle proliferation and collagen synthesis
- attenuation of endothelial cell tissue plasminogen activator binding sites
- activation of factor VIIa and V
- inhibition of protein C and heparin sulfate
- increased blood viscosity, and decreased endothelial antithrombotic activity due to changes in thrombomodulin function
- increased oxidative stress via production of free radicals
- increased platelet accumulation
- decreased Nitric oxide production leading to impaired endothelial vasodilation.

Causes of Hyperhomocytenuemia

An inadequate intake of B vitamins, as well as genetic factors that affect the body's absorption and use of folic acid, can lead to elevated homocysteine levels. Homocysteine can be elevated in the

Pathways of homocysteine metabolism



following conditions:

- Thermolabile variant of MTHFR (Methylene Tetra Hydrofolate Reductase) having low enzyme activity
- Intracellular cobalamin defects
- Vitamin B6 deficiency
- Vitamin B12 deficiency
- Folic acid deficiency

Plasma and urine concentrations of homocysteine are severely elevated in a rare autosomal recessive disorder; homocysteinuria or severe hyperhomocystenemia. It is clinically manifested as developmental delay, ocular abnormalities, thromboembolic disease and even osteoporosis.

Laboratory Diagnosis

75 to 85 per cent of homocysteine is protein bound, 15 to 25 per cent is acid-soluble free forms. Plasma homocysteine levels normally are between 5-12

$\mu\text{mol/L}$ and levels $>100 \mu\text{mol/L}$ are considered as severely high. An oral methionine challenge (100 mg/kg) can be given to patients suspected of hyperhomocysteinemia who have normal fasting homocysteine levels. It is more useful for patients with cystathionine-beta-synthase deficiency than for those with MTHFR reductase deficiency. The homocysteine concentration is measured on fasting plasma samples before the methionine challenge and four and eight hours afterward. The prognostic significance of the oral methionine challenge is uncertain. Correcting nutritional inadequacies will lower homocysteine levels. A diet rich in fruits, vegetables, and low-fat dairy products and low in saturated and total fat also can lower fasting serum homocysteine levels. Trials in primary and secondary prevention of the disease show that adequate intake of vitamin B complex, whether in diet or from supplements, prevents homocysteine-associated vascular disease.

Patient Based Quality Control: Hitting a Moving Target

Dr Hafsa Majid
Chemical Pathology

Clinical laboratories are required to ensure accuracy of every result reported; for that purpose since long quality control materials are used. The CLIA have recommended that minimum two level controls should be run each day. The question arises whether with increasing volumes this frequency of running controls would be enough for high volume laboratories? On the other hand if the controls run per day are increased it will directly impact the cost. As a solution patients' data can be utilized ('patient-based quality control') that monitors in real time the average patient value for any given analyte. The patients' data can be used in two ways for assessing systemic errors described below.

Average of Normals' (AON)

The AON method of quality control was first described by Hoffman & Waid in 1965. In AON, an error condition is signaled in an analytic process **whenever the average of selected consecutive patient data is beyond the control limits established for the average of the patient population.** Patient results included if they were within a "normal

range" that was determined from patient data. For control limits, Hoffman and Waid used the 95 per cent confidence limits for the stable patient mean. The AON can be done on relatively small sample like significant analytic error could be detected by averaging as few as ten consecutive values.

For establishing AON the laboratory needs to decide, outliers to be excluded (especially for average), age limits (eg 18 to 70yrs for serum creatinine), define location of sample received from (eg dialysis unit, surgical unit, outpatients etc) and if multiple results per patient would be allowed or only one result per patient

will be taken in calculation of averages. An example of AON graph for plasma glucose is shown in figure 1.

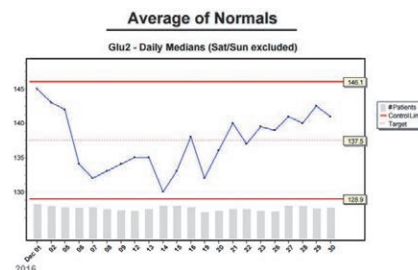


Figure 1: Inpatients' Average of Normal chart for plasma glucose for the month of December.

