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From the Editor's Desk

Dear Readers

The last issue of LABRAD for the year 2022 is in your hands. I hope you will enjoy the interesting and informative updates on the new advancements in our field. I look back with a sense of fulfilment and relief on the year just passed and look ahead at 2023 with new hope. The current year was the first "full" or "normal" year following the Covid 19 pandemic which transformed our world in so many fundamental ways. Our volumes in both pathology and Radiology not only returned to pre Covid level but in fact exceeded the pre pandemic levels. We expanded our services in multiple areas and required technologies and expertise required to remain at the cutting edge of our fields. Our quest for excellence was exemplified by our outreach services successfully emulating main Lab and receiving the coveted CAP accreditation. However, advancements in radiology and different specialties of pathology are occurring internationally at frenetic pace and we need to practice to keep ourselves abreast with these advancements and adapting them quickly and optimally. Only then can we hope to retain our premium and leading role in diagnostic services in Pakistan and providing best possible cure to the every patient. To make Labrad informative and updated we always encourage residents, technologists and faculty to come together and submit articles. In this issue we have some good reads contributed by small teams. Some such examples in the current issue of Labrad are " Clinical Usefulness of Biochemical Parameters in Prenatal Diagnosis of Down Syndrome In First and Second Trimester , Updates in new WHO Blue Book, Significance of Audit in Clinical Laboratory , Microsatellite Instability (MSI) Testing , Grading Of Soft Tissue Sarcomas , The Role of Immunohistochemistry in Histopathology, Contrast Enhanced Spectral Mammography , Pre-Analytical Variables Affecting Coagulation Testing ,Monkeypox Outbreaks vs. The COVID-19 Pandemic and others.

We value your opinion and feedback regarding topic selections, educational and resource materials and ideas on how we can better network and communicate using LABRAD.

So let us all look at the next year with hope and excitement as we are living in an exciting era of development in Pathology and Radiology . For now, happy reading.

Dr Sidra Arshad
Editor, LabRad

Case Quiz

Dr Hafsa Majid, Clinical Chemistry

A 26-year-old man was brought to the emergency centre after being found unresponsive by his family in the morning. The family revealed no significant history but the patient has been to a party with his friends last night.

Na	139 mmol/L	K	3.7 mmol/L
Cl	112 mmol/L	Bicarbonate	7 mmol/L
Urea	3.4 mmol/L	Creatinine	87 µmol/L
Glucose	4.1 mmol/L	Osmolality	340 mmol/kg

Questions 1: Calculate the Anion and Osmolal Gap.

Questions 2: What is the most probable diagnosis?

Questions 3: What methods can be used for measurement of colligative properties?

Questions 4: What will be the best option for measurement of serum osmolality in this situation and why? (2)

Contrast Enhanced Spectral Mammography

Dr Kulsoom Fatima
Department of Radiology

Introduction

Contrast-enhanced spectral mammography (CESM) is a relatively new technique in breast imaging which combines full field digital mammography (FFDM) with intravenous contrast utilization. Mammography has proven to decrease breast cancer mortality, however it has low sensitivity because of overlap of tissues which can obscure a true lesion especially in dense breasts. The underlying principle of CESM, like that of magnetic resonance imaging (MRI), is that tumors have angiogenesis, thereby, allowing visualization of the tumor relative to the normal breast parenchyma. The sensitivity of CESM for the detection of breast cancer ranges from 93-100 percent. CESM employs a dual energy exposure undertaken during a single breast compression, following the injection of an iodinated contrast agent (1.5 ml/kg body weight). The low-energy image looks like a conventional mammogram, and the recombined subtracted image displays the contrast medium uptake. The average glandular dose (AGD) for CESM, although a bit higher than standard full FFDM, is still within the accepted international dose limits for mammography. The examination time is about 10 minutes, similar that needed for a standard 4-view mammography examination, although the total "room-time" is slightly more owing to the time required to prepare and administer the intravenous contrast medium. As this technique requires iodinated intravenous contrast medium, there is a small risk of contrast medium reaction (<1 percent), which is usually mild and self-limiting. There is also a small risk of contrast induced nephropathy (one-two percent) and patients should be screened for impaired renal function.

Current Indications:

FDA approved the use of CESM in 2011. The technique is now available at main campus, Aga Khan University, Karachi. The FDA approved current indications are:

- ✓ Evaluation of extent of disease in newly diagnosed breast cancer- Accurate assessment of disease extent is crucial for treatment planning and management. CESM can identify multi-focal tumors and correctly estimate the size (Figure 1).
- ✓ Evaluation of response to Neo-adjuvant Chemotherapy (NAC) - NAC is used for locally advanced breast cancer with intent to reduce tumor size and enable breast conservation. Contrast-enhanced MRI is the current imaging gold standard for assessing response; however, MRI is expensive, and not possible in patients with certain implants, renal failure, or claustrophobia, requiring sedation to complete the examination. CESM proves efficacious in monitoring NAC response (Figure 2).
- ✓ Inconclusive Findings at Mammography- The diagnostic accuracy of FFDM is highly dependent on the density of fibro glandular tissue. Therefore, a dense mammogram may be difficult to read and may contain asymmetries, or focal distortions that may or may not be breast cancer. CESM serves as an excellent problem-solving tool when there are inconclusive findings on screening mammography and may obviate the need for spot compression views. It has been shown to have a sensitivity of 100%, specificity 88 percent, positive predictive value (PPV) 76 percent, and negative predictive value (NPV) 100 percent. Evaluation of symptomatic patients. CESM has the potential to be used as the first-line imaging test in patients presenting with clinically palpable abnormalities.

Indication which is not FDA approved but CESM has shown good performance is:

- ✓ Supplement to high risk Screening- Studies have found specificity and positive predictive values comparable to those of breast M RI in screening high risk women suggesting that CEM may be useful when MRI cannot be performed, whether because of patient contraindication or lack of access.

Limitations:

- Suboptimal assessment of chest wall invasion.
- Inability to target abnormal areas of

enhancement evident only on CESM for tissue diagnosis.

- Some low vascularity cancers like lobular carcinoma may not show enhancement leading to false negative examination.
- Benign lesions such as Fibroadenoma may give false-positive results.

Conclusion:
CESM is emerging as a valuable tool in the diagnosis, staging and restaging of primary breast cancer with much better performance compared to FFDM. Compared with MRI, it is cheaper and better tolerated by patients. The limitations, however, must be recognized as this new technique becomes more widely available.

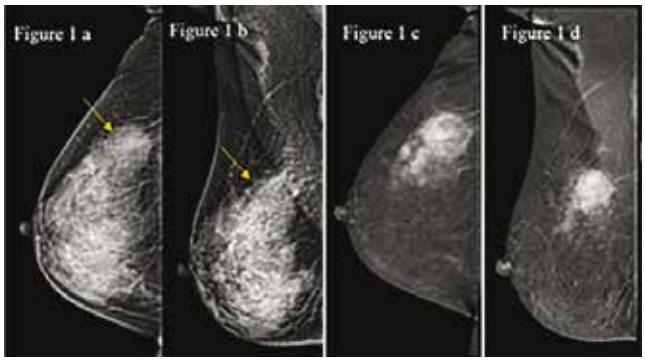


Figure 1 (a, b) Low energy CESM, Craniocaudal (CC) and medio-lateral oblique (MLO) view of right breast show a soft tissue density mass (yellow arrow) in the upper outer quadrant with ill-defined margins, inseparable from parenchyma. (c, d) recombined CESM, CC and MLO images clearly show mass along with its extension into adjacent parenchyma.

Figure 2 (a, b) FFDM, CC and MLO view of left breast show a biopsy proven malignant soft tissue density mass in the upper outer quadrant with partly ill-defined margins (black arrow), a clip has been inserted at the time of biopsy (blue arrow), partly covered enlarged axillary lymph node is also seen (black star). (c, d) post neo-adjuvant chemotherapy low energy CESM, CC and MLO images show interval reduction in size of the mass with partly ill-defined margins. (e, f) recombined CESM, CC and MLO view delineate the margins well for accurate size measurement.

Clinical Usefulness of Biochemical Parameters in Prenatal Diagnosis of Down Syndrome In First and Second Trimester

Hafsa Naseer
Clinical Chemistry

Down's syndrome, also known as Trisomy 21 is the most common autosomal chromosome aberration among live births. It is a congenital syndrome caused by all or part of trisomy chromosome 21, resulting in mental retardation and other abnormalities. The primary target of prenatal screening is the early detection of pregnancies at high risk for trisomy 21. Testing fluid from around the baby (amniocentesis) or tissue from the placenta (chorionic villus sampling) are the most specific tests for Down's syndrome detection. But both these tests are known to increase the risk of miscarriage as these tests involve inserting needles through the mother's abdomen. Rather than these diagnostic test, the screening of biochemical markers in mother's blood are more safe method for early detection of Down's syndrome but these tests can't determine with certainty whether a fetus is affected. Thus pregnancies identified at high risk of Down's syndrome using these screening tests require further testing using amniocentesis or CVS. Maternal serum screening relies on the measurements and quantification of multiple biochemical markers such as PAPP-A and free β-hCG in first trimester while Triple test (include AFP, HCG, unconjugated

estradiole) in second trimester

PLASMA ASSOCIATED PROTEIN-A: PAPP-A is a glycoprotein produced by the placental trophoblasts. In normal pregnancy, the concentration of PAPP-A in maternal circulation increases with gestational age until delivery. Low levels of PAPP-A are found in abnormal placental function which established the base for the first trimester screening of fetal Down syndrome. Other complications related with low PAPP-A are gestational hypertension, intrauterine growth restriction, preterm delivery, gestational hypertension with proteinuria

FREE β-HCG: Human chorionic gonadotropin is a glycoprotein hormone. hCG mediates multiple placental, uterine and fetal functions for the initiation and maintenance of pregnancy. Maternal serum hCG increases at 8–10 weeks and then decreases to reach a plateau at 18–20 weeks of gestation and remains constant in unaffected pregnancies. A Molecular biology studies have shown that trisomy 21 trophoblasts showed increase in β-hCG mRNA indicating that one of the causes of high hCG levels in maternal serum is the increased hCG production and secretion by the placenta.

TRIPLE TEST: The triple test is a second trimester screening test used to identify pregnant women whose fetus is likely to be affected by Down's syndrome. The name of the test is coined from the three proteins: alpha-fetoprotein (AFP), Human Chorionic Gonadotropin (hCG) and Unconjugated Estriol hormone (UE3). hCG increases in the first eight weeks and decreases gradually until 20 weeks. For detecting trisomy 21, an increased hCG level appears to be the most sensitive marker. Fetal yolk sac synthesized AFP in early gestation and fetal gastrointestinal tract and liver produced later. AFP concentration peaks in maternal serum after 12 weeks of gestation. Markedly elevated levels of AFP in maternal serum indicate that fetal integument is not intact while the lower levels indicate increased fetal size and fluid volume. Most of the circulating estriol is the joint product of fetus and placenta produced from a precursor synthesized in the fetus by the adrenal glands and transformed by the fetal liver and placenta into estriol. It increases gradually all through pregnancy. Decreased level of unconjugated estriol is a marker of trisomy 21 and trisomy 18.

The Science of Efficiency in a Clinical Laboratory

Iffat Arman
Clinical Chemistry

The challenge for a clinical laboratory in managed care is to ameliorate outcomes for both patient and health care system. Stack up this goal will require a clear percipience of the important performance measures to consider.

In order to encapsulate the specific aspects contemplate in our analysis we concisely delineate the functioning and the general structure of a clinical laboratory. The laboratory is segregated into different working areas as accessioning/specimen processing, biogenetic lab, Total lab automation, Endocrinology (Immunoassays), Drug toxicology, Specialized bench for Autoimmune disorder analysis.

Once the sample has been collected and redeemed, the laboratory applications can be accomplished. The clinical laboratory procedures can be deserted into three phases: Preanalytical, Analytical and Postanalytical.

The **Preanalytical operations** in general involve only specimen handling; a samples need to be prepared before the analysis. **Analytical phase** i.e. the evaluation of the specimen by an applicable instrument. **Post analytical** compulsions are enamored to the collection and transmission of results.

It is obvious that the stat samples shows a critical factor influencing the laboratory operations because they require discontinuity of workflow in the main laboratory routine work.

Laboratory operations depend on how the laboratory is staffed, equipped and organized. All these aspects are intimately related. To give an example, the laboratory automation is modifying the role of the clinical lab technologist. In fact, automated analytical systems reduce the requirement for skilled technicians in the different practical manual steps included in laboratory operations, but also strengthen the consultative and managerial roles of the clinical staff.

A decrease of the total idle time can be attaining also for the laboratory. Due to these changes the number of senior technicians (who usually are biologists or clinical chemists) is rapidly decreasing and the working role of these specialists eminently devoted to postanalytical tasks as keeping and transmitting test results, administrative work, managing telephone calls, and so on. Further we observe that technicians are replaceable due to the nature of the working tasks and to cross-training.

As it is reported the number of technicians assigned by our model compared with the number of technicians working in the laboratory. For the sake of clarity we note that this comparison is made without further refinements without considering the delicate issue of the crew resource management in a clinical context.

Updates in new WHO Blue Book

Saman Muhammad Amin and Dr Khurram Minhas
Histopathology

Updates in Follicular lymphoma:

Follicular lymphoma (FL) is a neoplasm of germinal center B-cells with varying proportions of centrocytes (CC) and centroblasts (CB) or large transformed cells and at least a partially follicular growth pattern. In rare cases with an entirely diffuse growth pattern, the neoplastic cells should still show germinal center B-cell morphology and immunophenotype.

Traditionally, FL has been graded as FL1, 2, 3A, and 3B based on the quantification of absolute numbers of CBs/transformed cells in 10 consecutive high-power fields. However, there is accumulating evidence of the lack of reproducibility in counting CBs, and thus of grading itself. Many studies have indicated no statistically significant difference in clinical outcomes between grades 1, 2, and 3A patients, who are treated similarly in modern clinical trials. Furthermore, there is compelling evidence of FL3A being biologically related to FL1/2, with similar immunohistochemical and genetic profiles and frequent co-existence in the same affected lymph node.

At present, there is no definite evidence to support the distinction between FL grades 1, 2, and 3A, and hence, to mandate grading. FL1/2 and FL3A together constitute the cFL subtype, which is defined by a mixture of CCs and CBs in various proportions, but in which CCs must be unequivocally present.

Grade 3B FL is now termed as follicular large B-cell lymphoma (FLBCL). It is commonly regarded as a particular subtype of FL with a close clinical and biological relationship to DLBCL. FLBCL is defined by the presence of a follicular pattern with follicles composed of ‘sheets’ of centroblasts and absence of centrocytes. BCL2 translocation is uncommon. FLBCL rarely coexists with cFL, but frequently coexists with DLBCL. Due to the extreme rarity of pure FLBCL, the possibility of concurrent DLBCL must be excluded by careful sampling. Therefore, a definite diagnosis of this entity should not be made on a needle core biopsy. A presumptive diagnosis of FLBCL should prompt additional investigations, such as BCL6 or MYC rearrangement. IRF4 FISH analysis is required if MUM1 is strongly expressed, to exclude LBCL with IRF4 rearrangement.

Solitary fibrous tumor in Central nervous system:

Solitary fibrous tumor is a fibroblastic neoplasm characterized by NAB2 and STAT6 gene fusion as well as STAT6 nuclear expression.

In 2016 CNS classification, hybrid term Solitary fibrous tumor/Hemangiopericytoma was used. The term “Hemangiopericytoma” has been retired in new classification with the tumor now termed only as Solitary fibrous tumor. This term now aligns with the soft tissue nomenclature. However, the grading scheme is different at both sites.

Grading in CNS is based on mitotic activity and necrosis.

CNS WHO grade 1: Less than 5 mitoses/10 high power fields (hpfs).

CNS WHO grade 2: 5 or more mitoses/10 hpfs without necrosis.

