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From the Editor's Desk

The establishment of the World Health Organization in 1948 marked the beginning of a journey to address global public health challenges, fostering collaborations, scientific advancements, and unity. As WHO celebrates its 75th anniversary, it presents a chance to reflect on the accomplishments that have enhanced our lives over the past seven decades. Additionally, it serves as a catalyst to inspire collective efforts in combating present and future health issues.

We dedicate this current issue of LABRAD titled "Role of Laboratory sciences and diagnostic imaging in advancing Public Health" to these tremendous efforts. Current issue includes various articles from the field of Laboratory medicine and radiology that are pertinent to public health. These include articles on predictive and prognostic biomarkers that are increasing used in the evolving era of personalized and precision medicine, cancer registry revival in Pakistan, which is cornerstone for advancements in cancer research and patient management, Urine cytology as a screening tool for neoplasms of urinary tract and role of Convalescent plasma therapy in COVID-19 patients. This issue also include articles highlighting the role of radiological imaging as a screening and diagnostic tool in public health setting such as use of coronary CT for coronary calcium scoring that can stratify cardiovascular risk, predict patient outcomes, and guide preventive therapy.

I hope that you find this issue of LABRAD worth reading and relevant to your health care practices. I would like to thank my entire team for a superb effort in compiling this issue. We would appreciate your valuable feedback and suggestion towards making future publications more informative and relevant.

Dr Sidra Arshad
Editor, LabRad

Toxicology In Public Health: Mitigating Risk, Fostering Well-Being

Dr. Yousra Sarfaraz
Clinical Chemistry

"What is there that is not a poison? All things are poison, and nothing is without poison. Solely the dose determines that a thing is not a poison."
(The Third Defense
(Paracelsus, Father of Toxicology)

We don't define only the toxic substance as poison but anything in excess can work alternatively causing toxic effects. Toxicology deals with the day-to-day exposure of harmful chemicals, such as, recreational drugs, smoking, or having peracute effect upon drug overdose, investigating pharmacodynamics and pharmacokinetics of drugs and drug-drug interaction, exposure to common household chemicals, harmful gases and organophosphate insecticides poisoning or dealing with heavy metals or radioactive substances at workplace.

Globally, the public health toxicology programs are responsible for identifying potential health hazards of chemical exposure and to recommend subsequent approach to dwindle the emanating health effects. In public health sector the risk or probability of chemical substance causing adverse effects under exposure of certain environment or condition within the population is being assessed in concern with four components, risk characterization (RC), hazard identification (HI), dose-response assessment (DRA), and exposure assessment (EA).

Public health encourages individual to adapt healthy lifestyle, it is interconnected with environment in direct and indirect manner. Within Pakistan, the day by day increase in pollution and increment in environmental hazards is quite evident. The

hazardous gases in environment, exposure of (esp.) farmers to organophosphates insecticides, common household poisoning, along with trend of multiple drug abuse is growing eminently. Keeping in accord that Pakistan still lacks in poison control centers, rehabilitation centers and laboratory testing as per the requirement and need of population. However, public health sectors are working to extend the limited boundaries and achieve the desired baseline facilities.

According to National Drug Survey conducted by United Nation Office on drugs and crime (UNODC) in 2012 and 2013, around 6.7 million people are into drug abuse, similarly, a study conducted by faculty of clinical chemistry section of AKU in 2008, stated that within two years of span (July 2006 – March 2008) around 17,714 tests were performed for screening urine toxicology. The percentage of positive case for drug of abuse was, benzodiazepine 39.5 percent, cannabinoids 7.8 percent, barbiturates 2.8 percent, opiates 2.4 percent, cocaine 0.3 percent and amphetamine 0.2 percent. When analyzed on gender biased stats, in positive cases of benzodiazepine 59.3 percent were female and 33.7 percent were male. Rest of the drugs positive in males were cannabinoids 8.1 percent, opiates 2.4 percent and barbiturates 2.1 percent. In females' positive cases, barbiturates 5.5 percent, cannabinoids 3.5 percent and opiates 2.5 percent was reported. Amphetamine and cocaine were found to be least positive drugs used by both genders. However current Statistics of shows that now Cannabinoids are the most commonly abused drug among Pakistani population.

Currently, Toxicology bench, Clinical chemistry section, department of Chemical Pathology and Laboratory Medicine, Aga Khan University Hospital offers testing for drug of abuse including, Amphetamine, Alcohol, Barbiturate, Benzodiazepine, Cannabinoids, Cocaine, Opioids in the panel. The screening test is performed by using enzyme-immunoassay technique while the newly introduced advancement is the drug of abuse confirmatory test performed on worldwide recognized gold standard technique LC-MS/MS. This test not only confirms the toxic substance in urine sample it further recognizes the metabolites and derivatives of the abused drugs providing a broader spectrum of information for the treatment of substance abuse addiction. It also enables clinician to monitor any additional abused drug intake which was not previously mentioned by the subject/patient. AKU also offer test to monitor the toxicity of heavy metals such as, Copper, Zinc and Lead by using spectrophotometric technique. Moreover, AKUH has further planned to extend this panel to provide maximum facility to the Pakistani population to aid them in, recognizing and seeking the desired help for the treatment of relative toxicity.

Several awareness programs are also working conscientiously to mitigate and manage the associated risk factors and provide the basic guideline to public, to stay cautious around controllable toxic hazards. Indeed, knowing the risk and avoiding it is the best first line of defense for the well-being of any personnel.

Predictive and Prognostic Biomarkers in Histopathology

Drs Alka Rani, Muhammad Raza, Nasir-Uddin
Histopathology

Biomarkers are biological molecules found in tissues, blood, and other body fluids, that are considered signs of disease. Biomarkers play a fundamental role in the comprehensive pathologic evaluation of malignancies. They can be of prognostic or predictive nature:

- A prognostic biomarker informs about the likely course and outcome (e.g., disease recurrence, disease progression, death) independent of treatment received.
- A predictive biomarker indicates whether a disease is likely to respond to a particular therapy.

The introduction of prognostic and predictive markers by immunohistochemistry (IHC) has made great impact on diagnosis and implementation of standard patient care. IHC methodologies have allowed the identification of specific and

highly selective cellular epitopes in formalin fixed, paraffin embedded tissues with an antibody and appropriate labeling system. Pathologists, particularly histopathologists, play a vital role in the validation, application, and reporting of these markers. This article will summarize the list of prognostic and predictive markers available by IHC technique. The following are commonly used markers.