Patient Moving Averages (PMA)

Moving averages is a simple arithmetic mean of predefined consecutive patient results. The number of consecutive results to be included are decided, average calculated and with each new patient result the window moves by one patient result while the oldest value drops out of view. The mean is recalculated and data point plotted on a Levey-Jennings chart.

In PMA calculation protocol two parameters need to be defined:

- a. The size of the error you wish to detect require control limits and n (number of consecutive results that need to be averaged). To detect
 - i. Large error; wider limits and large 'n'
 - ii. Even a small error; narrow limits and small 'n'. Also It is easier to detect a small shift for analytes that have a narrow range (Na, Cl), than for highly variable analytes (Triglycerides)
- b. Truncation limits: values that need to be excluded from the mean calculation, eg days when patient mix or volumes changes drastically like on weekend patients' volumes decrease so it can affect PMA of that day and if multiple samples are received from a camp for chronic kidney disease or hepatitis C patients can affect PMA for creatinine of LFTS. Such results should be excluded from calculations.

In monitoring AON or PMA the goal is to monitor the process and not the patients. In the absence of systematic error, the mean patient value doesn't deviate significantly from the historic patient mean. A mean exceeding predefined control limits thus indicates the possible presence of a systematic error. These tools are more useful when data is collected regularly (every week or every month), and for relatively high-volume tests ($n \geq 50$ samples per day).

Selection of Analytes for PMA Monitoring

Not every analyte is amenable to PMA monitoring. If daily test volume are less than the pre-decided 'n' for PMA calculation then there is no point in monitoring PMA. It is not suitable for analytes with great variability eg CK, LD. The PMA monitoring will not be effective for analytes such as troponin, as it is undetectable in most patients so for it monitoring moving median will be more practical.

Advantages of PMA Monitoring

Appropriately designed and implemented moving averages protocols can detect a systematic error hours before your next quality control run and will also decrease the frequency of control runs per day. The real challenge in setting up moving averages monitoring is defining your own protocol and taking corrective action to control the systematic error identified.

Diagnosing Alkaptonuria: A Rare Inherited Metabolic Disease

Dr Yusra Zaidi
Chemical Pathology

Alkaptonuria is a rare inherited metabolic disorder (IMD) due to deficiency of the hepatic enzyme Homogentisate 1,2-dioxygenase involved in tyrosine degradation pathway forming an intermediate metabolite homogentisic acid (HGA) and its oxidative products in various connective tissue. The true incidence of Alkaptonuria varies from 1:500,000 to 25:500,000, prevalence is higher in Middle Eastern and Asian countries compared to western countries owing to the high cousin marriage rate.

Life expectancy is not affected in patients with Alkaptonuria, but the quality of life is significant affected due to accumulation of HGA in the tissues giving rise to ochronosis (yellow to brown discoloration) in tissues especially cartilage, tendons, ligaments, at times even bone and makes them brittle, weak and susceptible to rupture.

Affected individuals may have dark urine or urine that turns black when exposed to air. However,

this change may not occur for several hours after urination and often goes unnoticed. Most common symptomatic presentation is arthritis especially in the spine and large joints (Ochronotic arthropathy), beginning early and progressing more rapidly in males than females. Additional symptoms that are seen less often include kidney stones, prostate stones and heart disease due to accumulation of HGA within the aortic or mitral valve.



Figure 1: Chromatogram showing Homogentisic Acid Peak (marked with red arrow).

Patients are diagnosed with Alkaptonuria based on increased HGA excretion, evident by presence of peak on urine organic acid chromatogram detected by gas chromatography-mass spectrometry analysis; as shown in figure 1. From 2013 till to date the Biochemical Genetics Laboratory of our section has reported nine cases of Alkaptonuria. All patients were diagnosed based on marked peaks of Homogentisic acid on urine organic acid. Male to female ratio was 2:1. Most common age of presentation was in third, fourth and fifth decade of life while two cases presented within first year of their birth.

Standard Treatment for Alkaptonuria includes symptomatic treatment for pain using anti-inflammatory drugs or narcotics may also be prescribed, if needed. Dietary protein restrictions are advised and vitamin C is prescribed, which prevent accumulation and deposition of HGA. Recently a drug, Nitisone is (a drug for Tyrosinemia) is under study for use in Alkaptonuria. It is shown to prevent significant accumulation of homogentisic acid but further research is needed to understand its safety and effectiveness in long term usage.