CNS WHO grade 3: 5 or more mitoses/10 hpfs but with necrosis.

Monkeypox vs. COVID-19

Dr Maria Owais and Dr Anila Rashid
Haematology & Transfusion Medicine

Joshua Lederberg, Nobel Prize laureate, had said that "the single best threat to man's continuous dominance on this planet is virus". We were struggling to fight against Polio, dengue, viral hepatitis, HIV; however, the advent of COVID pandemic has changed the everything. More recently World Health Organization (WHO) declared monkeypox to be a "public health emergency of international concern," its highest-level alert, on July 23, 2022.

Since the start of the monkeypox outbreak and as of 27 September 2022, 20 083 confirmed cases of monkeypox have been reported from 29 EU/EEA countries. In total, 58 cases have been reported from three Western Balkan countries and Turkey.

Summary of difference between Monkey pox and COVID 19:

	Monkey pox	COVID 19
Causative agent	Monkey pox virus (MPV)	SARS-CoV-2
Year of discovery	1958	2019
Genome	Double-stranded DNA	Single-stranded RNA
Variant and genotypes	2 clades	27 clades (till date)
Primary route of transmission	Direct, prolong contact with monkeypox rash, scabs or bodily fluids from infected person	Respiratory droplets
Potential reservoirs	Monkey, rodents and other small mammals	Bats, pangolins and minks

SARS-CoV-2 and Monkeypox Virus: Structure and Evolution

Structure and Genome

Structurally speaking, SARS-CoV-2 and monkeypox virus (MPV) are very different. SARS-CoV-2 like all coronaviruses, is an enveloped single-stranded RNA virus. It is small (100 nm diameter), spherical and decorated with a porcupine-like sheath of spike (S) proteins. S proteins bind to host cells via angiotensin-converting enzyme 2 (ACE2), a protein ubiquitously expressed by organs throughout the human body, to initiate infection.

MPV is a member of the Poxviridiae family—the virus is enveloped, brick shaped and large (220-450 nm long). Its double-stranded DNA genome is encapsulated in a core containing enzymes needed for replication and evasion of host immune defenses. Like SARS-CoV-2, MPV has surface proteins that facilitate its entry into host cells. However, rather than a single protein, poxviruses use 11 to 12 transmembrane proteins to fuse with host cells, likely binding glycosaminoglycans or laminin on the cell surface.

Evolution and Variants

The differences in the genomes of SARS-CoV-2 and MPV have important evolutionary ramifications. RNA viruses, like SARS-CoV-2, can be sloppy replicators. RNA polymerase, which copies the viral genome, lacks the ability to catch and fix replication errors. Unlike other RNA viruses, coronaviruses do have an enzyme (i.e., an exoribonuclease) with some proofreading ability. However, while this may slow the acquisition of mutations in SARS-CoV-2, it does not stop them altogether. As a result, random mutations develop that can, if beneficial for viral fitness, quickly become widespread. This has been apparent throughout the COVID-19 pandemic. In 2021, the SARS-CoV-2 Delta variant dominated the pandemic landscape. When 2022 rolled around, Omicron, which spreads easier from person-to-person, replaced Delta as the most dominant variant. The increased transmissibility of Omicron is tied to a slew of S protein mutations that regulate binding to ACE2 and promote the ability to evade host antibodies.

There are 2 known viral clades of MPV: the Congo Basin clade and the less virulent West African clade, which underlies current outbreaks in non-endemic countries. DNA viruses, like MPV, do not mutate as freely as RNA viruses. The enzymes involved in DNA viral replication (i.e., DNA polymerase) are better at proofreading and fixing errors than those in RNA viral replication (i.e., RNA polymerase). Poxviruses typically acquire about 1-2 mutations per year. However, evidence suggests that MPV has acquired nearly 50 mutations compared to strains detected in 2018-2019. If/how these genetic changes influence the spread of monkeypox is unclear. What

researchers do know is that most of the mutations bear the mark of a human antiviral enzyme, APOBEC3, which edits base pairs in viral genomes. The mutations, therefore, do not reflect the virus's random mutation rate, but seem to be indicative of time spent in humans (indeed, data suggest MPV may have been circulating among human populations in Africa and Europe for several years before the influx of cases began in May 2022). This differs from mutation patterns in SARS-CoV-2, which are largely tied to replication errors that may or may not become fixed in a population.

Reservoirs

Monkeypox and COVID-19 are both zoonotic diseases, meaning they are transmitted from animals to humans. SARS-CoV-2 is thought to have originated in bats, potentially hopping to another animal, such as pangolins or minks, before making the leap into humans. However, direct evidence supporting this chain of transmission events is still lacking.

MPV is endemic to countries in central and western Africa. Although monkeypox was first discovered in monkeys kept for research in the Democratic Republic of the Congo (DRC), they are not the main, or only, reservoir of the virus. Rodents, including rope squirrels and Gambian pouch rats, are believed to be reservoirs of MPV. Yet, MPV has only been isolated from wild animals on 2 occasions, including a rope squirrel in the DRC and a sooty mangabey in Côte d'Ivoire in 2012. As with SARS-CoV-2, more research is needed to understand the origins, reservoirs, and circulation of MPV among animal populations.

Transmission

SARS-CoV-2 is a respiratory virus—it spreads when an infected person breathes out small virus-laden droplets. If someone else inhales these droplets, or they land on their eyes, nose or mouth, the exposed individual can become infected. Because SARS-CoV-2 spreads efficiently through the air, it is particularly challenging to control—a single person has the potential to infect many others just by breathing. Moreover, people can spread COVID-19, even if they are asymptomatic.

While MPV can be transmitted through respiratory secretions, it is not a respiratory virus. Rather, it primarily spreads through direct (usually prolonged) contact with monkeypox rash, scabs, or body fluids from someone who is infected. It can also be spread congenitally, or by touching objects and surfaces that have been used by someone with monkeypox. Activities like trying on clothing at a store, however, pose a low risk—an individual would need to have extended contact with clothing that had come into prolonged contact with monkeypox lesions or sores to increase their risk of infection. This is more likely when living with a person with a confirmed case of monkeypox, but less relevant to the usual clothes-on, clothes-off routine of the fitting room.

Though MPV is transmitted through sexual contact, scientists are still investigating whether the virus spreads specifically through sexual transmission routes (i.e., semen or vaginal fluids), as well as whether the virus can be transmitted prior to symptom onset. What is clear is that, because MPV spreads primarily through close, prolonged contact, monkeypox is far less transmissible than COVID-19.

Symptoms and Disease Severity

COVID-19 symptoms appear anywhere from two to 14 days after exposure to SARS-CoV-2. They can include fever, chills, headache, sore throat and loss of taste or smell, among others. People usually feel better after a few days to few weeks, though some people have prolonged symptoms that continue for 3+ months (i.e., long COVID). COVID-19 can be fatal. Since the beginning of 2020, COVID-19 has caused over 6,400,000 deaths across the world, though rate of deaths has declined, in part due to the availability of vaccines and treatments. Risk for severe COVID-19 depends on several factors, including the SARS-CoV-2 variant causing the infection, vaccination status, age and whether a person has underlying conditions or is immunocompromised.

For monkeypox, it can take up to 3 weeks after exposure to MPV for symptoms to develop. Though it varies on a case-by-case basis, symptoms may mirror those of COVID-19 during the early stages of infection (e.g., fever, headache, chills). Clinically speaking, monkeypox differs from COVID-19 in that it is characterized by the development of a rash, which can be painful and itchy, and tends to be distributed on the face, extremities and genitals.

Most people recover from monkeypox after 2-4 weeks. It can be severe, even fatal, but the mortality rate is nowhere near that of COVID-19. According to WHO, there have been 12 deaths from monkeypox since January 2022, 5 of which occurred outside of the African region. Disease severity is tied, in part, to the strain of MPV causing infection. Like COVID-19, monkeypox severity also depends on age (young children are more likely to develop severe disease) and the presence of underlying conditions.

Diagnosis

Due to rapid antigen tests, people can test themselves for COVID-19 at home. Nucleic acid amplification testing (NAAT), such as polymerase change reaction (PCR), is also available. These methods are performed in laboratories or point-of-care facilities (e.g., pharmacies, school health clinics, among others) and involve isolating and amplifying the genetic material from patient specimens to detect SARS-CoV-2 RNA.

Currently, there are fewer options for diagnosing monkeypox. Confirmatory testing is only conducted via PCR on fluid from pustules or dry crust from scabbed lesions. Moreover, samples must be sent to a public health laboratory or 1 of 5 commercial labs for analysis—there are currently no options for testing at home or at point-of-care facilities. As monkeypox outbreaks evolve, and case counts rise, other diagnostic tools may be developed that promote the ease, accessibility and/or diagnostic capabilities of monkeypox testing.

Prevention and Treatment

There were no vaccines for COVID-19 at the beginning of the pandemic because SARS-CoV-2 was a novel virus when it was discovered in late 2019. Now, 4 vaccines have been approved for use in the U.S. COVID-19 vaccination, which protects against severe disease and hospitalization, is approved by the U.S. Food and Drug Administration (FDA) for people 6 months of age and older. There are also several antiviral and monoclonal antibody treatments available to treat COVID-19.

Unlike the early days of 2020, when COVID-19 first came onto the scene, there are already vaccines that protect against monkeypox. A live-attenuated vaccine, trademarked JYNNEOS, is currently being used for widespread vaccination efforts. JYNNEOS was developed to prevent smallpox and is also protective against monkeypox in adults 18 years and older (as of August 9, 2022, people younger than 18 years old, and at high risk for monkeypox infection, may also receive the vaccine under an Emergency Use Authorization).

While JYNNEOS is the preferred vaccine for monkeypox, according to the Centers for Disease Control and Prevention (CDC), there is a second smallpox vaccine, ACAM2000, that may be used as an alternative. ACAM2000 can be considered for people 1 year of age and older. However, because ACAM2000 has the potential for more adverse side effects, particularly people with weakened immune systems, the CDC recommends that individuals consult with their healthcare provider to determine which vaccine is best for them.

Monkeypox vaccination efforts are currently focused on people who have been exposed to monkeypox or who are more likely to get monkeypox, such as members of the men (i.e., people assigned male at birth) who have sex with men (MSM) community, who have been disproportionately affected by the disease. Right now, there are no specific treatments for monkeypox. However, tecovirimat, a drug that treats smallpox, may be considered for people with, or at risk for, severe disease.

For both COVID-19 and monkeypox, isolating infected individuals and maintaining proper hygiene (i.e., handwashing) and disinfection practices are important for preventing and slowing the spread of infection.

Monkeypox Outbreaks vs. The COVID-19 Pandemic

There are several important differences between monkeypox outbreaks and the COVID-19 pandemic. For one, SARS-CoV-2 was a novel virus when it emerged in late 2019, meaning it had never been seen before. As a result, the world didn't have vaccines or immunity to the virus, which allowed it to spread like wildfire. The rise of new SARS-CoV-2 variants, coupled with the virus's ability to transmit efficiently from person to person through the air, only fueled (and continues to fuel) this fire.

Monkeypox is not a new disease. Scientists know more about MPV than they did about SARS-CoV-2 at the beginning of the COVID-19 pandemic. Importantly, given that MPV spreads primarily through close contact, it is less efficient at spreading between humans. Vaccines are also already available and, while there have been supply chain challenges, are being administered to at-risk communities.

Still, MPV is spreading in ways not previously seen (i.e., through sexual networks). The virus has also acquired mutations with unclear function and significance—if MPV continues to circulate, it could develop mutations that help it better infect humans. As such, the world must remain vigilant and put the lessons learned from the COVID-19 pandemic to good use.

From the Diary of a Researcher- Notes on Understanding Beta-Thalassemia and Metabolic Bone Disease

Dr Arsala Jameel Farooqui
Clinical Chemistry

In October 2021, I started visiting Fatimid Foundation for my duties as a senior research assistant for the Department of Pathology and Laboratory Medicine. The project is headed by the Section of Chemical Pathology, with the collaboration of specialists from Haematology, Internal Medicine, and Dentistry at Aga Khan University Hospital, and the specialists and staff at Fatimid Foundation. It focuses on the assessment of metabolic bone disease in patients who are afflicted with beta-thalassemia major. These chronically transfused patients present with iron overload and are tested for various biochemical parameters of bone health, including serum calcium (Ca), Albumin (Al), phosphate (P), vitamin D (25-OHD and 1-25-OHD), parathyroid hormone (PTH) among others. My job is to recruit willing patients at the Foundation, conduct their thorough physical and dental examination, and then collect their blood and urine samples for laboratory testing.

Considering the seriousness of the project and the patients' conditions, I was nervous on my first day, but when I stepped into the thalassemia ward, I was surprised to sense a feeling of calm and lightness. The patients, staff and medical officers seemed at ease with each other, and I regretfully realised that I was probably the only person in the room who was mentally treating them with pity. Despite the physical and mental discomfort of the various difficulties that come with the disease, these children show remarkable maturity and understanding about their condition. Years of constant blood transfusions have also made them become fiercely independent, and I noticed that many of the older children come to get transfusions on their own.

However, I had to stay cognizant of the fact that there was merely a different circumstance of birth separating me from their inescapable fate – and it doesn't end with a disease diagnosis. It is important to recognize that managing such lifelong diseases pushes afflicted patients and their families further down the poverty line, and juggling the expenses of multiple children, transportation, and personal lives is a burden that needs to be eased. I cannot stress upon the gravity of having a life where a fortnightly blood report determines how you will stay alive. Dealing with these patients face-to-face made me realize that this public health problem has individuals at its core, and as such it must be dealt with tactfully, and all subsequent treatment and management plans must be catered to individual patient conditions.

For the research team, this became especially relevant when the test results came back. Although many of the patients were taking oral calcium and vitamin D supplements, the results revealed vitamin D (25-OHD) deficiency in most of the tested patients, and calcium deficiency in some, which were a probable sign of iron overload induced metabolic bone disease. Even though the affected patients are generally more aware of thalassemia-induced complications, they do not have a clear idea about the ramifications of bone disease. Perhaps it is because this area is not given due attention in practice, perhaps it is due to the lack of routine metabolite testing, or the treating physicians' limited awareness of its manifestations in beta-thalassemia. This remains to be confirmed. However, one thing is clear- by staying undetected, the vicious cycle of metabolic bone disease continues to roll, manifesting in untreated patients with increased bone fragility and bone pains.

But now that we, the researchers, knew that it was present, what could the next step be? After careful consideration of several age-specific guidelines, the investigators decided to provide the severely deficient patients (whose 25-OHD levels were less than 20 ng/ml) with an oral vitamin D supplement of 200, 000 IU, that was procured by the collaborators at Fatimid Foundation. However, for patients with vitamin D insufficiency, the challenges were different. In the absence of nutritional assessment, the team had to rely on subjective accounts of the patients' nutritional history and devise an individualized supplementation strategy that also considered the food insecurity

challenges that have arisen in the country in the wake of flood disasters.

In October 2022, the sample collection for this project is nearing its end. The incoming steps of data analysis and manuscript preparation will have their own challenges, but my job is to greet them with an open mind. Above all, I hope that our efforts help make a tangible difference in these patients' lives down the road.

Significance of Audit in a Clinical Laboratory

Dr Hamna Siddiqui
Clinical Chemistry

What do you mean by an audit in a laboratory? It's a procedure used by health professionals to assess, evaluate, and improve care in a systematic way that allows to measure existing practices against an established standard. It's done to examine the quality of medical care, including procedures used for diagnosis and treatment, resource use, and outcomes of patients and life quality. In a laboratory, this entails assessing the quality of pathology services in terms of their contribution to patient care, which involves both clinicians who use diagnostic services and laboratory personnel who provide them.