1) ER and PR (Estrogen and Progesterone receptor): These are steroid hormone receptors, physiologically expressed in mammary, endometrial and ovarian tissues, among others. Routinely referred to as hormone receptors, these are both prognostic and predictive. The proportion and intensity of nuclear expression is evaluated by IHC in tumors of breast and endometrium. Patients with ER-positive breast tumors have better survival than patients with hormonal negative tumors. Conversely, lack of ER and PR expression is associated with a higher risk of node metastases and poor prognosis. These also predict potential benefit from tamoxifen therapy.

2) Her2/neu: Is a membrane tyrosine kinase and oncogene, commonly overexpressed in breast and gastric cancers. Interpretation is based on intensity of membranous staining and proportion of strongly labelled tumor cells. It yields both prognostic and predictive information. Patients with Her2/neu-positive tumors are more aggressive and have a worse prognosis compared to Her2/neu-negative tumors. Her2/neu-positive tumors tend to respond better to platinum-based chemotherapy and all eligible to receive trastuzumab.

3) Ki-67: Ki-67 is a nuclear non-histone protein, present in all active phases of cell cycle, except the G0 phase. The nuclear expression of Ki-67 is strongly linked to high tumor cell proliferation and growth and is routinely evaluated as a proliferation marker in breast cancer. It is also used widely in many hematolymphoid malignancies, brain tumors, and pediatric tumors. In breast cancer, increased expression is associated with tumor progression, recurrence, reduced disease-free survival, and poor overall survival.

4) E-Cadherin: It is an important cell-cell adhesion molecule in epithelial tissues, with membranous staining. It is a prognostic marker as loss of expression in bladder, esophageal, gastric and oral tumors is associated with poor prognosis and advanced tumor stage.

5) MSI status: Inactivation of the DNA mismatch repair (MMR) system leads to instability of microsatellites and progressive accumulation of mutations. The most important MMR players include MLH1, PMS2, MSH2, and MSH6. The inactivation can occur due to either promotor methylation or mutations (germline and/or somatic). Lynch syndrome (LS) is a classical example, associated with cancers numerous systems. Generally, the MLH1 variant is correlated with the highest risk of colorectal cancer, while the MSH2 variant is correlated with the highest risk of other cancers. MSI status is confirmed using a series of tests usually starting with MMR IHC. These markers are of both prognostic (intact nuclear expression is associated with better prognosis and improved relapse-free survival) and predictive value (neoadjuvant/adjuvant treatment in colorectal cancer).

6) PD-L1: PDL-1 is a surface protein that protects expressing cells by interacting with PD-1 on lymphocytes, resulting in lymphocyte apoptosis and immune downregulation. Physiologically its role is to prevent undesired immune interactions. Many tumors use this mechanism to evade host cellular immunity. These tumors are potential targets for immune checkpoint inhibitors. PD-L1 overexpression is commonly seen in melanoma, non-small cell lung carcinoma, head and neck squamous cell carcinoma etc. It indicates worse outcomes and poor prognosis.

Additionally, there are numerous new and emerging biomarkers, revolutionizing the understanding and management of oncological diseases.

Cancer Registry and Public Health Surveillance - Role of Pathologists

Drs Sahar Suleman and Madiha Bilal Qureshi
Histopathology

Cancer surveillance is a continuous systematic collection and analysis of information on newly diagnosed cancer cases including screening tests, extent of disease, treatment, survival, and cancer related deaths with subsequent dissemination of cancer data and statistics worldwide. Cancer Registration Program plays an essential role in this regard by determining the incidence of cancer, type of cancer, regional distribution of different types/ variants, and in forming public policies related to cancer. Cancer registries serve as a reservoir of cancer data for researchers, students, hospital administration and physicians. They provide accurate and timely information regarding cancer incidence, survivorship, and treatment. As histopathologists are responsible for diagnosis and classification of most cancer types, their contribution to Cancer registries cannot be ignored. Pathology laboratories are a fundamental source of information.
There are different types of cancer registries:

Hospital-based cancer registries (HBCR): These registries have the task of collecting all cancer cases attending that healthcare facility with special interest in administrative processes. They provide the initial data for Population-based cancer registries.

Population-based cancer registries (PBCR): These registries record incident cancer cases within a defined geographic region/state in a specified period. They gather data from all pathology laboratories, hospitals, physicians, and other sources.

Special Purpose Cancer registries: These registries focus on a particular type of cancer or a special population and are more valuable and specific source of information.

Pervez et al in their comprehensive articles published in Asian Pacific Journal of Cancer Prevention' 2020 and Journal of College of Physicians and Surgeons Pakistan'2023 have meticulously described the process of formation of National Registry Programme in our country. Pakistan established its first Cancer registry at 'Armed Forces Institute of Pathology (AFIP)', Rawalpindi in 1960, which was followed by 'National Cancer Registry (NCR)' in 1970s till 1990s founded by 'Pakistan Medical Research Council (PMRC)'. However, this registry could not sustain due to funding issues. In the meantime, the 'Karachi Cancer Registry (KCR)' was conceptualized in January 1995 as the first Population-based cancer registry supported by the Government of Sindh, in collaboration with the Unit of Descriptive Epidemiology, International Agency for Research on Cancer (IARC) of the World Health Organisation (WHO). Subsequently, 'Punjab Cancer Registry (PCR)' was initiated from a Public Sector Hospital in Lahore but was later shifted to Private Sector Hospital 'Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH&RC)'. This registry is also a source of data to IARC (WHO) and 'Globocan 2018'.

In 2017, an initiative was taken to revive the lost Karachi Cancer Registry. The data were classified using ICD-10 (International Classification of Diseases-Oncology) and the information was provided by major healthcare centers in the city. The initial results were reported in 2020. Since then, the registry is successfully functioning well. A notable step taken to compile all cancer registries and datasets from all over Pakistan was just done recently, with intention to provide a universal and representative report regarding cancer patterns in Pakistan. A widespread data pool was published in the recent volume (June'2023-33(06)) of JCPSP. The open access article concluded that Breast cancer is the most common cancer in females touching epidemic proportions while 'oral cancer' which is the leading cancer in males ranks third in frequency in females. Other common cancers in Pakistan include liver cancer, lung cancer, and cervical cancer, which are largely preventable as showed by strong correlation with hepatitis B and C, smoking, and high-risk human papillomavirus. Cancer Registry at AKUH was established in January 2009. Over the last 14 years, it has evolved into a primary data source, contributing at National and International level with substantial data of more than 60,000 patients. The program uses CNExT Registry Software for standardized cancer reporting. The

team consists of representatives from departments such as Surgery, Paeds Oncology, Gynae Oncology, Histopathology, HIMS and Research office. Similar trends as with national statistics, were observed at our institution in 2022 with Breast cancer being the most common in females whereas Head and Neck malignancy as the most common among males. Top cancers in children included Leukemia followed by Brain/CNS and Bone Tumors.