Recently Described Soft Tissue Lesions: An Update

Qurratulain Chundriger and Nasir Ud Din
Histopathology

Soft tissue pathology is a rapidly changing subspecialty. Several soft tissue lesions have been recently described in the medical literature. Some are recently recognized, hence often underdiagnosed because of limited knowledge whereas others have been recognized for some time but can be viewed afresh in light of emerging molecular data. Here we discuss six of these recently described soft tissue tumours.

Angiofibroma of Soft Tissue

In 2012, Mariño-Enriquez and Fletcher described 37 cases of a distinctive benign fibrovascular tumour that arose most commonly as a soft tissue mass of the extremities and was characterized by a specific

translocation t(5;8) (p15;q13). These lesions were named as Angiofibroma of soft tissue. They are slow growing tumours of the subcutis or deep soft tissue most commonly arising in the extremities of adults (median age 49 years) with a slight female predominance (2:1). Most are well-circumscribed lesions with a median size of 3.5 cm (range: 1.2–12.0 cm).

Histologically, these tumours characterized by a proliferation of uniform bland spindle cells with present in a variably myxoid or collagenous stroma. The cells have ovoid nuclei and inconspicuous cytoplasm. A prominent network of small thin-walled and finely branched blood vessels is seen. Epithelial membrane antigen (EMA) is positive in nearly

half of cases, usually only in scattered cells but rarely diffusely. Focal staining for desmin, smooth muscle actin (SMA), or CD34 may be seen in a subset of cases, but S100 protein is negative. Main differential diagnoses include Myxoid Liposarcoma, Solitary Fibrous Tumour, Low grade fibromyxoid Sarcoma, Low grade myxofibrosarcoma and cellular angiofibroma. Angiofibroma of soft tissue is a benign tumour, but may occasionally recur locally.

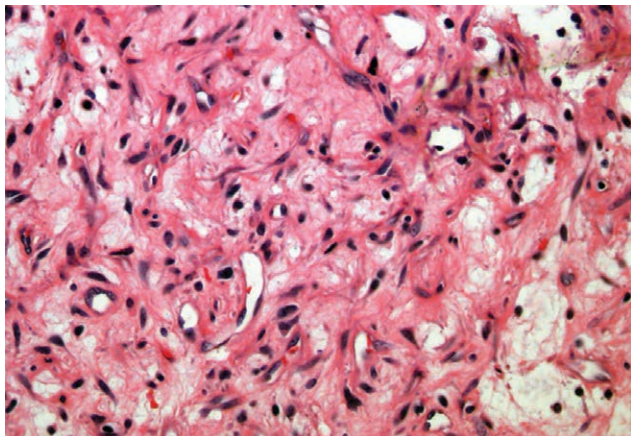


Figure 1. Angiofibroma of soft tissue with chicken wire type vessels, collagenous stroma and bland spindle cells

Fibrosarcoma-like Lipomatous Neoplasm

In 2013, Deyrup and colleagues revisited the concept of “spindle cell liposarcoma”. Based on their analysis of previous publications on this topic, this term in their opinion actually represents a heterogeneous group of tumours, including variants of well differentiated liposarcoma, myxoid liposarcoma, and even spindle cell lipoma. Excluding those entities by molecular analysis, they described 12 cases of a low grade adipocytic neoplasm with prominent spindle cell component which they termed “fibrosarcoma-like lipomatous neoplasm”. Most of these tumours arise in adults (mean age: 50 years). These tumours present as superficial or deep soft tissue masses with a wide size range (mean: 7.5 cm) arising in the groin and para-testicular region, as well as the buttock, thigh, flank, and shoulder.

Key histologic features include a uniform fibroblast-like spindle cells arranged in parallel bundles, myxoid background with arborizing thin vessels, wide range of lipoblasts lacking significant atypia, including signet ring and spindled univacuolated/bivacuolated cells. Univacuolated (“ice cream cone”) and bivacuolated (“hourglass”) spindled lipoblasts are often seen in fibrosarcoma-like lipomatous neoplasm. Main Differential Diagnoses include Well-differentiated liposarcoma/atypical lipomatous tumor

(WDL/ALT), Myxoid liposarcoma and Spindle cell lipoma. Fortunately, molecular analysis can usually easily exclude these possibilities.