Audits in the clinical laboratory are mainly concerned with providing feedback about the day-to-day aspects of laboratory services. It usually involves correlating the performance of laboratory services to predefined standards. These standards were ideally devised using evidence-based medicine principles. The practices are duly modified, and a re-audit is performed after a certain time period if necessary. Pre-analytical (request forms/specimens/phlebotomy services), analytical (range of investigations available/test method SOP/efficient use of staff/lab reports/safety guidelines /test usage), and post-analytical (reporting methods/reference ranges/interpretation/complaints and remedial action taken) phases are audited in the laboratory. Clinical data from the laboratory tests is medically significant in determining the course of disease, affecting up to 70 percent of the clinician's diagnosis.

Figure 1: Evidence-based laboratory medicine and audit.

What are its types?

- **External audits** are conducted by auditors and are used to assess the quality system of a healthcare facility based on predetermined standards. For instance, accreditation, certification, and so on.
- **Internal audits** are conducted by auditors from the hospital's own institution, along with healthcare professionals from a various department than the one being audited, to help make sure independent judgement. They are utilized to properly assess the quality system against standards and to prepare for external audits to constantly improve healthcare quality.
- **Clinical audits** are distinct from other types of audits in that they are typically initiated and carried out by healthcare professionals. This process occurs on a continuous basis as part of daily routines in which healthcare professionals collaborate to collect data and evaluate their own.

Figure 2: The audit cycle

Why is it important to conduct an audit?
It is crucial to enhance patient outcomes by improving professional practice and service quality. It virtually guarantees that the lab has quality systems in place to assist in the implementation

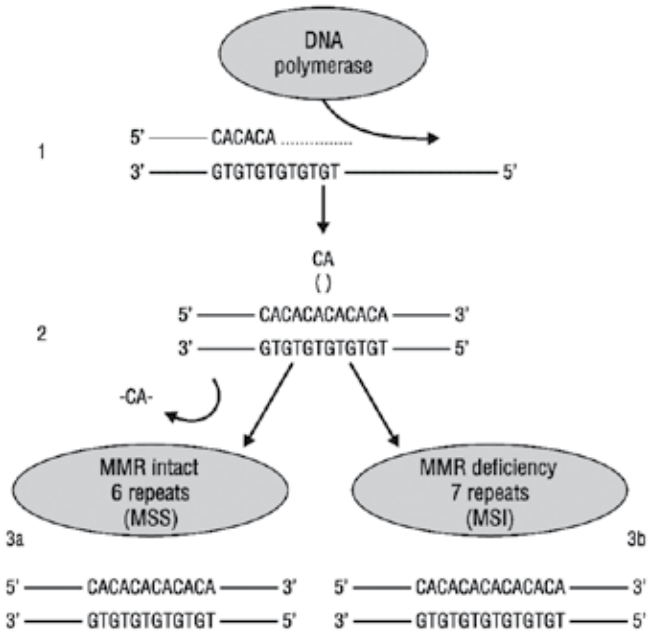
of good lab methods and in the generation of data of integrity and quality. It is a way to determine whether healthcare is being provided in accordance with standards and to inform healthcare workers about their service, what is working well and where patient care could be improved, allowing you to showcase the benefits of your training to others. It provides excellent use of clinical time to increase the number of contented patients and help you advance your practice. It eliminates unnecessary treatment and investigations by identifying patients who have persistent problems but have never been followed up on. Helps laboratory for an external audit, increase staff awareness of quality system requirements and identify the gaps that need to be corrected. It makes you understand where preventive or corrective action is needed. Helps to review documents and records to confirm accuracy and to measure the performance of laboratory services against established standards.

Solving problems associated with process or outcome. To monitor workload for controlling demand. It detects changes and introduction of new tests in a laboratory. Helps monitor compliance for best practice and analytical quality to ensure accuracy in a laboratory. An audit is a quality improvement process and is an important part of the quality assurance programme of a laboratory. There should be at least one complete audit in a year or parts of laboratory system in a month depending on a laboratory as it measures performance, practices standards, help to improve and significantly involves a re-audit after a specified time to ensure for sustained improvements. A complete audit should be performed at least once a year, or parts of the laboratory system should be audited once a month, depending on the laboratory, as it measures performance, practices standards, aids in improvement, and includes a re-audit after a specified time to ensure long-term improvements.

Microsatellite Instability (MSI) Testing

Samra Naz, Anum Ujala, Dr. Zeeshan Ansar Ahmed, Irma Aijaz, Nida Naz
Molecular Pathology

Each of our cells contains DNA with genes that provide instructions for our cells on how to grow, carry out specific activities, divide, or die. The DNA in our cells also contains segments of short repetitive



DNA sequences called microsatellites. Microsatellites are short stretches of DNA with a repetitive sequence of nucleotide, also known as Short Tandem Repeats (STR) 1-6 base pairs scattered throughout the entire genome. Microsatellite instability (MSI) is the condition of genetic hypermutability that results from impaired DNA mismatch repair (MMR). The DNA MMR system is composed of 4 MMR genes and their encoded proteins (MLH1, MSH2, MSH6, PMS2). The presence of MSI represents phenotypic evidence that MMR is not functioning normally.

(1) DNA replication.

(2) CA repeat wrongly incorporated into the chain of replicated DNA.

(3a) Maintained microsatellite stability (MSS) by an effective MMR system.

(3b) MMR system defect: lack of elimination of wrongly incorporated into DNA nucleotides and resulting MSI

Human mismatch repair genes and proteins

In order to mediate DNA repair, versatile proteins collectively called MSH and MLH/PMS have evolved in eukaryotes including mammals and humans. All of them fulfill their functions as heterodimers. Their names reflect the homology to the E. Coli system, which is why name MSH is short from muts Homolog, while MLH is derived from mutl Homolog of E. Coli. Our knowledge of the ways in which mammalian MMR system function comes primarily from in vitro studies thatshowed that MMR mechanisms involve the following steps: lesion recognition, repair initiation, lesion excision, and DNA resynthesis. Evidence shows that mismatch repair prefers actively transcribed genes. The MMR system consists of a group of proteins that interact as heterodimers capable of perceiving and repairing mispaired bases and small loops formed from insertions or deletions. MMR repair processes have diversified in human matching to the functional combination of proteins forming the dimers. Thus, MMR machinery in humans has 8 genes that code for its components. The homologs of E. Coli muts genes in humans are hmsh2, hmsh3, hmsh5, and hmsh6, while mutl homologs are hmlh1, hpms1 (hmlh2), hmlh3, hpms2 (hmlh4). Variations in the deficiency of DNA repair genes are important for specific tumor susceptibility. Loss of proper functioning of DNA damage repair proteins, whether through mutations or loss of translation, introduces genomic instability critical for tumor evolution.

Cancer related to MSI

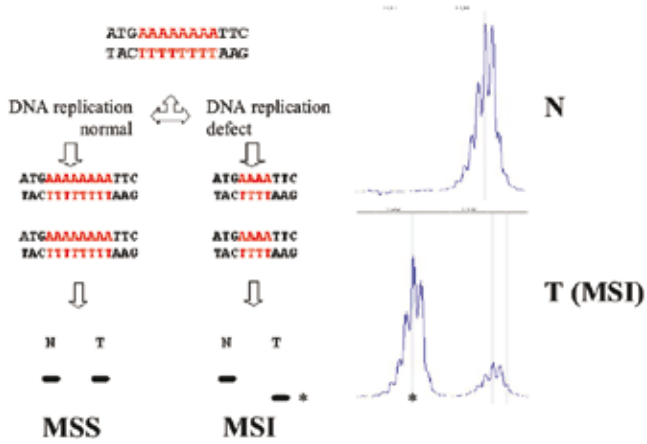
In order to be able to understand MMR's role in cancer we first need to discuss the so called "mutator hypothesis." This hypothesis has been proposed in order to rationalize the disproportion between heavily mutagenized tumor cells, and the number of mutations existing in normal cells. The usual incidence rate of spontaneous somatic mutations occurring during the lifetime of one individual does not match to the number of genetic alterations observed in tumor cells. We know now that this increased mutational extent is a consequence of genomic instability, a phenomenon that characterizes tumors. It is also well-known that carcinogenesis results from multiple sequential genetic changes. However, the mutator hypothesis relates primarily to the malfunctioning of MMR system that elicits the mutator phenotype characterized by the elevation of mutation burden. The outcome of a hypermutation phenotype is that microsatellite instability arises. Data suggest that the mutation frequency of normal human cells is much too small to explain the hundreds of genetic changes that occur above the random mutation rates. The responsibility lies in mutations of mutator genes often referred to as mutator mutations. The mutator gene usually comes from a group of genes responsible for DNA repair mechanisms, or from the group responsible for controlling DNA synthesis fidelity. If the mutator gene is himself hit by a mutation, this will lead to increased rates of mutation in an individual's genome that cannot be properly repaired. Therefore, cancer cells displaying increased rates of genomic instability are said to comprize of a mutator phenotype. Comprehensive sequencing studies of a variety of tumors substantially supported the mutator hypothesis. For instance, one of the largest studies supporting the mutator phenotype of human tumors is brought by The Cancer Genome Atlas (TCGA)—a project whose goal is to determine the number and type of mutations in specific tumors. TCGA reports that the number of mutations detected per tumor contrasts greatly not only to the number found in normal cells, but also between different types of tumors ranging from 500 to 100,000mutations.

It is generally believed that the acquisition of genetic instability is associated with progression and therefore occurs later in tumor evolution. However, there are contrasting viewpoints that suggest it may represent an early event responsible for the initiation of tumor formation. An ingenious paper showed that the overall number of mitoses, stem cells carry out within a certain tissue, correlates positively with the lifetime risk of developing cancer. This suggests that the probable number of mitoses of a particular tissue should, in addition to the number of genomic instability events, be considered when evaluating the role of MSI for specific tumors. The cumulative effect of MSI and the number of cell divisions could help us better understand the difference in MSI accumulation between tumor types, as well as the specificities of cancer progression. As they are responsible for correcting the mutational overload ,MMR genes acquired the role of tumor suppressor genes. In sporadic non-heritable cancers MSI is the consequence of either inactivating mutations in one of the MMR's genes, or epigenetic mechanisms of MMR gene expression including down regulation by micrornas. Several papers report on MSI-associated candidate mirnas. A comparison study between mirnas involved in colorectal cancer indicated that decreased levels of mir-552, mir-592, mir-181c, and mir-196b were observed in proficient MMR tumors as compared to increased levels of mir-625 and mir-31 in deficient MMR tumors.

Microsatellite instability in colorectal cancer

Microsatellite instability (MSI) is hallmark for some colorectal cancers (CRCs) in which short tandem

repeats are prone to mutations along with DNA sequence. It is due to DNA-mismatch- repair system deficiency because of germline/somatic mutations. The germline mutations lead to lynch syndrome while epigenetic mutations lead to sporadic CRC tumors. Colorectal cancer (CRC) is the third most commonly-diagnosed cancer in the world and ranked second for cancer-related mortality in humans. Microsatellite instability (MSI) is an indicator for Lynch syndrome (LS), an inherited cancer predisposition, and a prognostic marker which predicts the response to immunotherapy. High-level MSI (MSI-H) is of eminent clinical importance. It is the seminal molecular feature for the identification of individuals with Lynch syndrome, but it may also occur in sporadic cancers with CIMP phenotype, which arise from serrated precursor lesions. MSI-H status is a marker of favorable prognosis and may be used for outcome prediction, that is, molecular grading.



PCR based molecular detection for MSI For MSI analysis testing can be performed on fresh frozen paraffin-embedded (FFPE) tumor tissue and a normal genomic DNA using a fluorescence-based PCR assay. Alleles that are present in the test sample but not in corresponding normal sample indicate MSI. The MSI analysis includes fluorescently labelled primer for co-amplification of five mononucleotide repeat markers BAT-25, BAT-26, NR-21, NR-24 and MONO-27 for MSI determination and two pentanucleotide repeat markers Penta C and Penta D to detect potential sample mix-up. PCR products are followed by capillary electrophoresis.

For data analysis, GENE MARKER software is required. The software allows manual and automated analysis of the raw data and generates electropherograms with accompanying data tables displaying PCR fragment lengths (in base pairs) and peek height in (RFU)

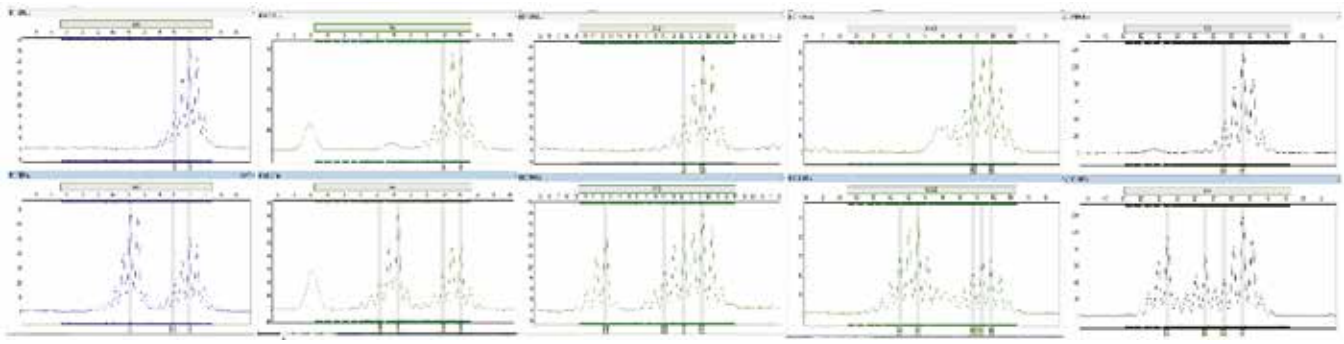


Figure 1: MSI-HIGH

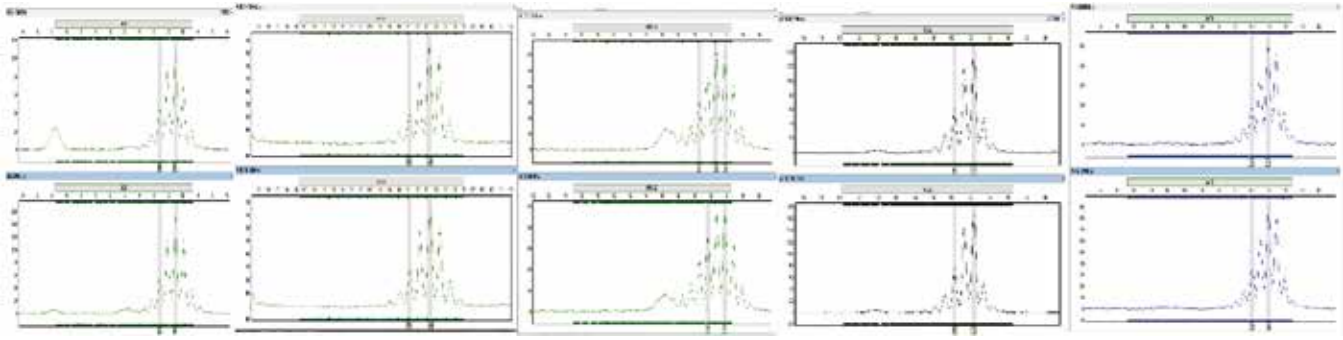


Figure 2: MSI-STABLE

Pre-Analytical Variables Affecting Coagulation Testing

Dr Hareem Alam
Haematology

Reliability of any laboratory test result depends on pre-analytical, analytical and post analytical factors. Pre-analytical variables refer to all procedures that occur prior to sample analysis, such as sample collection, transportation, storage and handling.