This cancer information can be used to assess the trends of tumors over time and to find peculiar patterns in diverse regions or populations, thereby showing whether screening, preventive measures and treatment are making a difference or not. The data is used to support different research ventures that help in developing advanced facilities for better treatment and management of cancer. Hence, registries play an integral role in cancer care and research by bridging the information gap and collecting a complete summary of data. The collaboration of histopathologists in this regard provides vital information and their participation should be appreciated and regarded.

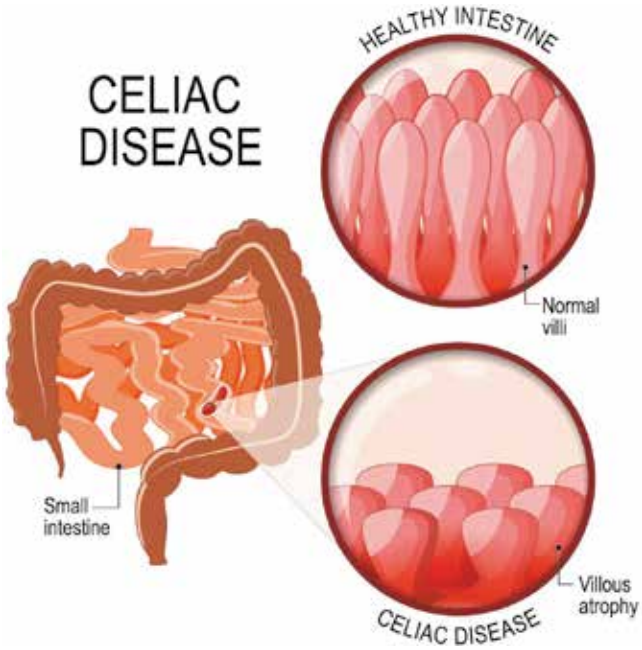
Diagnosis of Celiac Disease DQA1*02,05; DQB1*02,03:02 (DQ2&DQ8) in patients

Ms Sabira Sharif, Drs Asghar Nasir, Zeeshan Ansar
Molecular Pathology

According to Celiac Disease Foundation it is estimated to affect one in 100 people worldwide, but only about 30 percent are properly diagnosed. Celiac disease can develop at any age after people start consuming gluten. Left untreated, celiac disease can lead to additional serious health problems.

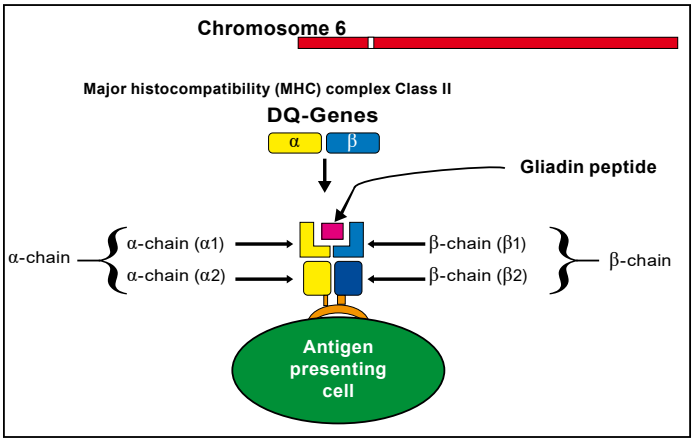
Also known as “gluten intolerance”, “non-tropical sprue” or “gluten-induced enteropathy, “Celiac disease (CD) is an autoimmune enteropathy triggered by ingestion of gluten that occurs in genetically susceptible individuals. Gluten along with environmental trigger starts an inflammatory reaction which results in damage to small intestine. Gluten represents a mixture of proteins found in the endosperm of cereal seeds (wheat, rye, barley, oats, etc.). And, among its components, the main one is gliadin, which is the toxic fraction and is directly involved in the pathogenesis of CD. The gliadin meets the transglutaminase tissue (TGt) in the intestinal lumen, thus forming a macromolecular complex which can be recognized as antigens by antigen presenting cells via allele of the major histocompatibility complex class II, namely HLA-DQ2 and HLA-DQ8.

When people with celiac disease eat gluten (a protein found in wheat, rye, and barley), their body mounts an immune response that attacks the small intestine. These attacks lead to damage on the villi, small



fingerlike projections that line the small intestine, that promote nutrient absorption. When the villi get damaged, nutrients cannot be absorbed properly into the body.

Genetics and epigenetics in Celiac disease



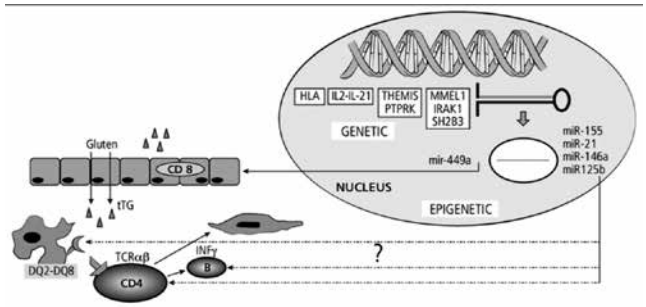
Class II *HLA-DQ*: The genes encoding for HLA molecules are found in the major histocompatibility (MHC) complex on the short arm of chromosome 6 (6p21.3). HLA molecules involved in celiac disease are encoded in a region known as class II by genes known as -DQ.*HLA-DQA1* gene encodes the α chain (α1 and α2), while *DQB1* encodes the *β chain (β1 and β2) of HLA-DQ* protein. Both chains are associated as heterodimers on the surface of antigen-presenting cells and form a cleft that binds antigens and presents them to T-cells.