No recurrence or metastasis was identified in any of these patients (mean follow-up time: 68 months; range 9 month to 20 years), suggesting that perhaps “liposarcoma” is not an appropriate term for this tumor, based on current data.

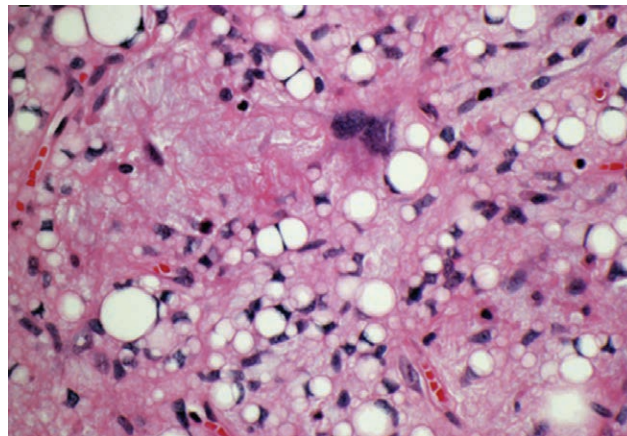


Figure 2. Fibrosarcoma-like lipomatous neoplasm exhibiting a wide ranging of adipocytic differentiation (uni and bivacuolated lipoblasts)

Biphenotypic Sinonasal Sarcoma

Sarcomas arising in the sinonasal region are in general very rare. Biphenotypic sinonasal sarcoma a.k.a low-grade sinonasal sarcoma with neural and myogenic features is a recently described entity based on review of sinonasal sarcomas from several decades of cases and consults. The clinical presentation of biphenotypic sarcoma is features of sinusitis, difficulty in breathing, facial pressure, congestion, and, rarely, facial pain. The age range is 24 to 85 years (mean: 52 years), and there is female predominance in the original series (21 women, 7 men). The tumour most commonly affects multiple sites, including the nasal cavity proper (54 per cent) and ethmoid sinus (57 per cent); extension into the orbit and cribriform plate are common. The key histologic features include poor circumscription, infiltrative growth of highly cellular, bland spindle cells, in vague “herringbone” pattern, without atypia or frequent mitoses, along with benign proliferation of respiratory epithelium with variable admixture with tumor cells. These tumours show dual positivity for S100 (neural marker) and Smooth muscle actin and muscle specific actin (muscle markers), thus called biphenotypic. A recent study by Wang and colleagues found PAX3 rearrangement by FISH in 96 per cent of these cases (n=25); 79 per cent of these cases were positive for PAX3-MAML3 fusion

by RT-PCR. Main Differential Diagnoses include Fibrosarcoma, Malignant peripheral nerve sheath tumour and Monophasic Synovial sarcoma. Local recurrence is common, but metastases or mortality from disease have not yet been reported.

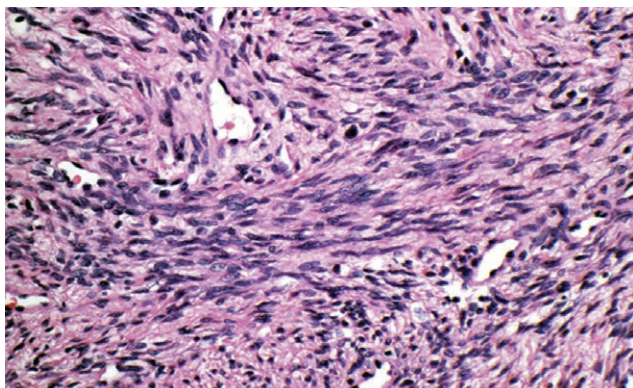


Figure 3. Biphenotypic sinonasal sarcoma showing fascicles of bland spindle cells.