Several preanalytical variables may affect the results of routine coagulation assays. To improve the precision and accuracy of laboratory testing, it is critical to identify these variables and their potential impact. Following is the summary of factors affecting coagulation testing:

Pre-analytical variable	Effects on coagulation	Recommendation
Patient specific factors		
Stress	Both physiological and psychological stress may artifactually alter coagulation tests, especially for Von Willebrand factor (VWF) and platelet function studies.	Patient should be in a relaxed and smoking free state prior to phlebotomy.
Physiological state	Physiological state such as pregnancy may affect components of the haemostatic pathway e.g., Protein S levels are low during pregnancy.	Results should be interpretated in context of physiological states.
Lipemic samples	High levels of lipids may lead to turbid plasma which can interfere with optical density measurements used to determine the end point in tests, such as clotting factor assays and platelet aggregation studies.	Samples should be collected from a fasting and drug naive patients.
Hemolyzed samples	Hemolysis increases the spectrometric absorbance of the plasma sample Hemolysis increases the spectrometric absorbance of the plasma sample Hemolysis increases the spectrophotometric absorbance of the plasma sample thus affect the accuracy of test results.	If possible, grossly hemolyzed specimens should be rejected. If possible, grossly hemolyzed specimens should be rejected. If possible, grossly hemolyzed specimens should be rejected. If possible, grossly hemolyzed specimens should be rejected. If possible, grossly hemolyzed specimens should be rejected. Grossly hemolyzed samples should be rejected.
Specimen collection		
Venipuncture technique	Prolonged tourniquet application will induce increased vessel pressure, hypoxia, and lower pH below the tourniquet, thereby potentially masking mild deficiencies in VWF, FVIII and other endothelial associated coagulation proteins.	Atraumatic phlebotomy with minimal tourniquet use which should not exceed more than 1 minute.
Anticoagulant	3.2% trisodium citrate is the anticoagulant of choice in a ratio of 1 part Anticoagulant:9 parts blood	
Correct sample volume	Underfilling or overfilling of blood collection tubes is the predominant cause for falsely elevated PT, INR, and aPTT results.	Tubes that are overfilled or underfilled are unacceptable for testing and should be rejected.
Hematocrit adjustment	When the patient hematocrit is significantly elevated (> 55%), the excess citrate in the plasma sample would potentially inhibit clot formation producing artifactually prolonged clotting time values.	Patients with elevated hematocrits require reduced volume of citrate prior to collection for accurate measurement of PT and APTT.

Transportation
Coagulation whole blood samples should not be transported or stored on ice.
The PT, in whole blood, is stable for 24 hours at room temperature while aPTT is stable for up to 4 hours at room temperature.
For other tests, unless otherwise indicated from manufacturer, the stability in whole blood is 4 hours.
Specimen processing
With the exception of whole blood testing and platelet function studies, platelet-poor plasma (PPP) is the sample of choice, which is defined as < 10,000 platelets/μL.
Sample storage
For samples not tested within the recommended room temperature stability limits, PPP should be stored at –70°C, or colder for 6 months or at –20°C for 2 weeks.
Sample vials are rapidly thawed in a 37°C water bath, before processing.

Grading of Soft Tissue Sarcomas

Drs. Manahil Khan, Madiha Bilal Qureshi and Nasir Ud Din
Histopathology

Introduction:

Soft tissue sarcoma (STS) is a heterogenous group of malignant tumors of mesenchymal origin. It accounts for about 1 percent of all adult and 12 percent of pediatric malignancies. Approximately 80 percent of sarcomas originate from soft tissue and rest from bone. STS has minimal male predominance and frequency increases with age.

Localization and frequency of STS:

Although STS can involve any portion of the body, but lower extremity is the most common site (45 percent), followed by trunk (30 percent), upper extremity (15 percent), head and neck (9 percent) and others (1 percent).

Etiology of STS:

- **Previous radiation exposure:**
- No sooner than 3 yrs.
- 1-3 percent risk.
- Are high grade sarcomas.
- Seen at field edges commonly.
- **Chemical exposure:**
- Arsenic, plastic.
- **Chemotherapy exposure:**
- Alkylating agents in childhood for ALL.
- Chronic lymphedema:
- Stewart-Treves syndrome.
- Filariasis.
- **Genetic:**
- Li-Fraumeni syndrome increases the risk to almost 50 percent by the age of 35 yrs.
- **Viral infections.**
- **Immunodeficiency.**

Clinical features:

- Depend on site of origin and involvement:
- Extremities and upper trunk:
  - Painless mass.
- Retroperitoneum:
  - Abdominal mass with pain (50% of cases).
- Viscera's: Depend on involved organ.
- Head & neck: Mechanical problems secondary to compression or invasion of adjacent structures like:
  - Nasal obstruction
  - Cranial nerve dysfunction
  - Proptosis

Diagnosis:

- History and examination.
- Plain radiographs:

  - Calcification, or bone invasion.
- Ultrasound:
  - Solid vs Cystic.
- MRI / CT:
  - Location, extent, operability, mets.
- Biopsy: Tissue diagnosis.
- PET scan: Can be used to rule out metastasis.

Staging:

- The TNM staging system for soft tissue tumors of the AJCC and UICC is recommended.
- T stage mainly depends upon size of the tumor, N on involvement of regional nodes and M on distant metastasis.

Grading:

Grading of soft tissue sarcomas relies on intrinsic features of primary tumor. Grading of STS was initially proposed by Broders et al, but a more effective way to use grade and stage in sarcomas was published by Russell et al in 1977, which emphasized the role of grade in predicting outcome of patient. Since then, multiple grading systems have been reported using different histologic parameters. The two most commonly used grading systems are NCI (National Cancer Institute) and FNCLCC (French Federation of Cancer Centers Sarcoma Group) grading systems. However, the FNCLCC grading system is more accurately defined and more reliable in predicting prognosis and outcome of disease. Moreover, the FNCLCC grading system is also preferred by College of American Pathologists (CAP) and American Joint Committee on Cancer (AJCC). FNCLCC grading has greater efficiency with reference to prognostic prediction and reduction of cases that are assigned intermediate grade.
Precise grading requires adequate sample of tissue, prior to therapy. Core biopsies cannot be reliably graded, but effort should be done to categorize into at least low or high grade. Three independent prognostic factors are used in FNCLCC grading system: degree of differentiation, mitoses and necrosis. A particular score is given to each of the factors and grade is calculated by summing up the three attributed scores (Table 1).

FNCLCC Grading

Histologic grade	Tumor differentiation, Mitotic count, Necrosis
Tumor Differentiation Score	
Score 1	Sarcomas closely resembling normal, adult mesenchymal tissue and potentially difficult to distinguish from the counterpart benign tumor (eg, well-differentiated liposarcoma, welldifferentiated leiomyosarcoma)
Score 2	Sarcomas for which histologic typing is certain (eg, myxoid liposarcoma, myxofibrosarcoma)
Score 3	Embryonal sarcomas and undifferentiated sarcomas, synovial sarcomas and sarcomas of doubtful tumor type
Mitotic Count Score	1HPF = 0.1734 mm2
Score 1	0 to 9 mitosis / 10 HPF
Score 2	10 to 19 mitosis / 10 HPF
Score 3	>19 mitosis / 10 HPF
Necrosis Score	
Score 0	No tumor necrosis
Score 1	<50% tumor necrosis
Score 2	≥50% tumor necrosis
Histologic grade	Sum of 3
Grade 1	2 or 3
Grade 2	4 or 5
Grade 3	6 to 8

Tumor Differentiation Score is assigned to particular tumors according to Histologic Type in the Updated Version of the French Federation of Cancer Centers Sarcoma Group System:

Grade 1:
Atypical lipomatous tumor / Well-differentiated liposarcoma
Well-differentiated leiomyosarcoma
Malignant neurofibroma
Well-differentiated Fibrosarcoma

Grade 2:
Myxoid liposarcoma
Conventional leiomyosarcoma
Conventional fibrosarcoma
Myxofibrosarcoma

Grade 3:
High-grade myxoid (round cell) liposarcoma
Pleomorphic liposarcoma
Dedifferentiated liposarcoma
Pleomorphic Rhabdomyosarcoma
Poorly differentiated/pleomorphic leiomyosarcoma
Biphasic / monophasic / poorly differentiated Synovial sarcoma
Mesenchymal chondrosarcoma
Extraskeletal osteosarcoma
Extraskeletal Ewing sarcoma
Malignant rhabdoid tumor
Undifferentiated pleomorphic sarcoma
Undifferentiated sarcoma, not otherwise specified
Grading of tumors including alveolar soft part sarcoma, embryonal, alveolar rhabdomyosarcoma, angiosarcoma, extraskeletal myxoid chondrosarcoma, epithelioid sarcoma and clear cell sarcoma is not recommended. Grading of malignant peripheral nerve sheath tumor is debatable.

Mitosis Count: The mitotic count should be calculated in the most mitotically active area, sparing areas adjacent to necrosis. Count should be done in either 10 consecutive high-power fields at 40X (1 HPF x 400 = 0.1734 mm2) or covering 1 mm2 area. Mitotic count close to cut offs should be reassessed. The WHO Classification of Tumours (5th Edition) has changed the denominator of 10 HPFs to an area of 1mm2 to standardize the area included in mitotic count.

Necrosis: Necrosis should be assessed on gross examination and confirmed microscopically.

Limitation: The biggest limitation for FNCLCC grading system is the tumor differentiation score which is subjective among pathologists. The system cannot be applied to all histologic types. Some specific histotypes are associated with distinct clinical behaviour and in such cases, histotyping becomes significant than grading. Core needle biopsies should be reported cautiously in terms of grade since limited tissue may not contain high grade areas.

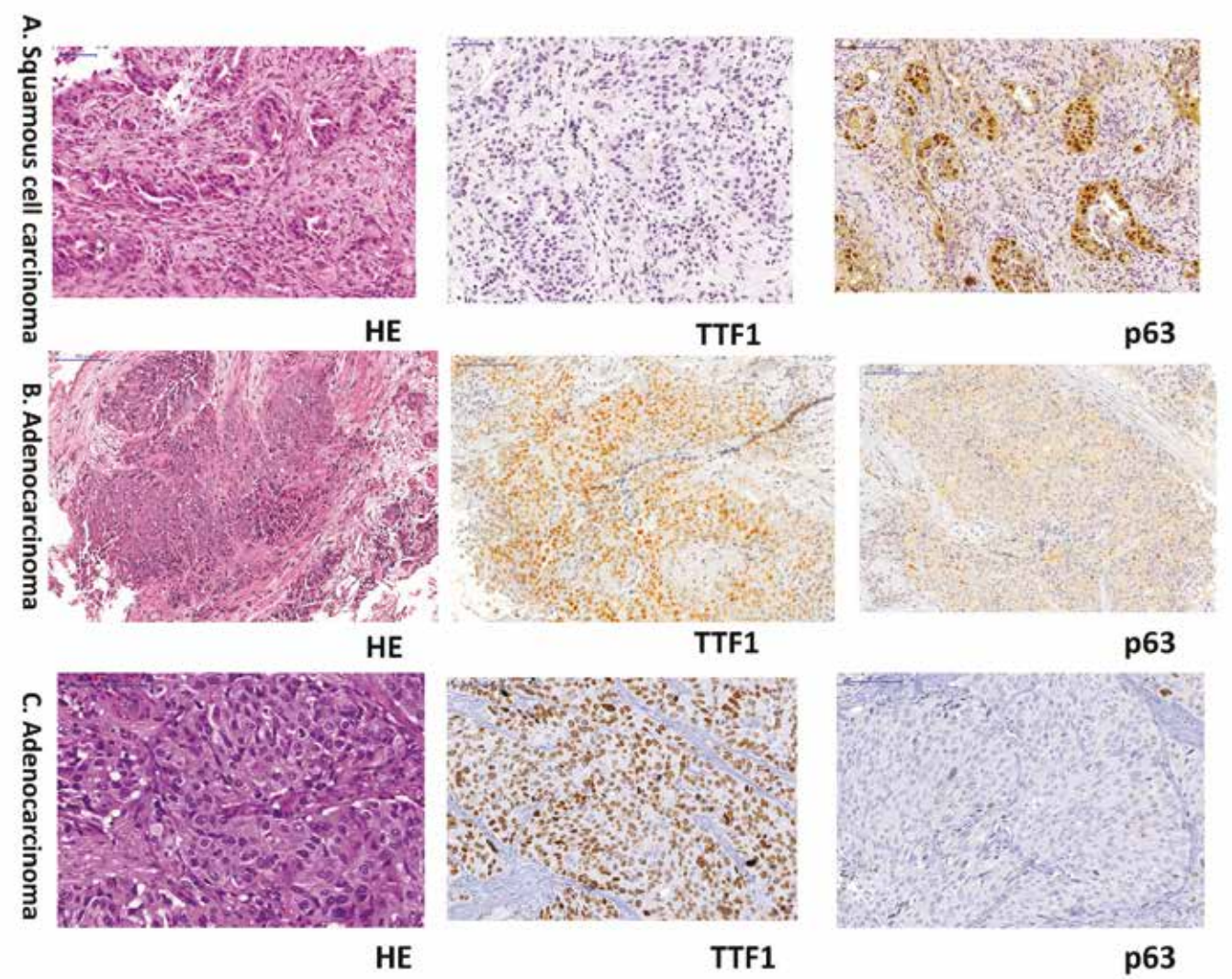
The Role of Immunohistochemistry in Histopathology:

Zeba Anwer and Dr Qurratulain Chundriger
Histopathology

What is Immunohistochemistry?

Immunohistochemistry (IHC) is a very sensitive technology that enables for high-resolution antigen localization within a cell or tissue. The method is based on the use of a primary antibody that binds to its complementary antigen specifically. A variety of approaches, such as colorimetric end points, can then be used to visualize the bound antibody.

Why Immunohistochemistry is play an important role in Histopathology?



IHC staining for the diagnosis of Lung Cancer. Fig. A shows Squamous cell carcinoma, which does not stain with TTF-1 but shows positive P63 stain. Similarly, B & C both show examples of Adenocarcinoma of lung, which shows nuclear positivity for TTF-1 and negative P63 staining.

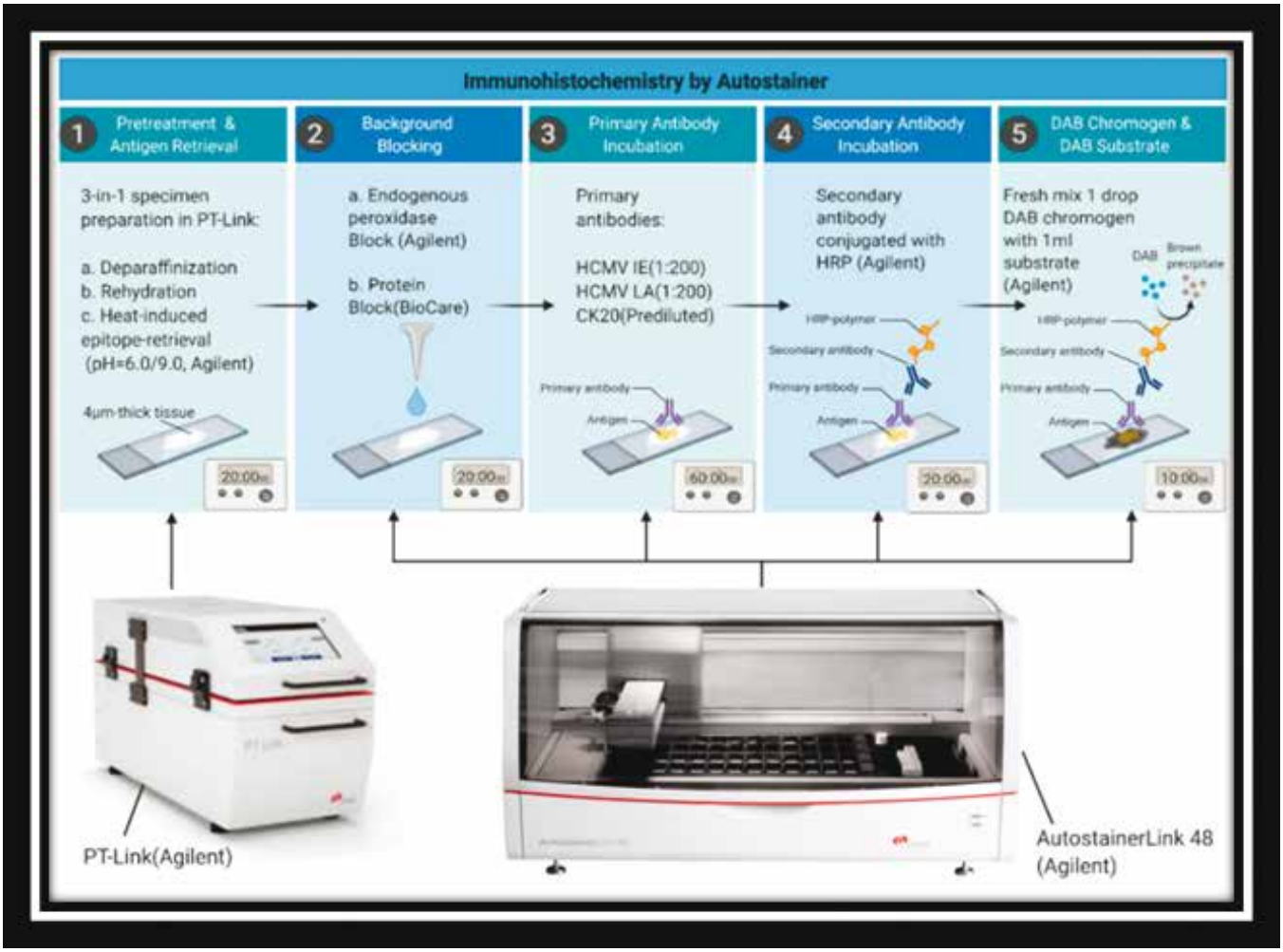
Future directions of immunohistochemistry:

The use of Genomic immunohistochemistry will aid in the discovery of underlying molecular alterations that can be employed for both diagnosis and treatment. More accurate results will be obtained by using automated computerized picture collection and analysis systems. The creation of more specific antibodies from recombinant antibody fragments will result in molecules with ultra-high affinity, stability, and potency. Tissue microarrays [TMA] as a high-throughput technology allow for cost-effective evaluation of sample use and reagent expenses.