Methodology

Genomic DNA is isolated and amplified by primers specific to DQ2 and DQ8 alleles. Subsequently, amplicons were detected by gel electrophoresis and analyzed by Score software (Olerup, USA). This method is easy to perform and detect DQA1*02XX,05XX; DQB1*02XX,03: XX genotypes in blood samples. Results are compared with patient's family history, hematological parameters and other clinical and laboratory findings.

Pathogenesis

After the deamidation of gluten peptides by the tissue transglutaminase (tTG), those peptides bind to HLA DQ2-DQ8 and presented to the CD4 T lymphocytes. CD4 T lymphocytes are responsible of amplify the response through the release of cytokines and/or activation of other immune system cells, accounting for many of the signs and symptoms of CD. There are other loci that would give susceptibility to CD, each of which is associated with a low risk for developing the disease. miRNAs regulate the proliferation and differentiation of epithelial cells. The expression of miRNAs associated to inflammation and AIDs, may modulate protein expression or target molecules involved in this pathology.



The diagnostic method for CD involves serological or intestinal biopsy, but genetic test could be implemented. HLA typing precludes the need for further diagnosis, and it has high negative predictive value.

Magnifying the Clinical Value of Expert Review and Reassessment

Drs Bushra Zafar Sayeed, Madiha Bilal Qureshi and Muhammad Raza
Histopathology

Laboratory medicine plays a critical role in the delivery of healthcare by contributing to 70 percent of clinical decisions. Some of the factors required for optimal delivery of care include accuracy, reproducibility, timeliness, cost-effectiveness, professional staff, equipment and quality management. Through our adherence to these principles, we are trusted as the final say throughout the region and receive many cases for expert opinion.

What is a PBCR specimen?
It is a case sent to us for a second opinion. These are usually cases of high diagnostic complexity and require extended work-up. The paraffin-embedded tissue blocks accompanied sometimes by the remaining specimen, along with the previous biopsy report are provided. We process the specimen according to our protocol and provide the concluding diagnosis.

Why are we sent PBCR specimens?
The reason behind patients opting for us for the absolute verdict on a case is the trust, quality and resources which we provide. We have the largest number of highly skilled, experienced, and reputable personnel. Furthermore, our resources to adequately diagnose an array of conditions, are present in only a few institutions in Pakistan.

How many PBCR specimens do we receive?
PBCR specimens account for approximately three percent of our work-volume. This is a huge number given the fact that these are highly resource-intensive cases, due to frequent fixation and processing artifacts, limited amount of remaining tissue available for interpretation and more importantly, the strong selection bias towards cases with high degree of diagnostic complexity. It is mostly difficult to optimally evaluate these cases without the extensive help of ancillary resources, such as immunohistochemical markers.

What novel IHC markers are available at our institution?
There is a wide range of IHC markers available at AKUH. Some of the novel markers include of those used in diagnosis of tumors of adult and pediatric brain, soft tissue and bone, kidney, male and female genital tracts, skin, and head and neck. We also have a wide range of lineage specific markers for tumors of unknown origin, lymphoma markers for up-to-date classification, and prognostic and predictive markers for guiding oncological management. Mismatch repair protein analysis is a good example, used for screening and management of cases suspected hereditary cancer predisposition syndrome.

How does it impact the community?
Accurately diagnosing a difficult case has a huge impact on the community. PBCR specimen processing affects the community by providing precise data for most of the reportable neoplasns occuring in a geographical region. It allows the identification of the possible causes of cancer in the community and to assess the impact of cancer control activities. The pace of development, resource availability and awareness is slower in low middle income countries, because of human and financial restrictions. However, the importance is undebatable as diagnosing the cases effectively changes the dynamics for research, treatment and prevention. We are playing a significant role in this aspect to better serve our community. As exhaustive as it may be, it is the determination of our leadership and the commitment of our employees that has made this possible.

Urine Cytology: A Screening and Surveillance Test

Drs Ummiya Tahir, Madiha Bilal Qureshi and Saira Fatima
Histopathology

Urinary cytology is used as a screening test for neoplasms of the urinary tract. The microscopic examination of exfoliated cells in the urine is a very effective method to detect urinary tract malignancies. It is a cost-effective method with minimally to non-invasive approach and has high specificity and positive predictive value. Urologists use cytology as an adjunct to the routine radiographic and endoscopic findings to ascertain that a potentially life-threatening urothelial malignancy is reliably detected.

Urinary cytology is not a usual screening test for the general population. Its utility is for symptomatic patients with new onset, unexplained hematuria and selected populations with increased risk of development of urothelial carcinomas. Such risk factors include smoking, industrial or chemical exposure like chlorinated hydrocarbons, aromatic amines and polycyclic aromatic hydrocarbons including benzidine. Urinary cytology is also important for surveillance of patients with known prior urothelial malignancy including patients treated with different therapies.

Samples for urinary cytology include:

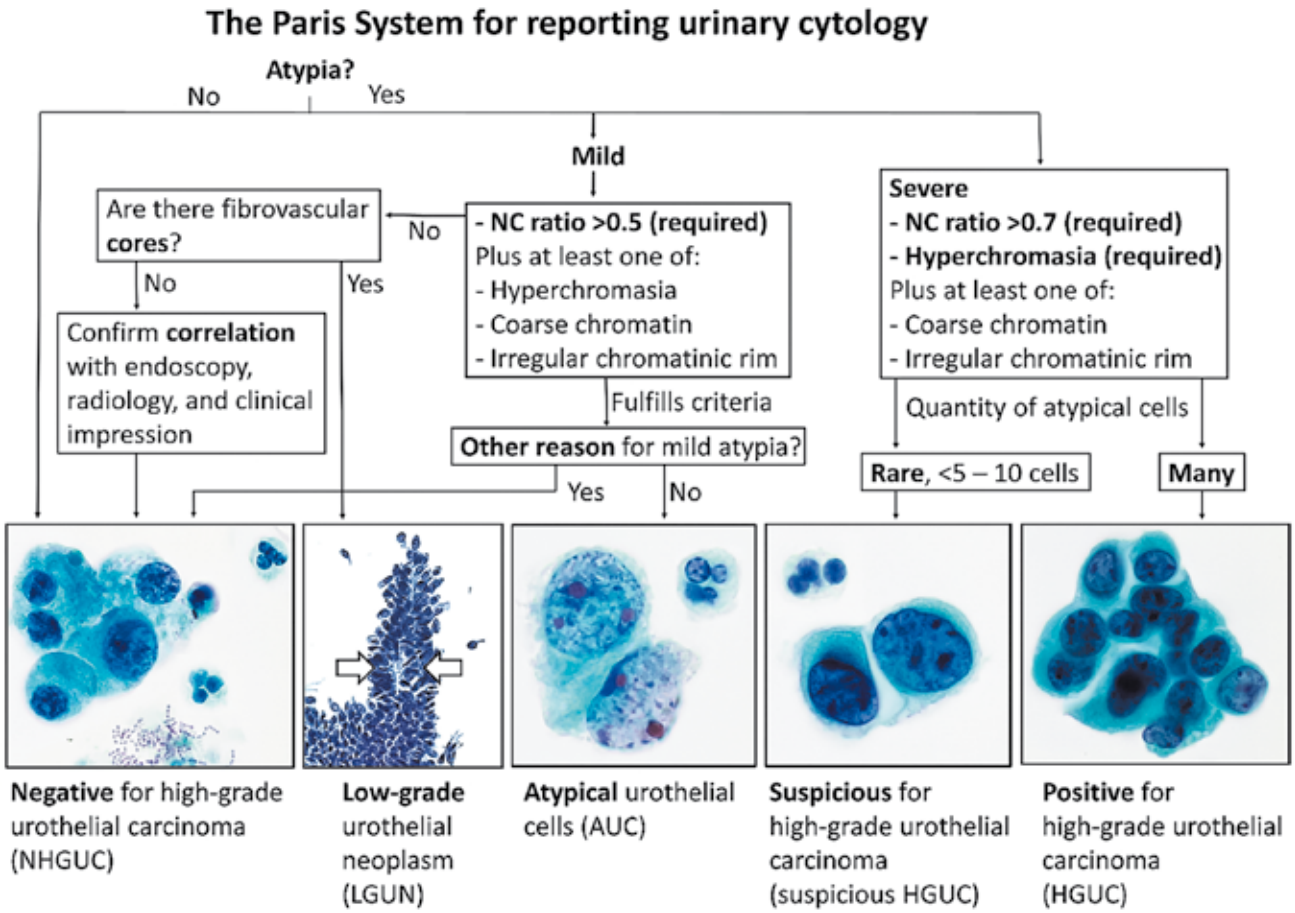
- **Voided urine:**
  - Easiest non-invasive method
  - three second morning voided midstream urine samples obtained over three consecutive days

- **Instrumented urine:**
  - Obtained via:
  - Irrigation of bladder/ Bladder washing
  - Catheterization of bladder
- **Ileal conduit/Neobladder urine:**
  - Used in patients that have undergone cystectomy
  - Ileal conduit: A part of ileum is anastomosed with the ureters to the skin or to the urethra
  - Neobladder is bladder reconstruction from bowel

An adequate specimen is a specimen representative of what is sampled. Adequacy relies on the quantification or at least semi quantification of the number of cells and/or the volume of voided urine. Presence of 2,600 cells or two well-visualized urothelial cells/ HPF in 10 consecutive HPFs can be used as an objective measure of adequacy in instrumented urine. Specimens more than 30 ml are more probable to be adequately cellular/satisfactory. If a sample contains even a few abnormal cells regardless of the volume, cellularity or specimen type, the specimen is considered satisfactory. In cases where urothelial cells are completely obscured by inflammatory cells or lubricant, the specimen is considered unsatisfactory.

Urine specimen is a relatively tougher cytology specimen that a pathologist encounter because of interobserver variability. The interpretation of a urine specimen hugely depends on the expertise of the examiner. The cytology reports should be accurate and understandable, clearly describing the cytopathologic findings and outcome probability to guide clinicians selecting the optimal management options for the patient. In this regard, The Paris System for Reporting Urinary Cytology (TPS) has led to standardization of the reporting system and has improved the screening and surveillance potential of urine cytology. The AKUH urinary cytology reporting is based on TPS. The presence of skilled Cytopathologists and Cytotechnologists along with utilization of TPS has reduced rates of indeterminate diagnoses and improvised the sensitivity of the test and risk stratification for patients with urothelial malignancies.

Approach to Diagnosis in urinary Tract Cytology According to Paris System



An Overview of Convalescent Plasma for The Treatment of COVID-19

Dr Maria Owais
Haematology & Transfusion Medicine

The COVID-19 epidemic had been a serious obstacle for the medical community and researchers around. Finding efficient cure and treatment was crucial to reducing the spread of the virus as the number of patients increased. Convalescent plasma therapy was one remedy that was found to be useful.

Convalescent plasma treatment includes giving plasma from COVID-19 patients who have recovered to others who are presently battling the illness. Antibodies in the plasma can aid in virus neutralization and improve the recipient's immune response. Although convalescent plasma therapy is not a new medical procedure, it has garnered popularity as a potential COVID-19 treatment.

The effectiveness of convalescent plasma treatment in treating COVID-19 patients has been investigated in several trials. Convalescent plasma treatment was linked to lower mortality rates and better clinical outcomes in patients with severe COVID-19, according to a comprehensive evaluation of 19 research published in 2020. Additionally, a randomized controlled trial conducted in India on patients with COVID-19 who were hospitalized discovered that the early treatment of convalescent plasma therapy slowed the disease progression.

While some research has produced encouraging outcomes, others have revealed contradictory information. Convalescent plasma therapy did not reduce mortality rates or enhance clinical outcomes in hospitalized COVID-19 patients, according to a randomized controlled trial conducted in Argentina. These contradictory findings could be the result of variances in the convalescent plasma therapy timing, dosage, and source, as well as variability in the patients' individual characteristics.

Convalescent plasma therapy is still an effective treatment for COVID-19 patients despite these difficulties. Under the terms of an Emergency Use Authorization (EUA), the US food and drug administration has approved the use of convalescent plasma therapy for the treatment of COVID-19 patients. The EUA permits medical professionals to treat COVID-19 patients outside of clinical trials using convalescent plasma treatment.

Convalescent plasma therapy came out to be one of the viable COVID-19 treatment. However, more research is required to properly understand its efficacy and safety.

Role of Imaging in Public Health Care

Dr Shayan Sirat
Department of Radiology

Medical imaging plays a crucial role in modern medicine, but have you ever considered its significance? It has revolutionized the healthcare industry, enabling practitioners and researchers to gain unprecedented insights into the human body.