Malignant Gastrointestinal Neuroectodermal Tumour

Malignant gastrointestinal neuroectodermal tumor (GNET) is a rare, aggressive tumour of the gastrointestinal (GI) tract that has previously been called as “clear cell sarcoma-like tumour of the GI tract with osteoclast-like giant cells”. Stockman and colleagues proposed this name after a review of 16 cases which showed histologic, immunohistochemical, and molecular findings similar to Clear cell sarcoma of soft tissue, but with distinct differences, including no evidence of melanocytic differentiation. GNET typically arises in young to middle aged adults and most often involves the small intestine. The tumour is typically centered within the wall of the bowel, with secondary involvement of the mucosa and serosa. Histologically, the tumours grow in solid sheets, pseudopapillary formations and alveolar formations, generally without the well-formed nests that characterize soft tissue-type clear cell sarcoma. The cells are epithelioid to polygonal with variable amount of eosinophilic cytoplasm and vesicular nuclei with chromatin margination and scattered intranuclear cytoplasmic inclusions. The tumour cells are positive for S100, SOX10 and neuroendocrine markers; however, markers of melanocytic differentiation, including Melan A and HMB45 are negative. These tumours show EWSR1 rearrangement with numerous fusion partners. Main differential diagnoses include GIST, monophasic synovial sarcoma, melanoma, soft tissue clear cell sarcoma and Epithelioid Malignant Peripheral Nerve Sheath tumour.

Prognosis may be good if the tumour can be completely removed; however, metastases are common (over 50 per cent), often to liver and lymph nodes.

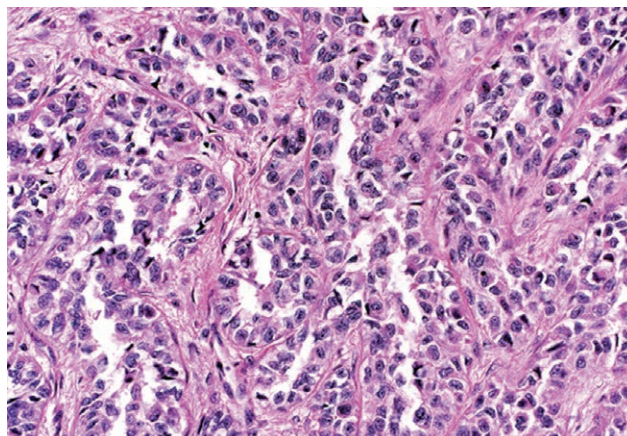


Figure 4. Polygonal cells with a pseudovascular spaces seen in GNET.

Superficial CD34 Positive Fibroblastic Tumour

In 2013, Carter and colleagues described 18 cases of a tumour with borderline malignant potential, having unique characteristics i.e. striking nuclear pleomorphism, paradoxically rare mitotic activity, indolent behavior, and diffuse CD34 expression. They termed it as “Superficial CD34 positive fibroblastic tumour”. Awareness of this entity is important, as there is a tendency for overdiagnosis as a pleomorphic sarcoma in light of the nuclear atypia, even though most cases do not recur or metastasize. The tumour presents in adults as a slow-growing supra-fascial mass with a mean size of 4.1 cm, most commonly in the lower extremity. Histologically it shows striking pleomorphism but low mitotic rate (<1 per 50 HPFs). The cells are spindled to epithelioid with abundant eosinophilic often granular cytoplasm and are arranged into hypercellular sheets or fascicles. Most tumour cells display marked nuclear pleomorphism with hyperchromasia and multiple large inclusion-like nucleoli as well as cytoplasmic nuclear pseudo-inclusions. Xanthomatous foamy tumour cells are commonly seen, and mixed inflammation is often present. There is diffuse strong CD34 expression (always), focal cytokeratin expression (often). FLI-1, ERG, S100 protein, desmin, smooth muscle actin, and TP53 are all negative, Ki-67 proliferative index is less than one per cent, and nuclear INI-1 (SMARCB1) expression is retained. Differential diagnoses include Undifferentiated pleomorphic sarcoma, Myxofibrosarcoma, Atypical fibroxanthoma, Myxoinflammatory fibroblastic sarcoma and Pleomorphic hyalinizing angiectatic tumor.