How IHC is being done in Histopathology:

Productivity is high. Quick processing with a short turnaround time Reduce diagnostic time by processing a full batch of 48 slides in less than 3 hours. DAKO AUTOSTAINER 48 LINK PLATFORM is used to support a patient-case workflow by staining up to 24 slides in less than 2 hours.

The distinctive feature that distinguishes IHC from many other laboratory procedures is that it is performed without destroying histologic architecture, allowing for the assessment of a molecule's expression pattern in the context of the microenvironment. Pathologists are probably the best at co-analyzing both the target molecule and its subcellular, cellular, and intercellular relationships, and the importance of this co-analysis is increasingly recognized in biomedical research fields such as new drug development and prognostic/predictive biomarker investigation.



IHC Staining by DAKO AutostainerLink 48

Antibodies are generally divided into two categories. Monoclonal and polyclonal.

Comparison between monoclonal and polyclonal antibodies

Antibody	Advantage	Disadvantage
Monoclonal: Monoclonal antibodies react to a single epitope in an antigen.	Great epitope specificity and lower background	Less sensitivity or reactivity to masked epitope in a formalin fixed paraffin embedded sample
	Better reproducibility	
Polyclonal: These antibodies bind to multiple different epitopes in a single antigen.	Higher sensitivity (recognizing multiple epitopes)	Lesser reproducibility due to batch to batch variability.
		Higher background due to natural antibodies limited production.

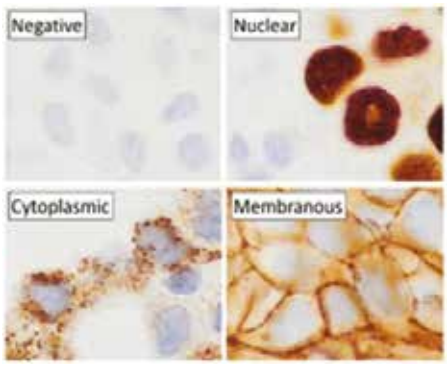
DAKO Antibodies are intended for laboratory use to qualitatively identify by light microscopy antigens on or in cells from either tissue or cell preparation samples. Positive and negative results aid in the classification of normal and abnormal cells and tissues and serve as an adjunct to conventional histopathology. The clinical interpretation of any positive staining or its absence is complemented by morphological and histological studies with proper controls. Evaluations is made within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. In general, IHC staining

techniques allow for the damasking and visualization of antigens first by pretreatment with proteolytic enzymes or heat retrieval (if required), followed by the sequential application of a specific antibody to the antigen (primary antibody), a secondary antibody to the primary antibody (link antibody or labelled polymer), an enzyme complex, and a chromogenic substrate with interposed washing steps. The enzymatic activation of the chromogen results in a visible reaction product at the antigen site. The specimen is then counterstained and cover slipped. Results are viewed using a light microscope, and interpreted by a qualified pathologist to aid in the diagnosis.

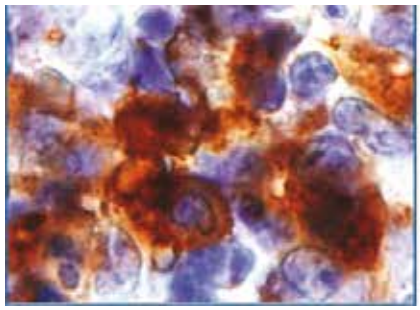
Quality control in Immunohistochemistry:
Regular controls need to be performed according to the guidelines of the College of American Pathologists (CAP) for Immunohistochemistry.

Positive Tissue Controls:
Positive tissue control specimens is fixed, processed and embedded in the same manner as the patient sample. One positive control tissue for each set of test conditions is included in each staining run. Tissues used for positive control testing must give positive staining in order to detect subtle changes in the primary antibody sensitivity. Refer to the product specific package insert, Performance Characteristics Section for normal tissue specimens is used for a positive control tissue. Known positive control tissues is utilized for monitoring the correct performance of processed tissues and test reagents, NOT as an aid in formulating a specific diagnosis of patient samples. If the positive control tissues fail to demonstrate positive staining, results with the test specimen is considered invalid.

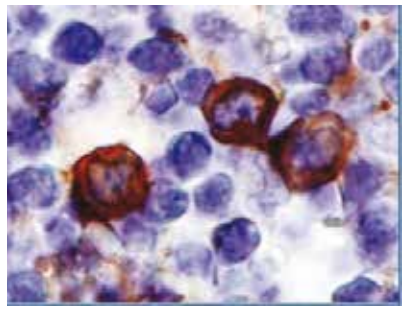
Negative Tissue Controls: Negative tissue control use a normal tissue known to be negative for the antigen being tested (refer to the product specific package insert, Performance Characteristics Section) that is fixed, processed and embedded in a manner identical to the patient sample(s) with each staining run to verify the specificity of the primary antibody and to provide an indication of background staining. The variety of different cell types present in most tissue sections offers internal negative control sites (this should be verified by the user). If specific staining occurs in the negative control tissue, patient specimen's results should be considered invalid.



Pattern of Staining with respect to reactivity



A: Nonspecific Staining



B: Specific Staining

Purpose of Daily Quality control

Tissue: Fixed & Processed Similar to Patient Sample	Specific Antibody & Detection System
Positive Control: Tissue or cells known to contain target antigen to be detected (could be located in patient tissue). Tissue which exhibits weakly positive staining is most sensitive to antibody or detection system degradation.	Controls all steps of the analysis. Validates reagent and immunostaining procedures.
Negative Control: Tissues or cells expected to be negative (could be located in patient tissue or positive control tissue)	Detection of unintended antibody cross-reactivity to cells/cellular components.
Patient Tissue	Detection of specific staining.

Recent Updates in 2022 WHO Classification of Thyroid Neoplasms

Dr Rabia Qureshi and Dr Saira Fatima
Histopathology

A better knowledge of the cell of origin, pathological characteristics, molecular classification, and biological behavior is now possible through 5th edition of the WHO Classification of Endocrine and Neuroendocrine Tumors, which has separated follicular cell-derived thyroid tumors into a number of new groups.

Follicular cell–derived neoplasms

Most thyroid neoplasms are formed from follicular cells. They are now separated into benign, low-risk, and malignant neoplasms in this new classification.

1. Benign tumors:

In addition to follicular adenomas, benign tumors also include follicular adenomas with papillary architecture, oncocytic adenomas, and thyroid follicular nodular disease.

For the first time, a thorough description of the multifocal hyperplastic/neoplastic lesions that frequently develop in the context of multinodular goiter is available; this peculiar condition is referred to as **thyroid follicular nodular disease (FND)**. This category avoids labelling a lesion as either hyperplastic, neoplastic, or the contradicting "adenomatous hyperplasia". The condition that is most frequently linked to this clinical scenario is a disorder that manifests as multiple thyroid lesions made up of follicular epithelial cells with a highly variable architecture. These lesions can be very small or very large, range from colloid-rich macrofollicular nodules to cellular microfollicular nodules, be poorly or well defined, and have absent, well-defined, or incomplete capsules.

Follicular adenoma with papillary architecture is a benign, non-invasive encapsulated follicular cell-derived neoplasm that is distinguished by a distinctive "centripetal" intrafollicular papillary architecture that is more organized than PTC, lacks nuclear features of PTC, and is frequently linked to autonomous hyperfunction.

In the classification, ***oncocytic follicular adenomas*** now have their own unique position. The phrase "Hürthle cell" should not be used. It is generally known that follicular adenomas can exhibit focal oncocytic alteration; in this classification, the description **">75 percent oncocytic cytology"** is employed.

2. Low-risk neoplasms:

Low-risk neoplasms are borderline tumors that are clinically and morphologically intermediate between benign and malignant tumors. Despite the relatively low rate of metastasis, these neoplasms have the capacity to do so. They are divided into three groups histologically, including **non-invasive follicular thyroid neoplasm with papillary—like nuclear features (NIFTP), thyroid tumors of uncertain malignant potential (T-UMP), and hyalinizing trabecular tumor (HTT).** To avoid overtreating these low-risk neoplasms, the word "tumour" was designated.

NIFTP: two subtypes of NIFTP are described in the 2022 WHO classification. At least 75% of the cells in ***"oncocytic NIFTP"*** are oncocytic. Although any tumour with a diameter of <1 cm can be described as "sub centimeter NIFTP," tumors with a diameter of ≤ 2 mm typically cannot be diagnosed as such.

T-UMP: two subtypes are described. ***Follicular tumour of uncertain malignant potential (FT-UMP)***, which lacks PTC-like nuclear features (nuclear score of 0-1), and ***well-differentiated tumour of uncertain malignant potential (WDT-UMP)***, which has pronounced PTC-like nuclear features (nuclear scores of 2-3. It is not advised to use the term "atypical adenoma." Controversial capsular or vascular invasion distinguishes UMP tumors from follicular adenoma and NIFTP.

HT: are well circumscribed nodules with nuclear alterations resembling those seen in PTCs, trabecular architecture, and prominent intratrabecular hyaline substance that has accumulated because of the production of an active basal membrane type of protein. Although the intratrabecular eosinophilic hyaline material mimics amyloid, Congo red stain is negative for it. The collagen IV-immunoreactive hyaline material is diastase-resistant but stains with the periodic acid-Schiff stain. When combined with a positive follicular markers' expression and MIB1 antibody membrane staining, an HTT diagnosis can also be confirmed.

3. Malignant neoplasms:

Follicular variant of PTC (FVPTC) has two variants; infiltrative and & encapsulated follicular variant of papillary thyroid carcinoma with capsular and/or vascular invasion. Infiltrative FVPTC is BRAF-like tumour and a member of the PTC family, according to molecular research, but invasive encapsulated FVPTC is a RAS-like neoplasm and is more like follicular thyroid cancer (FTC) than to PTC.

Based on the type, and/or degree of invasion, both FTC and FVPTC are subtyped. Each of these tumour types has a different prognosis and can be minimally invasive (tumour capsular invasion alone), invade blood vessels (angioinvasive FTC or FVPTC) or widely invasive. Angioinvasive FTC or FVPTC can be diagnosed by the presence of a single focus of angioinvasion (vascular invasion) in the absence of extensively invasive growth.

Papillary Thyroid Carcinoma (PTC) The term "variant" has been changed to "subtype" in the new WHO classification of thyroid cancers to maintain consistency with existing WHO tumour classification schemes and to avoid confusion with the term "genetic variant" used in molecular diagnostics. As opposed to the fourth edition, the revised standard for diagnosing the tall cell subtype is tumor cell height of at least 3 times the breadth and tumour is composed of 30 percent tall cells. It is advised that "PTC-microcarcinoma" not be considered as a different subtype in the 5th edition of the WHO classification of thyroid neoplasms. This is also consistent with clinical care recommendations, which construct individualized risk stratification protocols for patients with PTC based on various pathologic characteristics rather than just size.

Oncocytic carcinoma as a distinct entity, oncocytic carcinoma refers to neoplasms produced from oncocytic follicular cells (>75 percent oncocytic cells) that lack high-grade features and the typical nuclear features of PTC (those would be oncocytic PTCs).
Follicular-derived carcinomas, high-grade Two categories of high-grade non-anaplastic follicular cell-derived carcinomas with intermediate prognostic risk are recognized by the new WHO classification. Anaplastic features are absent in both subtypes.
Differentiated high-grade thyroid carcinomas (DHGTC) are invasive, high-grade, and still distinguished because they retain the unique cytologic and/or architectural characteristics of well-differentiated histotypes of carcinoma of follicular cell derivation, such as nuclear features and/or architecture of papillary carcinoma and follicular growth pattern of follicular carcinoma. Minimum requirement is one of the following two features: Mitotic count ≥5/ 2 mm, tumor necrosis. **Poorly differentiated thyroid carcinoma (PDTC)** is poorly differentiated exhibiting solid, trabecular, and insular growth patterns (or combinations of these), high-grade, and invasive follicular cell-derived carcinomas. Minimum requirement is one of the following three features: Mitotic count ≥3/ 2 mm, tumor necrosis, convoluted nuclei.

Anaplastic thyroid carcinoma Squamous cell carcinoma of the thyroid is now categorized as an anaplastic thyroid cancer morphologic pattern. The emphasis on quick and early testing of all anaplastic carcinomas for the presence of the BRAF V600E mutation is another significant improvement to the anaplastic carcinoma section. Since the combination of BRAF and MEK inhibitors was proven to be effective against BRAF V600E-mutated anaplastic cancer, this testing is required.

Thyroid C-cell–derived carcinoma
Medullary thyroid carcinoma
The addition of a grading system called "The international medullary thyroid carcinoma grading scheme" is the most significant change for medullary thyroid carcinoma in this WHO edition. High-grade tumors are defined in this as having at least one of the three features of tumour necrosis, a mitotic count of

five per two mm2, and/or a Ki67 proliferation index of 5 percent.

Other updates

Mixed tumors made up of both C cells and any follicular cell-derived malignancy have their own section. Based on cytogenesis, several rare thyroid neoplasms have been classified into new sections. The term **"salivary gland-type carcinomas of the thyroid"** now cover both mucoepidermoid carcinoma and secretory carcinoma of the salivary gland type. **Thymic tumors within the thyroid** include thymomas, thymic carcinomas, and spindle epithelial tumors with thymus-like elements. Sclerosing mucoepidermoid carcinoma with eosinophilia and cribriform-morular thyroid carcinoma are two other cancers whose cell lineage is unknown and are labelled as **thyroid tumors of uncertain histogenesis.** Cribriform-morular thyroid carcinoma is no longer classified as a subtype of PTC. **Embryonal thyroid neoplasms** including Thyroblastoma, associated with DICER1 mutations, is a significant addition. **Mesenchymal and stromal tumors, hematolymphoid neoplasms, germ cell tumors, and metastatic malignancies** are all covered separately like other fifth edition of the WHO books.