When diagnosing illnesses, physicians often rely on diagnostic scans such as x-rays, CT scans, and MRIs. While medical expertise forms the foundation of healthcare diagnoses and decisions, medical imaging is essential for confirming diagnoses. It also assists in making treatment decisions and planning future care. With advancing technology, medical imaging can reveal internal problems that are not visible during a basic external examination. In ongoing illnesses, medical imaging is vital for monitoring the effectiveness of treatments and making necessary adjustments. The detailed information provided by medical imaging enhances the overall care provided to patients.

Ultrasound imaging is particularly valuable for expectant mothers. Recent advancements in ultrasound technology have resulted in high-resolution sonogram images, offering detailed insights into the baby's health and development in the womb. This early detection capability allows obstetricians to identify any concerns during pregnancy. Ultrasound is also widely used to examine various body parts, such as the neck, breasts, abdomen, pelvis, and extremities. It serves as a guide for soft tissue biopsies and certain treatments.

Another benefit of medical imaging lies in its role in preventive care. Recommended screenings like mammography aid in the early detection of breast cancer, contributing to a significant reduction in breast cancer-related deaths since 1990.

Surgeons also utilize medical imaging as a valuable tool during surgical procedures. For instance, in endoscopic sinus surgery, CT scans allow close examination of the extensive sinus network before the procedure, aiding in surgical planning. CT scans are increasingly used during surgeries to guide physicians through intricate internal operations. They are also useful for detecting issues within bones and joints, assisting doctors in determining appropriate treatment methods.

MRI is an alternative to CT scans when studying organs or soft tissues. It offers superior differentiation between different types of soft tissues and between normal and abnormal tissues. Additionally, MRI procedures pose no risk of radiation exposure.

Newer applications of MRI, such as magnetic resonance angiography (MRA), help evaluate blood flow in arteries and detect aneurysms and vascular malformations. Functional MRI (fMRI) is used to identify specific functional centres in the brain, aiding in surgical planning for brain disorders.

A combination of positron emission tomography and computed tomography (PET/CT) provides the benefits of both techniques in a single procedure. PET scans reveal areas of increased metabolic activity, while CT scans offer detailed anatomical information. This combination allows doctors to accurately pinpoint the location and significance of high metabolic activity regions, often used to monitor the effects of disease treatment.

Endovascular surgery, an alternative to open surgery, offers advantages such as shorter recovery times, less discomfort, and smaller incisions. It is particularly beneficial for patients at high risk of complications due to other medical conditions. Procedures such as angiography, angioplasty, embolization, gastrostomy tube placement, stent placement, foreign body removal, IVC filter insertion, and catheter insertions are common in endovascular surgery.

Medical imaging truly serves as a vital component of the healthcare field, facilitating diagnostics, treatment, and prevention. As technology continues to advance, we can expect further growth in medical imaging, leading to earlier detection of health issues, improved treatment outcomes, and enhanced preventative care.

MRI Liver with Hepatocyte-Specific Contrast Agent

Dr Shayan Sirat
Department of Radiology

Introduction:

Magnetic resonance imaging (MRI) is a well-established diagnostic test used for evaluating focal liver lesions. However, in cases of cirrhotic liver or lesions smaller than 1.0 cm, MRI may not accurately identify or characterize malignant nodules in approximately 60 percent of cases. To address these limitations, liver-specific contrast media have been developed to enhance the sensitivity and specificity of MRI in assessing focal liver lesions. Among the liver-specific contrast media available, gadoxetic acid (Gd-EOB-dTPA) is approved for clinical use in Pakistan. Gadoxetate disodium, a hepatospecific gadolinium-based contrast agent, is exclusively used for liver imaging in MRI.

Physical and chemical properties, as well as bioavailability of Gadoxetate disodium:

- Gadoxetate disodium exhibits combined properties of both extracellular and hepatocyte-specific agents.
- It has a high affinity for hepatocytes, with approximately 50 percent of the contrast agent taken up by the liver.
- Approximately 50 percent of the contrast agent is excreted by the liver, while the remaining 50% is excreted by the kidneys, resulting in complete elimination within 24 hours.

Clinical benefits of Liver Specific MRI:

- Significantly improved detection of hepatocellular carcinoma and metastases compared to CT or unenhanced MRI.
- Enhanced detection of lesions smaller than 1 cm in diameter.
- Superior differentiation between benign and malignant focal lesions.
- Improved accuracy in lesion characterization.

Indications for Liver Specific MRI:

- Evaluation of focal nodular hyperplasia (FNH) versus adenoma.
- Assessment of hepatic metastases.
- Post-liver transplant evaluation (excluding cases involving hepatic artery assessment or suspected abscess).
- Hepatocellular carcinoma (HCC) surveillance.

Disadvantages:

- Not recommended for use immediately after hepatic ablation.
- Suboptimal for evaluating hemangiomas or inflammatory diseases.
- Should be avoided in patients during the perioperative liver transplantation period.
- Caution should be exercised when administering to patients with renal impairment (GFR < 30 ml/min) due to the risk of nephrogenic systemic fibrosis.

Preparation for Liver Specific MRI:

- Claustrophobic patients or those experiencing significant discomfort while lying on their back for more than 10 minutes should consult their referring physician for appropriate medication to alleviate these issues. The radiology physicians will not prescribe these medications.
- Fasting for six hours before the appointment.
- Notification to the radiologist or technologist if the patient has any allergies or sensitivities to medications, contrast dyes, or iodine.
- Informing the technologist(s) if the patient is pregnant, nursing, or has any implanted devices such as aneurysm clips, pacemakers, bone growth stimulators, pain pumps, or other electronic implants.
- Before the examination, a thorough medical history will be taken. Patients aged 60 years or older and those with a history of renal disease, diabetes mellitus, or multiple myeloma will require a creatinine level blood test. The creatinine results are acceptable if obtained within the last seven days.

Contrast dosage: Liver-specific MRI is performed following an intravenous injection of 0.1 ml/kg body weight as a bolus, followed by a saline flush.
Duration of examination: Liver-specific MRI typically takes approximately 20 minutes longer

than a regular liver examination. This additional time is necessary to acquire delayed images in the hepatobiliary phase. The approximate scan time is 60 minutes.

Examination Protocol:

- Arterial phase: 20-30 seconds.
- Portal venous phase: 60-70 seconds.
- Equilibrium phase: 3-5 minutes.