Epithelioid Inflammatory Myofibroblastic Sarcoma

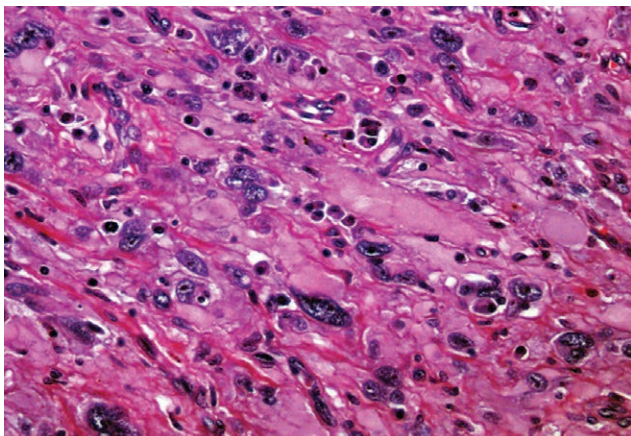


Figure 5. Large polygonal cells with glassy cytoplasm and prominent nucleoli and rare mitosis; features seen in Superficial CD34 positive fibroblastic tumour.

Inflammatory myofibroblastic tumour (IMT) is a mesenchymal tumour of intermediate malignant potential composed of spindled myofibroblasts with admixed inflammation. A rare epithelioid variant of IMT with distinctive nuclear membrane or perinuclear ALK staining was recently described and termed “epithelioid inflammatory myofibroblastic sarcoma” (EIMS) in light of its more aggressive behavior than conventional IMT. EIMS has a marked male predominance and wide age range (7 months–63 years, median 39 years). These tumours are almost exclusively found in the abdominal cavity. The lesions range in size from 8 to 26 cm. Histologically the tumors show epithelioid cells with vesicular nuclei and prominent nucleoli present in a myxoid stroma. Inflammatory infiltrate of either

predominantly neutrophils or lymphocytes is seen intermixed with tumour cells. Immunohistochemical stain for ALK protein shows positivity in unique nuclear membrane or perinuclear cytoplasmic pattern. The diagnosis of EIMS may be very challenging given its rarity and lack of histologic similarity to typical IMT. Main differential diagnoses include Anaplastic large cell lymphoma (ALCL), Epithelioid leiomyosarcoma, Rhabdomyosarcoma and Dedifferentiated liposarcoma.

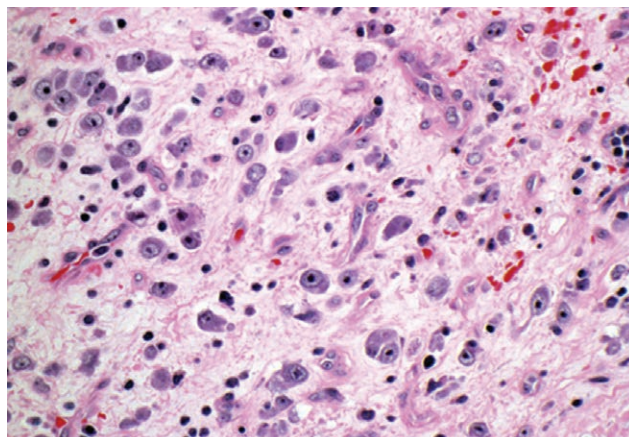


Figure 6. Scattered epithelioid cells with abundant eosinophilic cytoplasm, vesicular nuclei containing prominent nucleoli seen in epithelioid inflammatory myofibroblastic sarcoma.

Conclusion

Owing to rapidly updating classification schemes in histopathology, the pathologists as well as the clinicians should be able to update their knowledge, in order to be able to better understand the tumour biology and therefore avoid over or under treatment of patients.

A Snapshot of the Standards of Medical Care -2017: American Diabetes Association Guidelines

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Diabetes Mellitus (DM) is one of the most widespread diseases with an increasing incidence globally. Statistically, Pakistan is ranked seventh among the list of countries with a reportedly high prevalence of DM making it a major public health concern. In order to promote Diabetes care and management every year the American Diabetes Association

(ADA) releases the Standards of Medical Care in Diabetes. This document is the output of the ADA’s multidisciplinary professional practice committee that systematically searches MEDLINE to revise or clarify recommendations. Feedback from the larger clinical community is also incorporated. The recommendations are summarized as follows:

	Diabetes Mellitus Type 1	Diabetes Mellitus Type 2	Latent Auto Immune Diabetes of Adults (LADA)	Maturity Onset Diabetes of the Young (MODY)	Gestational Diabetes Mellitus (GDM)
Definitions	Diabetes due to autoimmune B-cell destruction, usually leading to absolute insulin deficiency	Diabetes due to a progressive loss of B-cell insulin secretion frequently on the background of insulin resistance	LADA is defined as initially non-insulin requiring diabetes diagnosed in adults with antibodies to GAD - glutamic acid decarboxylase.	MODY is an inherited form of diabetes mellitus. It is caused by mutations in an autosomal dominant gene disrupting insulin production	Diabetes diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes
Diagnostic Criteria	A1C $\geq 6.5\%$. OR FPG ≥ 126 mg/dL (7.0 mmol/L). OR 2-h PG ≥ 200 mg/dL (11.1 mmol/L) during an OGTT. OR Random glucose ≥ 200 mg/dL (11.1 mmol/L).		To discriminate LADA from type I and/or type II DM, diagnosis of LADA has been based on three criteria as given by The Immunology of Diabetes Society: <ul style="list-style-type: none"> • Adult age of onset (> 30 years of age); • Presence of at least one circulating autoantibodies (GADA/ICA/IAA/IA-2); and • Initial insulin independence (for the first six months). 	Individuals with a strong family history of diabetes, presenting from the second to the fifth decade, should be evaluated further through sequencing of the suspected gene (HNF1A, GCK etc) and detecting a mutation.	Perform a 75-g OGTT The diagnosis of GDM is made when any of the following plasma glucose values are met or exceeded: <ul style="list-style-type: none"> • Fasting: 92 mg/dL (5.1 mmol/L) • 1 h: 180 mg/dL (10.0 mmol/L) • 2 h: 153 mg/dL (8.5 mmol/L)
Age at Diagnosis	Most commonly in childhood	Most commonly in adults	Usually age ≥ 30 yrs	< 25 years	> 25 years

Criteria For Screening For Diabetes In Asymptomatic Adults

Testing should be considered in overweight or obese (BMI ≥ 25 kg/m² or ≥ 23 kg/m² in Asian Americans) adults who have one or more of the following risk factors:

- A1C $\geq 5.7\%$ (39 mmol/mol), impaired glucose tolerance, or impaired fasting glucose on previous testing
- First-degree relative with diabetes
- High-risk race/ethnicity (e.g., African American, Latino, Native American, Asian American, Pacific Islander)
- Women who were diagnosed with GDM
- History of CVD
- Hypertension ($\geq 140/90$ mmHg or on therapy for hypertension)
- HDL cholesterol level < 35 mg/dL (0.90 mmol/L) and/or a triglyceride level > 250 mg/dL (2.82 mmol/L)
- Women with polycystic ovary syndrome
- Physical inactivity
- Other clinical conditions associated with insulin resistance (e.g. severe obesity, acanthosis nigricans)

When to Screen? For all patients, testing should begin at age 45 years.

How often to screen? If results are normal, testing should be repeated at a minimum of 3-year intervals, with consideration of more frequent testing depending on initial results (e.g., those with prediabetes should be tested yearly) and risk status.

Criteria For Screening For Diabetes In Asymptomatic Children

Overweight children (BMI > 85 th percentile for age and sex, weight for height > 85 th percentile, or weight $> 120\%$ of ideal for height) plus with any two of the following risk factors:

- Family history of type 2 diabetes in first- or second-degree relative
- Race/ethnicity (Native American, African American, Latino, Asian American, Pacific Islander)
- Signs of insulin resistance or conditions associated with insulin resistance (acanthosis nigricans, hypertension, dyslipidemia, polycystic ovary syndrome, or small-for-gestational-age birth weight)
- Maternal history of diabetes or GDM during the child's gestation

When to Screen? 10 years of age or at onset of puberty.

How often to screen? If results are normal, testing should be repeated at a minimum of 3-year intervals.

Criteria For Screening For GDM

An association between several maternal-fetal outcomes and the level of maternal hyperglycemia has been reported hence screening for GDM is essential in every pregnancy.

High risk group for GDM

- Strong family history of diabetes
- Prior history of GDM
- Morbid obesity
- Other manifestations of glucose intolerance

How to Screen? 2-h Plasma Glucose after 75-g OGTT

When to Screen?

- At the 1st prenatal visit in those with risk factors.
- At 24–28 weeks of gestation in women not previously known to have diabetes.
- Screen women with GDM for persistent diabetes at 4–12 weeks postpartum, using the OGTT.

How often to screen? Women with GDM history should have lifelong screening for development of diabetes or pre-diabetes at least every 3 years.



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