MMR and MSI testing in malignancies

Dr Tamanna Asghari
Histopathology

Cancer is fundamentally a genetic disease, and mutations are crucial to its etiology and progression. Carcinogenesis develops by accumulation of several genetic and epigenetic abnormalities.

In individuals with hereditary cancer syndrome, the initial cancer-causing mutation is inherited through the germline, hence it is already present in every cell of the body.

Lynch syndrome is a highly penetrant autosomal-dominant syndrome in which several individuals in the family are affected with colorectal cancer (CRC) or extracolonic tumors such as cancer of endometrium, stomach, small bowel, ureter, renal pelvis, ovary, and hepatobiliary tract. It occurs due to loss-of-function of the mismatch repair (MMR) mechanism for genomic replication errors.

Genes encoding molecules involved in genome repair are referred to as DNA repair genes, and as "caretaker tumor suppressor genes" . This MMR system was recognized in 1961.

What is Microsatellite Instability (MSI)

- MSI-Stable (MSS)
- MSI-Low (MSI-L)
  - 1-29% of the National Cancer Institute (NCI) or mononucleotide markers exhibit instability
  - 1 of the NCI or mononucleotide markers exhibits instability
- MSI-High (MSI-H)
  - Greater than equal to 30% of the NCI or mononucleotide markers exhibit instability
  - 2 or more of the NCI or mononucleotide markers exhibit instability

Mismatch Repair Testing: Microsatellite instability and Immunohistochemistry

Patients with a MSI-H phenotype that indicates mismatch repair deficiency in their cancer may have a germline mutation in one of several DNA mismatch repair (MMR) genes (eg, MLH1, MSH2, MSH6, or PMS2) or an altered EPCAM (TACSTD1) gene.

An MSI-H phenotype is more frequently observed in sporadic colorectal cancer (about 15% of cases) due to somatic abnormalities, usually hypermethylation of the MLH1 gene promoter.

The specificity of MSI testing can be increased by using it primarily on at-risk populations, such as colorectal cancer patients younger than 50 years,

or patients with a strong family history of Lynch-associated tumors (eg, colorectal, endometrial, gastric, or upper urinary tract urothelial carcinoma)

MSI testing of tumor DNA is generally performed with at least 5 microsatellite markers, generally mononucleotide or dinucleotide repeat markers
Recent data suggests that dinucleotide repeats may have lower sensitivity and specificity for identifying tumors with an MSI-H phenotype. Consequently, there has been a move towards including more mononucleotides and fewer dinucleotides in MSI testing panels

Many laboratories now use a commercially available kit for MSI testing that utilizes 5 mononucleotide markers. MSI testing is frequently done in conjunction with immunohistochemical (IHC) testing for DNA MMR protein expression (ie, MLH1, MSH2, MSH6, and PMS expression). (Table 1)

Mutation of MMR genes	IHC staining			
	MLH1	MSH2	MSH6	PMS2
MLH1	−	+	+	−
MSH2	+	−	−	+
MSH6	+	+	−	+
PMS2	+	+	+	−

Table 1: immunohistochemical (IHC) testing for DNA-MMR protein expression

Intact expression of all 4 proteins indicates that MMR enzymes tested are intact but does not entirely exclude Lynch syndrome, as approximately 5% of families may have a missense mutation (especially in MLH1) that can lead to a nonfunctional protein with retained antigenicity.

Loss of expression of MLH1 may be due to Lynch syndrome or methylation of the MLH1 promoter region (as occurs in sporadic MSI colorectal carcinoma)
Genetic testing is ultimately required for this distinction, although a specific BRAF gene mutation (V600E) is present in many sporadic cases, but not familial cancers
PMS2 loss is often associated with loss of MLH1 and is only independently meaningful if MLH1 is intact
MSH6 is similarly related to MSH2
Defective mismatch repair in sporadic colorectal cancer is most often due to inactivation of the MLH1 gene promoter by hypermethylation (epigenetic silencing)

Genes responsible for Lynch syndrome

Lynch syndrome (hereditary nonpolyposis colorectal cancer-HNPCC) is caused by germline mutations in DNA mismatch repair (MMR) genes. Currently, four types of MMR genes, MLH1, MSH2, MSH6, and PMS2, are used in the clinic applications related to Lynch syndrome. (Table 2, Figure 1)

Gene	MIM	Locus	No. of exons	CDS (nt)	Product no. of AA	Product MW (kDa)	Function of product
MLH1	*120436	3p22.2	19	2271	756	84.6	Heterodimerizes with PMS2 to form MutLα, a component of the post-replicative DNA mismatch repair system (MMR)
MSH2	*609309	2p21	16	2805	934	104.7	Forms two different heterodimers: MutSα (MSH2-MSH6 heterodimer) and MutSβ (MSH2-MSH3 heterodimer) which binds to DNA mismatches thereby initiating DNA repair
MSH6	*600678	2p16.3	10	4083	1360	152.8	Heterodimerizes with MSH2 to form MutSα, which binds to DNA mismatches thereby initiating DNA repair
PMS2	*600259	7p22.1	15	2589	862	95.8	This protein forms heterodimers with MLH1 to form the MutLα heterodimer

Table 2: Genes involved in Lynch Syndrome

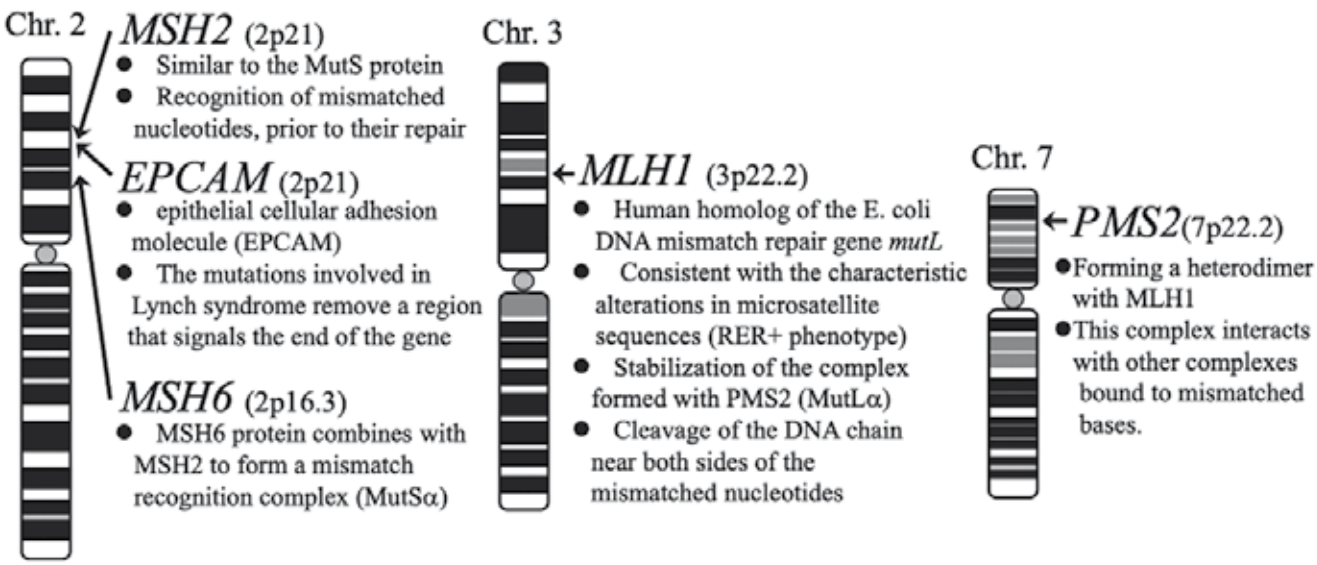


Figure 1: Genes involved in Lynch Syndrome

KEY POINTS

If two of the five markers show instability, the tumor is evaluated as MSI-high (MSI-H).

If one of the markers shows instability, the tumor is considered as MSI-low (MSI-L)

Any positive reaction in the nuclei of tumor cells is considered as intact expression (normal), and it is common for intact staining to be somewhat patchy

An interpretation of expression loss in tumor cells should be made only if a positive reaction is seen in internal control cells, such as the nuclei of stromal, inflammatory, or nonneoplastic epithelial cells

Loss of MSH2 expression strongly suggests Lynch syndrome.

One should also keep in mind that nucleolar staining or complete loss of MSH6 staining has been described in colorectal cancer cases with prior radiation or chemotherapy

For MSI testing, sensitivity ranged from 66.7 to 100.0% and specificity ranged from 61.1 to 92.5%, whereas for IHC staining, sensitivity ranged from 80.8 to 100.0% and specificity ranged from 80.5 to 91.9%

Approximately 10–15% of sporadic colorectal cancers show MSI-H findings. The cause is mostly the loss of MSH1 protein due to methylation of the MLH1 gene promoter region

About half of MSI-H sporadic colorectal cancers show BRAFV600E mutation, which is not detected in colorectal cancers from patients with Lynch syndrome

Final genetic testing for Lynch syndrome is performed using DNA sequencing in selected cases excluding sporadic colon cancer from all colorectal cancers

Multigene panel testing using next generation sequencing for hereditary colorectal cancer has been evaluated as a feasible, timely, and cost-effective approach compared to single gene testing

If the results of DNA MMR IHC and MSI testing are discordant (eg, MSI-H phenotype with normal IHC or abnormal IHC with MSS phenotype), then the laboratory should make sure that the same sample was used for MSI and IHC testing and that there was no sample mix-up.

Reporting of IHC:
MLH1 Result
- Intact nuclear expression
- Loss of nuclear expression
- Cannot be determined

MSH2 Result
- Intact nuclear expression
- Loss of nuclear expression
- Cannot be determined

MSH6 Result
- Intact nuclear expression
- Loss of nuclear expression
- Cannot be determined

PMS2 Result
- Intact nuclear expression
- Loss of nuclear expression
- Cannot be determined

Interpretation of IHC
- No loss of nuclear expression of MMR proteins: low probability of MSI-H.
- Loss of nuclear expression of MLH1 and PMS2: testing for methylation of the MLH1 promoter and / or mutation of BRAF is indicated.
- (The presence of a BRAF V600E mutation and / or MLH1 methylation suggests that the tumor is sporadic and germline evaluation is probably not indicated; absence of both MLH1 methylation and of BRAF V600E mutation suggests the possibility of Lynch syndrome and sequencing and / or large deletion / duplication testing of germline MLH1 may be indicated)
- Loss of nuclear expression of MSH2 and MSH6: high probability of Lynch syndrome (sequencing and / or large deletion / duplication testing of germline MSH2 may be indicated and, if negative, sequencing and / or large deletion / duplication testing of germline MSH6 may be indicated)
- Loss of nuclear expression of MSH6 only: high probability of Lynch syndrome (sequencing and / or large deletion / duplication testing of germline MSH6 may be indicated)
- Loss of nuclear expression of PMS2 only: high probability of Lynch syndrome (sequencing and / or large deletion / duplication testing of germline PMS2 may be indicated)

Constitutional mismatch repair deficiency syndrome

Constitutional mismatch repair deficiency syndrome (CMMR-D) is caused by biallelic homozygous or compound heterozygous pathogenic germline mutations of MMR genes and is a distinct childhood cancer preposition syndrome with an autosomal recessive inheritance.
In biallelic germline mutation carriers of MMR genes, hematological malignancies, brain/central nervous system (CNS) tumors and Lynch syndrome-associated carcinomas develop frequently
The median age at diagnosis of hematological malignancies and brain/CNS tumors was, respectively, 6.6 (age range 1.2–30.8) and 10.3 (age range 3.3–40) years. However, Lynch syndrome-associated tumors developed later [median age at diagnosis 21.4 years (age range 11.4–36.6)], and are mostly colorectal cancers
Various non-neoplastic features are related to CMMR-D including Cafe au lait spots (NF1 like), skin hypopigmentation, mild defects in immunoglobulin class switching recombination, agenesis of the corpus callosum, cavernous brain hemangioma, capillary hemangioma of the skin, combination of various congenital malformations, and Lupus erythematosus.

Rare Lymphoid Malignancies: Two Rare Case Presentations in Young Adults

Drs. Madiha Bilal Qureshi, Muhammad Raza and Arsalan Ahmed
Histopathology

T Cell Prolymphocytic Leukemia:

Introduction: T cell prolymphocytic leukaemia is an aggressive leukaemia of post-thymic mature T cell phenotype. It accounts for two percent of mature lymphocytic leukaemia and is associated with clonal T cell receptor gene rearrangements (TCL1A, TCL1B or MTCP). It usually affects the adults and elderly (age range 30-94 years) and involves the peripheral blood,

bone marrow, lymph nodes, liver, spleen, and skin. Patients usually present with hepatosplenomegaly, excessive lymphocytosis, and generalized lymphadenopathy. It is characterized by diffuse and perivascular proliferation of small to medium-sized monomorphic T cells. Standard treatment includes alemtuzumab (anti-CD52) and variable allogeneic bone marrow transplant. Prognosis is poor with median survival of one-two years.

History: A 45-year-old female presented with cervical lymphadenopathy and hepatosplenomegaly. Excision of cervical lymph node was done.

Diagnosis

Macroscopy: The specimen received was coded as "Cervical lymph node" and contained two incised pieces of lymph node that measured 2.4 x 1.6 cm in aggregate. Cut surface was tan, firm, homogeneous.

Microscopic Pathology: Microscopy showed a lymph node with diffuse effacement of architecture by sheets of medium-sized cells with scant basophilic cytoplasm showing cytoplasmic blebs, monotonous hyperchromatic cleaved nuclei, coarse chromatin, and small conspicuous nucleoli (Figure 1A). The lymphoid follicles were spared and there were prominent high endothelial venules with tumor cells present intraluminally, in the wall and adjacent paracortex. Brisk mitotic activity was appreciated. Immunohistochemical stains were performed which showed positivity for Pan T (CD3), CD4 and CD5 stains (Figure 1B) whereas immunostains Pan B (CD20), CD8, Tdt, CD30, CD10, Cyclin D1 and CD23 were negative (Figure 1C). Mib-1(Ki-67) proliferative index was 25-30% (Figure 1D). Based on combined morphological and immunohistochemical profile, the tumor was best diagnosed as T cell Prolymphocytic leukaemia.