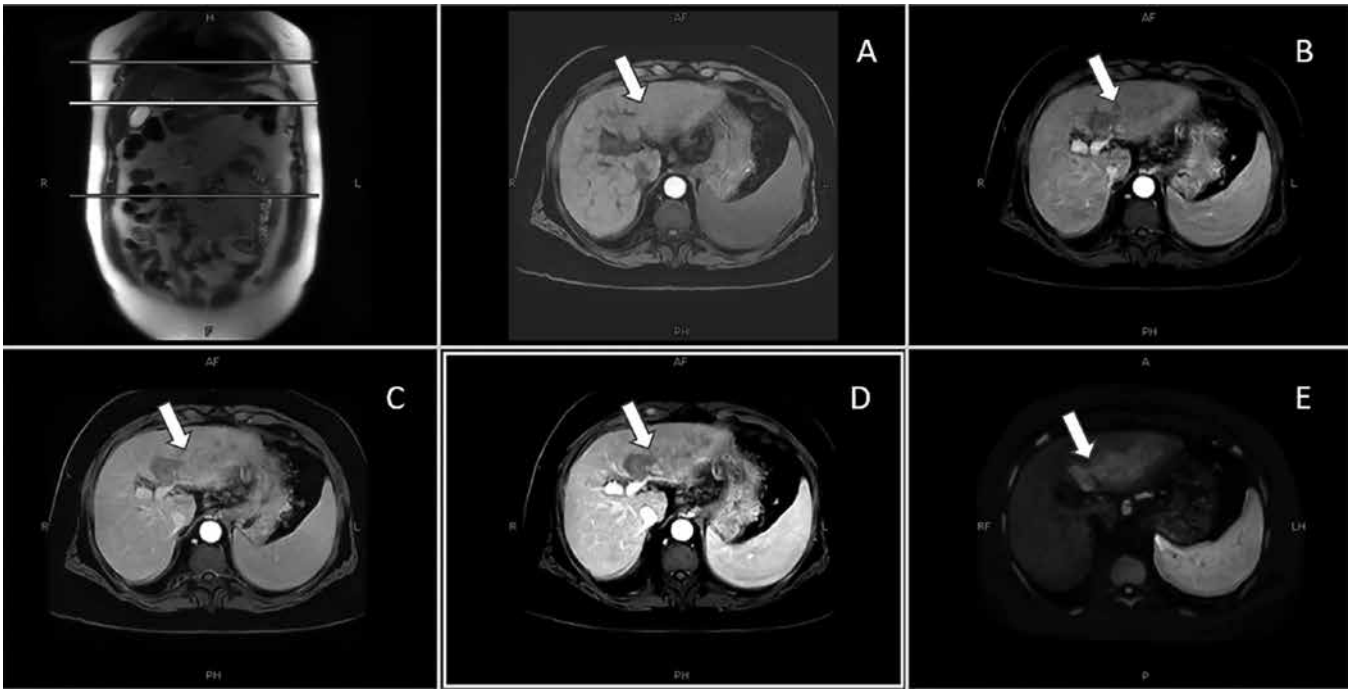


Figure 1: MRI Liver with Liver Specific Contrast Showing Atypical Hepatocellular Carcinoma

Dynamic contrast enhanced study of hepatobiliary system with liver specific contrast (Gadoxetate disodium) Early arterial phase (A) late arterial phase (B) portovenous phase (C) delayed phase(D) and diffusion weighted sequence (E) . Infiltrative lesion in left lobe of liver extending into left branch of portal vein, showing poor arterial enhancement and significant wash out on porto venous and delayed phases, also demonstrating diffusion restriction. (arrow)

Basics and Role of Cytopathology

Drs Fatima Safdar and Saira Fatima
Histopathology

Introduction:

Cytopathology deals with diagnosis and study of disease by examining tissue and fluid samples obtained using minimally invasive techniques. It is simple, cost-effective, standardized, low-technology diagnostic/screening tool useful in classifying a substantial subset of lesions into infectious etiology, non-infectious benign or malignant and helps in guiding further management strategies. It leads to a reduction in invasive surgical procedures in cases of benign diseases.

Sample collection and processing:

The samples received are broadly divided as gynecological samples including cervical cytology and non-gynecological samples from sites including thyroid, lymph nodes, respiratory tract, body fluids and urine etc. Its reduced invasiveness has led to the development of new diagnostic techniques, such as endoscopic ultrasound-guided fine needle aspiration

(EUS-FNA) of pancreatic and other abdominal lesions, Endobronchial ultrasound guided fine needle aspiration (EBUS-FNA) from mediastinal lymph node.
The four methods used for sample preparation commonly are direct smear, cytospin, paraffin embedded block, and liquid based thin layer preparation method. Common staining methods include Pap, Diff-Quick and Hematoxyllin and eosin stain. The complete clinical history of the patient is required for correlation with the findings of cytology.

Gynecological samples:

It includes pap smears collected by conventional methods and by Liquid based cytology (LBC) SurePath. LBC allows for easier processing, potential for automation (for cervical pap tests), fewer slides required per case, less screening time compared with conventional / direct smears and molecular-based HPV genotyping in conjunction with cytology. The Bethesda System is used for reporting samples and its classifications into further categories that are associated with certain malignancy risk and recommends further clinical management. The benign changes including infections are more clearly identified as "Negative for intraepithelial lesion or malignancy." HPV test is the most powerful independent risk factor for the development of both cervical dysplasia and invasive cancer. Immunohistochemical stain p16 is used for identification of HPV associated malignancies.

Non-Gynecological Sample:

1. **FNA:** It has been used for any lesion in the body which includes following major areas:

• Palpable lesions: Including thyroid, lymph nodes and parotid swellings.
• Non-palpable lesions: The non-palpable lesions are usually done with the help of image analysis (CT scan guided, ultrasound-guided, fluoroscopy-guided, and recently endoscopic ultrasound-guided fine needle aspiration, EUS-FNA).

The Bethesda system is used for reporting Thyroid samples, and it divides into six categories. The Milan System is recommended for Reporting Salivary Gland Cytopathology. The lymph node FNA helps in ruling out infectious etiology especially Tuberculosis, metastasis, or any other primary malignancy.

2. **Effusions:** The samples commonly included are pleural fluid, pericardial fluid and peritoneal fluid cytology. These are sent mainly to detect malignancies and infections. Cell block preparations from the sediment are very helpful in confirming malignancy using immunohistochemical stains. In many cases further characterization and primary site of origin can also be determined by immunohistochemistry.