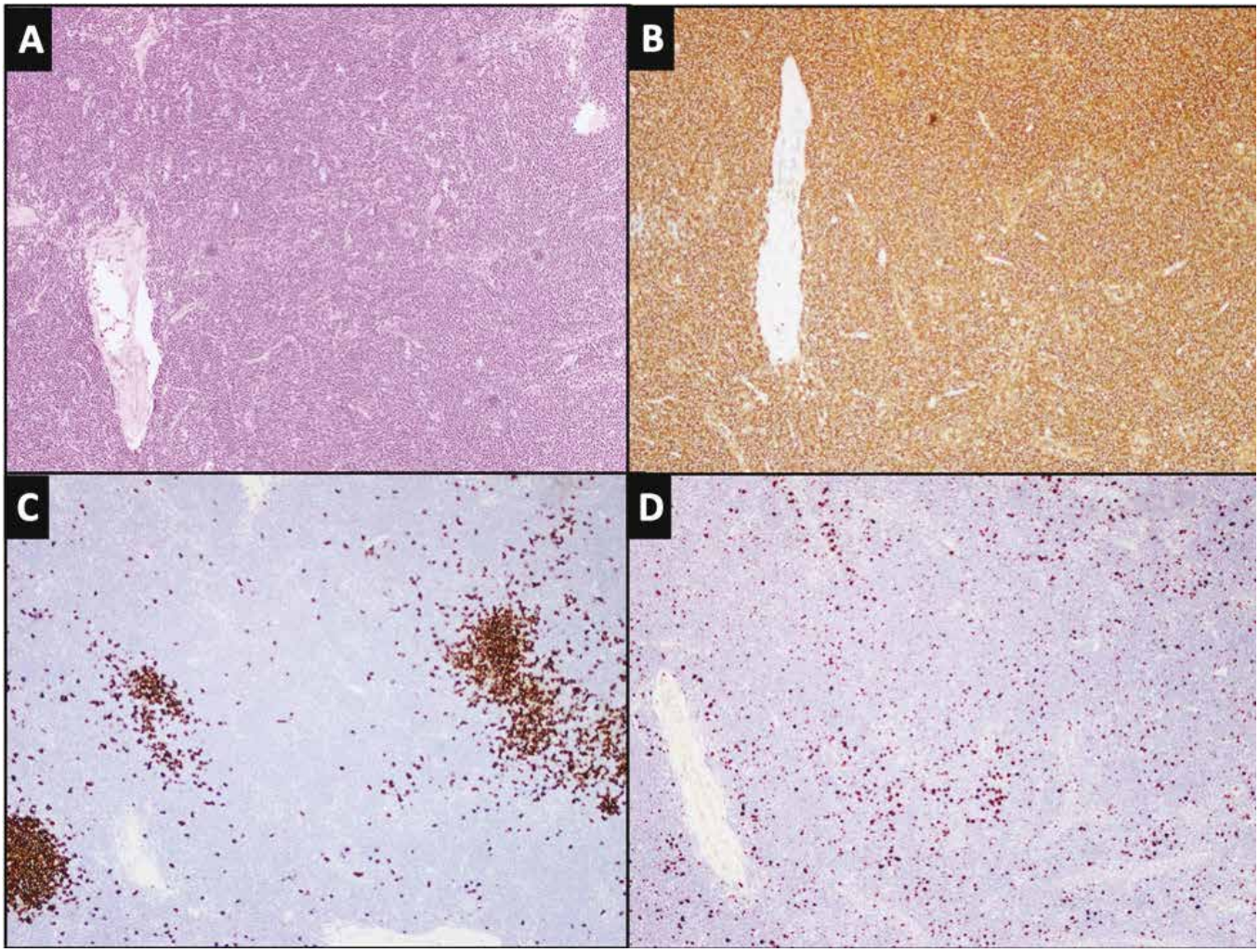


Figure 1 A: *Sheets of monotonous cells, also showing perivascular arrangement.* ***B:*** *Positive CD3 immunostain. C: CD20 highlights residual lymphoid follicles, negative in tumor.* ***D:*** *Proliferative index (Ki-67) is 25-30 percent.*

Conclusion: T cell Prolymphocytic Leukaemia is a very rare aggressive T cell leukaemia that should be kept in differential diagnoses when a lymph node is evaluated with clinical history of generalized lymphadenopathy, marrow involvement, excessive lymphocytosis, hepatosplenomegaly, and T-cell phenotype. Besides histologic features and immunoprofile, Flowcytometric and FISH studies are helpful.

Primary Cutaneous Follicle Center Lymphoma

Introduction: Primary cutaneous follicle center lymphoma (PCFCL) is a low-grade B cell lymphoma of follicular center B cells without any systemic/nodal involvement. It accounts for 10 percent of all primary cutaneous lymphomas. It primarily affects middle-aged adults with solitary or localized involvement of skin of head and neck and trunk. PCFCL is associated with deletion of 14q32.33 and c-REL amplifications. The lesions typically present as firm papules, plaques, or nodules. Histology shows follicular, follicular, and diffuse and diffuse patterns. Treatment includes surgical excision and/or radiotherapy, and rituximab in generalized cases. Prognosis is excellent with a 5-year disease-specific survival of >95 percent.
History: A 44-year-old male, who had been previously diagnosed with PCFCL of scalp one and a half years earlier, presented with a nodular swelling in his left shoulder for two months. There was no lymphadenopathy or systemic involvement on radiology.

Diagnosis:

Macroscopy: The specimen received consisted of a single skin covered fibroadipose tissue that measured 1.5 x 1.0 x 0.6 cm. Cut surface had a grey white to pale yellow appearance.
Microscopic Pathology: Microscopic examination revealed skin covered fibroadipose tissue exhibiting a lesion in the dermis showing follicular growth pattern with nodular infiltrates present throughout the dermis and extending focally into the subcutis (Figure 2A). These nodules were composed of a population of follicle center cells with admixed centroblasts and immunoblasts. The follicles lacked tangible body macrophages and had attenuated mantle zones. Perivascular and periadnexal involvement was present. The nodular infiltrate showed positive expression of CD20 (Figure 2B) and BCL6 in a meshwork of CD21 positive follicular dendritic cells, whereas immunostains for CD3, CD10 and BCL2 were negative. Proliferative index (Ki-67) was low (Figure 2C). A diagnosis of Primary Cutaneous Follicle Center Lymphoma was made based on the immunomorphological features and clinical history. The patient has been followed up for three years during which he developed two more similar lesions on the chest wall. Systemic workup is still negative.

Conclusion: PCFCL, irrespective of the growth pattern, number of blasts, BCL-2 expression, or the presence of localized or multifocal skin disease, has an excellent prognosis. Relapses are observed in 30% cases. However, they do not usually represent progression of disease.

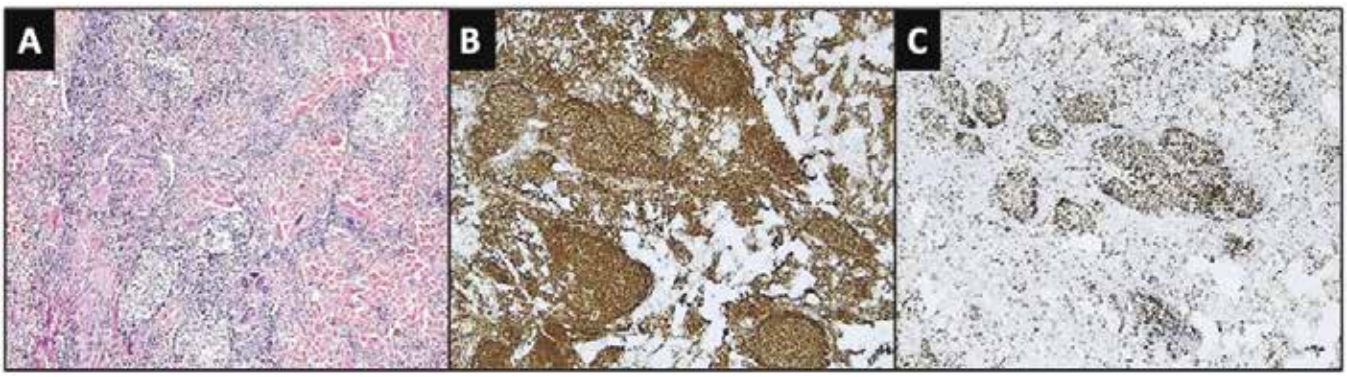


Figure 2 A: *Follicular growth pattern with nodular infiltrates throughout the dermis.* ***B:*** *CD20 positive in follicles.* ***C:*** *Low Ki-67 proliferative index.*

Transforming Pedagogical Framework of Pathology through Social Media (#Pathtwitter)

Dr Sahar Suleman
Histopathology

Pathology twitter has been active since quite some time and is an easily accessible platform for pathologists around the world to share educational content, take consultations, present journal club, collaborate for peculiar cases and arrange case series. Lack of lines of authorities and non-hierarchical structure enables rapid real-time global interactions possible that may not have been otherwise.

The 21st century has labeled Twitter as a free communication microblogging tool engaged in constant, instantaneous information exchange, a portal that is online 24 hours. A survey has pointed out twitter as useful pedagogical tool for pathology as it's an "image-based field", the microscopic images are easily shared followed by a lengthy discussion linked/threaded into a sequence of tweets called "Tweetorial". These educational tutorials would some time also have polls (image A) for a limited time to assess baseline knowledge in turn engage and attract audiences and facilitate assessment of learning. Furthermore, short videos or animations, links to journal articles/websites and other similar tweets can be included within the same thread assisting in dissemination of information. People can add their own takes on a topic, debate, praise, critique, network and make new allies.

How has it benefitted the low resource countries with limited pool of pathologists mostly having expertise in general pathology?

To answer this question Dr Olaleke Folarnmani a surgical pathologist in Nigeria rightfully pointed out in one of his discussions that Nigerian pathologists who are active on Twitter continue to leverage this opportunity and access to experts who volunteer to share their comments/opinions or approach (image B) in turn resulting in them being more confident and comfortable when signing out rare cases. This networking also provides mentorship and inspires excellence in one's practice.

Here are, our very own examples:

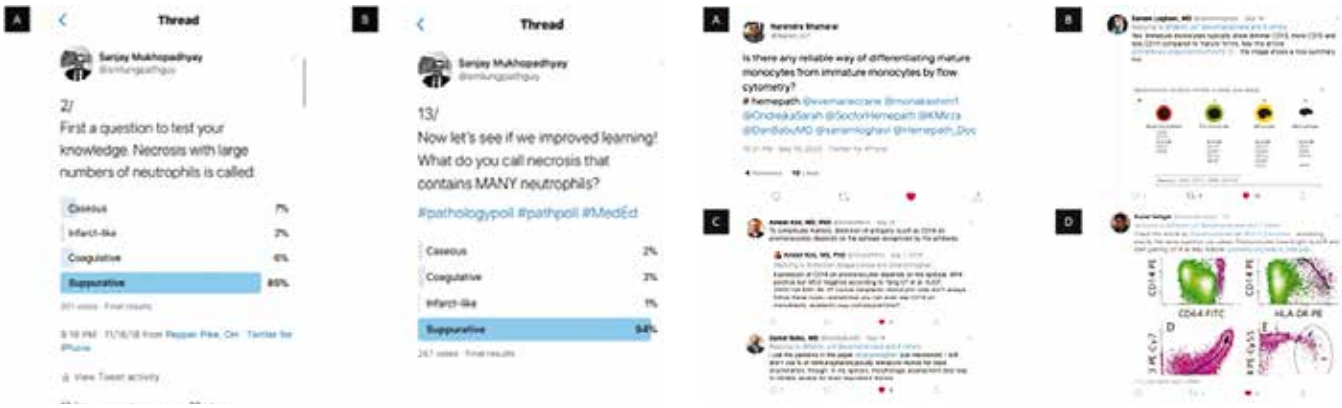
Dr Asad Diwan (Ex-chief resident and graduate of Histopathology residency program) stated, "Path twitter had a major effect on my routine recall during daily signouts and FCPS part II exam preparation. The superb schematic diagrams/algorithms by well-known pathologists around the world like Drs Pranav, Abhijit, Karen Pinto etc. made recalling trivial facts handy and just a click away via cell phone. I bookmark every thread/tweetorial and in future and I am planning to compile all these resources into a hardcopy for my junior colleagues".

Dr Sabeehuddin Siddique (Assistant Professor Histopathology) pondered upon the impact of twitter and stated, "Liver pathology is one of the most challenging sub-specialty in the domain of histopathology. Considering ourselves novices in this field with limited experiential knowledge, Dr Zoonish and I aimed to collaborate with an experienced liver pathologist who could share their practical insights with us and help us diagnose challenging and unusual liver biopsies. In our pursuit, we had been following a Twitter group **#GIPath** where one day we saw a tweet by Dr Stephen Lagana, Associate Professor, Department of Pathology and Cell Biology, University of Columbia. Dr Lagana, with expertise in the sub-specialty of Liver and GI Pathology, invited pathologists to a virtual meeting. He aimed to encourage pathologists across the globe to share and discuss the cases of GI and Liver pathology that either posed diagnostic difficulty or had an academic perspective. Considering this as a God-sent, we immediately responded to Dr Lagana through a tweet, expressing our intent to join the group. So earlier this year, the virtual Zoom meeting titled "GI Pathology Second Opinion Club" **(#gipsoc)** was launched.
To our excitement, other big names in the field, Dr Raul S. Gonzalez (Associate Professor at Beth Israel Deaconess Medical Center) and Dr Andrew M. Bellizi

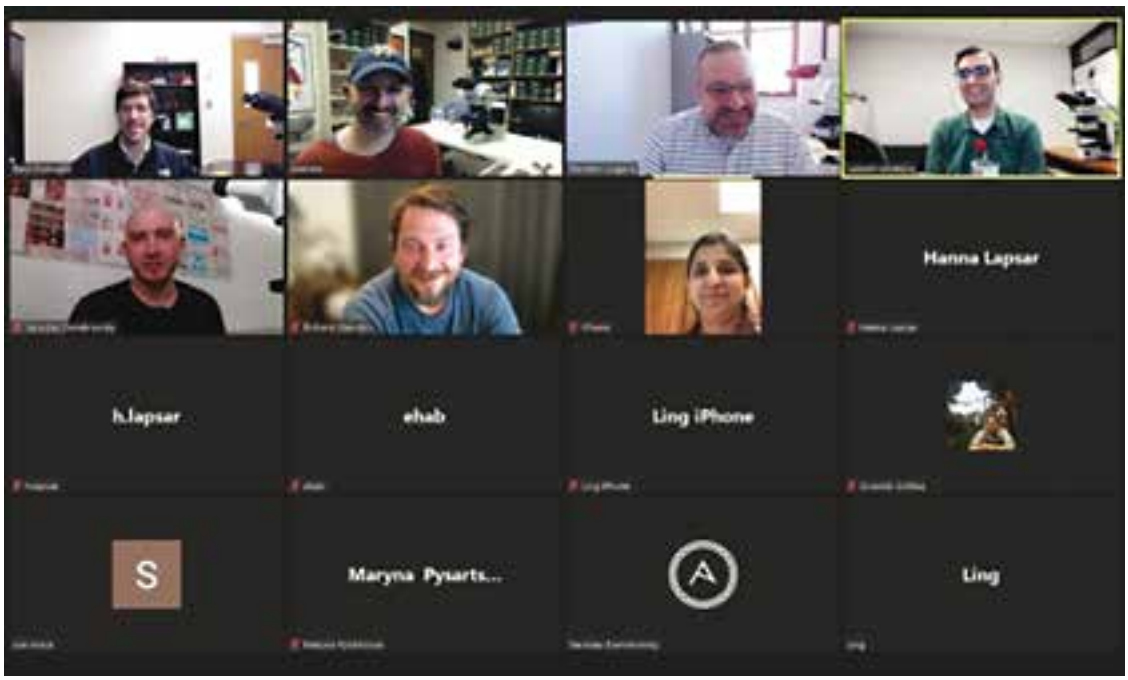
(Clinical Professor at the University of Iowa), also joined this group. This meeting has now become a regular bi-monthly event attended by a diverse group of pathologists who help one another by contributing their unique practical experiences through discussions on challenging GI and Liver pathology cases." (Image C)

In this age, the power and influence of social media is undeniable, nobody can run away from it. Department of Pathology and Laboratory Medicine at Loyola University Chicago Stritch School of Medicine, has conceived a "Digital Communications Fellowship" which is in its second year of running, their objective is to "Facilitate trainees to define a successful digital presence (a core competency of the future)."

In conclusion, I would urge all the laboratory professionals to experience this communication to garner innumerable opportunities for education, research, and networking.



Tweetorial showing pre and post quiz + poll1



Screenshot from the launch session of #gipsoc

Best of the Recent Past

Radiologist #Vascularintervention #Followtheirlead

Interviewee: Prof Muhammad Azeemuddin
Interview recorded by Dr. Shayan Anwar

1. **Considering your entire time as an intervention radiologist at your organization, can you recall a time (any AHAA moment) when you felt most alive or most excited about your involvement in the organization?**

   Being an Interventional Radiologist, we get a lot of opportunities to perform lifesaving procedures and doing any such procedure gives us a very satisfying feeling of accomplishment. Alhamdulillah there have been many such moments in my career in this organization and I really feel proud to be a part of this system. Furthermore, AKU has given us a very conducive environment to train young doctors in IR and when these trained persons perform well whether within or outside the organization, make me feel proud and excited to be a part of this organization.

2. **Please briefly share your initial phase of journey i.e., from medical graduate to consultant.**

   I did my MBBS in 1989 from Dow Medical College. After doing my internship in Surgery and Medicine at Civil Hospital Karachi I decided to pursue Radiology as my specialty and was lucky enough to get inducted in MCPS program in Diagnostic Radiology at Liaquat National Hospital Karachi. The department was chaired by the renowned radiologist Dr Rashid Ahmed who wasn't only an authority in Diagnostic Radiology but also amongst the pioneers of Interventional Radiology in the country. It was he who gave me passion for the Interventional Radiology and after doing my MCPS and later FCPS I went for a fellowship in IR to Singapore at Singapore General Hospital. After my return when Dr Rashid Ahmed decided to leave LNH, I also joined Aga Khan Radiology in Sept 2021 as an assistant professor and since then I am honored to be a part of this esteemed institution.

3. **3. Let's consider for a moment the things you value deeply. Specifically, the things you value about yourself and the nature of your work, what is the single most important thing your work has contributed to your life?**

   My nature of work is Interventional Radiology which is a small but rapidly growing field. I entered in this field when there were only a few trained Interventional Radiologists in the country so I was amongst those few who were performing a wide range of minimally invasive procedures for various conditions. If I would have been doing only diagnostic radiology I would not have a chance to interact with the patients directly whereas this interaction is really valuable for those who love to be in direct contact with the patients. This has given me an extraordinary respect and honor and practicing IR in an institute like ours has added further in this regard.

4. **As a senior intervention radiologist of the country, please share your experience of development of intervention radiology practices in Pakistan and its future in next 10 years.**

   Over the past two decades IR has grown significantly in our country. This is due to multiple factors. These includes:

   1. Increased awareness amongst health care professionals regarding importance of IR
   2. Increased numbers of trained IR personals. These predominantly were trained locally

as well as in Singapore. Over the years few trained Interventional radiologists from abroad also returned to Pakistan.
3. Establishment of IR sections in different institutes.

Currently the CPSP is in the process of recognition of IR as a separate sub-specialty with initiation of FCPS degree in IR from 2023. This is going to have a very positive impact on IR in future with training of more and more doctors in IR.
The main limitation in further progress of IR is ever rising cost and sustainable availability of consumables. Out of country shifting of trained IR due to socioeconomic reasons has also contributed negatively.

5. **Any advice for Junior Radiologist?**

This era has become more competitive and there are new threats and challenges especially after the introduction of Artificial Intelligence. They now have to prove their worth by increasing their clinical knowledge and skills. They should not only be good at interpretation of images but must be interpreting images against clinical background. Similarly, in IR they should take care of patients complete clinical issues rather than just performing a certain procedure. Furthermore, the tremendous pace of the advancements in technology though very beneficial for radiology but still poses challenges too. The young radiologists must keep themselves updated with all these advancements. Overall the future of radiology seems promising.

Meet the Managers of Pathology

September was celebrated as the Managers Appreciation month across the globe. It is reported that Appreciation improves workplace morale and when any employees know their hard work is appreciated and recognized, they become motivated to maintain and improve their great performance. Here we introduce managers of Department of Pathology and Laboratory Medicine.

Mr. Akhtar Shah, Manager Section of Clinical Chemistry

By Dr Lena Jarfi

Meet the super star of our Section, Mr Akhtar Shah (Twitter handle: @AkhtarShah3456) who has been working as Manager at the Section of Chemical Pathology, Department of Pathology & Laboratory Medicine since May 2019. He holds my Section steady without breaking a sweat.

His one of the major achievements has been bringing the first LCMSMS in the institute and initiating clinical testing on it. He has been a mentor to many residents and technologists and has always been keen in teaching lab-skills to his juniors and peers. Some of his initiatives being “Toxicology and Therapeutic Drug Monitoring Program”, Expansion of Newborn Screening Program to Secondary Hospitals of AKUH and introduction of >30 new biochemical tests in the Section despite the stress of COVID 19 in past couple of years. He was instrumental in CAP re-accreditation and was able to efficiently deploy artificial intelligence-based error detection techniques in our laboratory information system.

Rain or shine, Mr Akhtar keeps our Section of Chemical Pathology, which gets high volume, high velocity, and high variety data, functioning smoothly. His tenacity and unflinching dedication to his job inspire many. Be sure to thank him if you catch him in sight!

Ms. Asima Shahid, Assistant Manager Section of Microbiology

By Dr Joveria Farooqi

Ms. Asima Shahid became Assistant Manager of the section of Microbiology at Aga Khan Clinical Laboratories in 2018. She did MSc Microbiology from Karachi University in 2007, and immediately after she completed her traineeship at AKU clinical laboratories in 2009, she began her professional career here as Assistant Technologist. It is a credit to our technologist training program that she is now leading one of the busiest sections of the clinical laboratories.

Microbiology lab utilizes a mix of classical and advanced techniques, with a lot of manual work as well as automation. Asima had her hands full as she was initially assigned responsibilities of a Training Coordinator when the CAP checklist was introduced: ensuring all staff were familiar with all aspects of their areas of expertise before they could be considered competent, all while maintaining this documentation. Her superior grasp of all things related to CAP resulted in her becoming responsible for introduction of new tests, especially their validation, verification, SOPs and maintaining indicators, developing Internal QC Plans. She was assigned the molecular assays being performed in the section of microbiology, especially those in the mycobacteriology lab, and the new multiplex panels for meningitis and respiratory infections. Meanwhile, she also continued her work in the implementation of automation of antibiotic susceptibility testing on Vitek.

Her most exacting task during her tenure as Assistant Manager has been the smooth shifting of BSL-III laboratory (mycobacteriology subsection) from Juma to Soparivala Building. She also supervised the transition of Infectious serology test to Microbiology: all the tests are transferred from preanalytical phase to post analytical phase. Both transitions of operations were planned and executed perfectly with no impact on clinical reporting or biosafety. Similarly, managing operations smoothly despite staff shortage and increased workload during the COVID pandemic and Dengue outbreaks is another feat that Asima achieved without batting an eyelid.

Asima's whole-hearted approach to delivering quality results, even when faced with less than ideal situations has landed her in this leadership position. She is sincere to her work and colleagues, and passionate about continuous improvement in the lab. She is approachable yet firm, a trait most valuable in a leader.

Ms. Nazneen Islam, Assistant Manager, Section of Hematology

By Dr. Zeeshan Ansar

Ms. Nazneen Islam has joined Molecular Pathology section in year 2004. Since 2013 she is performing job responsibilities as Assistant Manager.

She has extensive experience in daily laboratory operations management, process mapping, regulatory compliance, personnel management, and quality assurance in all testing phases with a passion for continuous process improvement and advancement in Molecular Testing techniques.

As other part of the world SARS CoV2 testing was big challenge for our laboratory, her strategies were found remarkable in managing tests workload along with staffing, inventory and supplies management.

I would like to acknowledge her hardworking and dedication. Keep up the great management and TEAM work!

Ms. Shahmina Sadaf, Assistant Manager Section of Hematology

By Natasha Ali & Muhammad Shariq

I have known Shahmina for more than a decade. From a technologist to Assistant Manager level she has sailed smoothly in her career. She is an honest and a responsible person. Due to her excellent managerial qualities, faculty and senior administrative staff trust her a lot and never hesitant to assign crucial laboratory tasks to her.

She had been an integral part of several JCIA/CAP audits along with the launch of total laboratory automation in Haematology (the first in Pakistan). She is constitutively involved in laboratory quality assurance program ranging from timely reporting of test results to staff competency. These are essential components of laboratory management and Shahmina has always been a key role player. Her ability to absorb suggestions/recommendations for improvements in the section has yielded in astounding growth of the section of haematology.

Shahmina has been a team player from the start. As the saying goes – "Talent wins games, but teamwork and intelligence win championships" and in Shahmina's leadership we are destined to win many, many championships in the future.

Imdad Hussain, Assistant Manager – Transfusion Medicine

By Dr. Natasha Ali

When Imdad was handed over the leadership position, he was eager to bring his abilities and skills to this new responsibility. He has been a key role player in the development of transfusion services at Aga Khan University. He has led the

section from the front in all CAP accreditations, developed robust quality control standards, promoted team building among the staff and has guided them professionally for combined achievement of sectional goals. Imdad's dedication has been imperative for the growth of our section and I thank him for all his efforts!

Ms. Bushra Rizvi, Assistant Manager – Hemostasis and Thrombosis

By Dr. Natasha Aly

Bushra has been the pillar strength of the section of hemostasis and thrombosis in Haematology. Under her leadership, the section's test menu has expanded to the capacity of placing AKU as a reference laboratory for thrombophilia testing. This was also acknowledged in our recent CAP inspection by the audit team. Bushra's commitment to best practices and her attention to the tiniest details makes her exemplary and unique. Her determination is admirable and her dedication and willingness fuel our mutual efforts towards the success of the section. Thank you for your support and hard work that you always put in, every single day.

HAPPENINGS IN PATHOLOGY

CAP Accreditation of Main Lab and Outreach Laboratories – A Standardized Approach Towards Quality Management

Ms. Mashhooda Irfan
Manager

The clinical Laboratories of Aga Khan University Hospital are the largest laboratory network in Pakistan. This network is based on a hub and spoke model having the main laboratory in Karachi and 13 outreach laboratories in 9 cities of Pakistan. Approximately 291 collection units are also associated with the main and outreach laboratories. AKUH follows the guiding principle of IQRA (Impact, Quality, Relevance, Access) and so does the clinical laboratories. As obvious, Quality is one of the guiding principles on which the institution has a greater focus. To standardize the approach, accreditations from recognized institutions are the best means, and the CAP Accreditation program is one of them.

College of American Pathologist (CAP) has offered Laboratory Accreditation Program to laboratories worldwide since 1961. It is a comprehensive discipline-specific program that covers the entire lab operations in terms of their clinical function, quality management, safety, and physical facility. All the labs having a gold seal of CAP accreditation is a symbol that they are working on the same level of excellence. The accreditation cycle of AKUH Clinical Laboratories started in the year 2016 when the Main Lab in Karachi gets the accreditation followed by the accreditation of four outreach labs namely Lahore, Rawalpindi, Peshawar & Faisalabad in the year 2018. In the year 2022 four new labs of Multan, Sukkur, Hyderabad, and Clifton Medical Services (CMS) get their accreditation. So, all, eight outreach laboratories and the main campus lab are now CAP Accredited.

As the COVID-19 pandemic has impacted the accreditation cycles of CAP around the world so CAP decided to resume it through in-person and virtual audits. The same model was used for the AKUH Clinical Laboratories.

After completion of the inspection cycles of eight outreach laboratories, a Summation Conference was held on 6th December 2022 in Karachi. The program was organized in Multipurpose Hall A of the University Center Building of the AKUH-Karachi campus. The program was honored by the esteemed presence of AKU President Dr. Sulaiman Shahabuddin along with the CAP Inspection Team Leader Dr. Aaron Han. Other valued guests were VP Finance and CFO, Ms. Shagufta Hassan, Dr. Farhat Abbas, CEO Health Services Pakistan, Chief Medical Officer and Chief Nursing Officer of AKUH-Pakistan and COO Secondary Hospitals Karachi. Representation from the lab includes the chairperson, CAP Director, Director Operations, Section Heads, Quality Assurance Group, Sectional Managers & senior staff, and representatives from outreach labs (Virtually). The program was moderated by Dr. Sidra Arshad. The AKU President congratulated the teams involved in achieving this milestone. He further added that our lab network's widespread impact throughout Pakistan has contributed to AKU becoming a

national institution. Dr. Aaron Han also expressed his satisfaction and delight in the efforts poured in by the teams. He lauded the high-quality standards throughout AKUH's lab network and state us as the strong leader of lab systems throughout Pakistan.

All in all, it was a moment to cherish and was a result of extreme dedication, resulting in a standardized practice across all laboratories under the umbrella of AKUH.

PAP Meeting 2022

#PAP, #HCSP, #PSH, #PSI, #PSCP, #PSM, #AMP

Dr Qurratulain Chundriger
Histopathology

The last week of November 2022 saw the 43rd Annual conference of the Pakistan Association of Pathologists (PAP) and the 8th joint conference of societies of Pathology. It marked 75 years since the inception of the PAP society. It was a 3-day event, accompanied by pre- and post-conference workshops in all societies. These workshops took place at various venues, including the College of Physicians and Surgeons of Pakistan, the Indus Hospital, Sindh Institute of Urology and Transplant and the Pearl Continental

Hotel, the last also being the venue of the conference itself. The organizing committee included mostly faculty from AKUH.

The conference was attended by many participants, more than 1200 people, from all fields of pathology, mostly on site. It was also broadcast live on YouTube. Many international speakers were also attendees, both virtually as well as in person. It was well received after COVID related virtual events, which have become a norm in the past couple of years. It served as a platform for the exchange of ideas as well as social interaction between the most legendary minds in pathology and the new generation of pathologists,

Microbiology workshop being conducted at the Multidisciplinary Lab at Aga Khan University Karachi

inspiring and encouraging them.

Along with the oral presentations on updates in classification and free papers, a poster display

Chemical Pathology Society

was also organized, which portrayed around 500 posters depicting original work from all societies of pathology. Prizes were awarded to the best ones, both oral and posters. In addition, a Bukhari Gold medal was awarded to the best presenter among all the societies. It also featured a display of the latest advancements in technology by the vendors.
Molecular Pathology workshop at CPSP Karachi.
Histopathology and Cytology Society of Pakistan.
Up: Left to right: Dr. Shahid Pervez (AKU), Dr. Naila Kayani (AKU), Dr. Nasir-Ud-Din (AKU), Dr. Anil Parwani (USA). Down: Participants from across the country including faculty and residents.

Molecular Pathology workshop at CPSP Karachi.

Polaroid

Dr. Atta-Ur-Rahman inaugurating the PAP societies Conference, along with Dr. Shahid Pervez (President PAP) and Mr. Masood Ahmed (member organizing committee)

Polaroid

Microbiology Society members (standing from left to right), including our faculty Dr. Erum Khan (Chair department of Pathology and Laboratory medicine, AKU, 2nd from left) next to Dr. Afia Zafar, Dr. Imran Ahmed, Dr, Joveria Farooqui, Dr. Kausar Jabeen, Dr. Seema Irfan and Dr. Rumina Hassan with others.

Molecular Pathology faculty and members, including Dr. Zeeshan Ansar, Dr. Zahra Hassan, Dr. Najia Ghanchi, Dr. Asghar Nasir, Miss Nazneen Islam and others.

Polaroid

Chemical Pathology Society members, including our faculty Dr. Hafsa Majid, Dr. Aisha Habib Khan, Dr. Lena Jafri, Dr. Sibtain Ahmed and others.

Closing session with faculty from all societies.

Polaroid

The 43rd Annual Conference od Pakistan Association of Pathologist and 8th joint Conference of Societies of Pathology including Pakistan Society of Chemical Pathology, Association of Molecular Pathology Pakistan, Histopathology and Cytology Society of Pakistan, Pakistan Society of Hematology, Pakistan Society for Immunology and Medical Microbiology and Infectious Diseases Society of Pakistan was held at the Pearl Continental Hotel, Karachi fron 25-27th November 2022

Polaroid

Key of the Case Quiz:

Key Q1: Anion Gap - ([Na] + [K]) – ([Cl] + [HCO3]) = 23.7
Calculated osmolality = 2[Na] + [urea] +[glucose] = 285.5 mmol/kg
Osmolal Gap = measured osmolality – calculated osmolality = 54.5 mmol/kg

Key Q2: Ethylene glycol poisoning

Key Q3: Boling Point elevation, freezing point depression, vapour pressure depression, colloidal osmotic pressure.

Key Q4: Best will be freezing point depression, suspecting alcohol intoxication, which is volatile cannot be measured by vapour pressure depression or boiling point elevation method. And colloid osmotic pressure is for viscous fluid and serum cannot be measured by that method.

hospitals.aku.edu/Karachi/clinical-laboratories