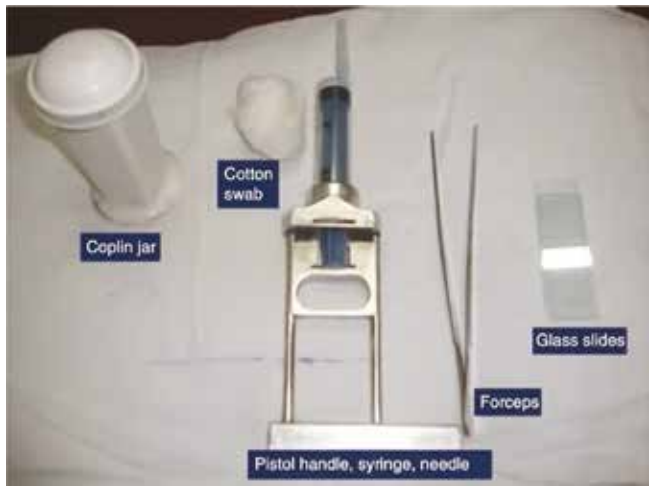


Figure 1: Essential equipment to do FNAC

3. **Respiratory Cytology:** It includes bronchial washing, sputum, bronchoalveolar lavage, and bronchial brushing cytology. These are commonly used to detect pulmonary infections and malignancies.

4. **Urine:** Urine cytology represents a significant portion on non-gynecologic cytology specimens in daily practice and its significant impact on management. It includes Urine cytology, bladder washing, and brushing cytology. Paris System is used for reporting urinary cytology as it standardizes terminology with standardized cytomorphologic criteria for reporting urine cytology.

5. **CSF:** The samples are mainly sent to rule out infections in immunocompromised patients including fungal such as cryptococcus and malignancies.

6. **Cell Blocks,** IHC, Molecular, ER And PR: The cell block prepared is used for performing ancillary studies if required including Immunohistochemical workup for further characterization of lesion. Recent advances in Next Generation Sequencing analysis using LBC specimens from which DNA could be extracted, will contribute to improving in the assessment of malignancies and the application of molecular-targeted drugs.

Limitations:

The main limitation is the small amount of material retrieved, which requires optimal processing to perform additional ancillary studies. When necessary, immunocytochemistry (ICC), electron microscopy, and molecular biology techniques are used to differentiate between reactive lesions and malignancy. Depending on their availability, flow cytometry, IHC, molecular analysis, genetic studies, culture, and polymerase chain reaction (PCR) for infections can all be performed on the FNAC material and increase the diagnostic value of the FNAC.

Conclusion:

In conclusion, cytopathology has developed rapidly in recent past years and achieved significant results. Quality control is important to improve the diagnosis. Although ancillary techniques are required for improving accuracy, cost is still a major issue.

Bone Tumors: From Timely Diagnosis to Improved Patient Outcome

Drs Madiha Bilal Qureshi, Muhammad Raza and Nasir Ud Din
Histopathology

Malignant bone tumors are rare. The overall annual incidence of bone sarcomas is around 0.75 to 2.0 per 100,000 population. However, specific bone sarcomas have age-related incidence rate. The common malignant primary bone tumors are Osteosarcoma, Ewing sarcoma and Chondrosarcoma. Earlier diagnosis of malignancy and timely management can lead to improved patient outcome. The potential for malignancy must be carefully assessed in an unusual bone lesion since misinterpretation of a lesion or delay in diagnosis may have limb and life-threatening consequences.

The clinical symptoms of malignant bone tumors include worsening non-mechanical pain (pain not affected by movement, positioning or activity) and night pain disturbing sleep. Swelling at the site becomes obvious once there is cortical breach and the tumor spreads beneath and beyond the periosteum. Other non-specific symptoms may appear like symptoms of musculoskeletal injury. A patient with non-mechanical bone pain or swelling/ non-mechanical pain in a limb (specifically around knee) must be referred for radiographic plain x-ray assessment.

Most malignant bone tumors have a metaphyseal location excluding Ewing sarcoma that is diaphyseal. Radiologic features like wide zone of transition, bone/matrix production, cortical breach, periosteal elevation/reaction, or associated soft tissue swelling should prompt further investigation. Lack of awareness of the seriousness of clinical presentation by the patient or low suspicion by physician may lead to delay in diagnosis and treatment.

Diagnosis of patients with worrisome features also depends on age of the patient. The possibility of metastatic tumor increases with age and is more common in older adults besides myeloma, whereas osteosarcoma is the most common bone malignancy in children. Around 56 percent of malignant bone tumors in children occur around knee. A non-resolving painless or painful swelling around knee in a child should always be investigated. Pelvic location is also a common site for tumors like Ewing and Chondrosarcoma. Such tumors are usually detected late owing to long duration non-specific symptoms. However, referred pain to leg or knee or night pain should raise alarm for an underlying tumor.

Patients with suspected malignant bone tumors should be dealt by an experienced multidisciplinary team including radiologist, orthopedic surgeon, oncologist and histopathologist. Imaging should be done before biopsy since biopsy may change the radiologic features. An orthopedic surgeon should decide the surgical approach and plan. The biopsy of such lesions should be performed at a reference centre for bone sarcoma by the orthopedic surgeon. A definitive histologic diagnosis requires experienced orthopedic pathologist and correlation with radiologic findings. Moreover, a histopathologist should be given complete clinical information including patient's age, site and

size of tumor, any underlying bone disease or family history. A biopsy report should include the histologic type of tumor and provisional grade.

Positive prognostic factors for bone sarcomas include localized disease, surgically achieved clear margins, and > 90 percent therapeutic response to chemotherapy. Poor prognostic factors include older age, axial skeleton location, metastatic disease, large tumor size, high grade, incomplete surgical resection and poor response to chemotherapy. Timely diagnosis and treatment in specialized centres with experienced multidisciplinary team can improve patient outcome. The Aga Khan University Hospital in this regard has an experienced musculoskeletal tumor multidisciplinary which helps patients in timely and appropriate management of bone sarcomas.

Differentiating Between Dengue Fever and Malaria Using The Hematological Parameters in Complete Blood Count

Drs Anila Zafar and Bushra Moiz
Haematology and Transfusion Medicine

Introduction:

Dengue fever and malaria are major public health problems in Pakistan. Infection timings may overlap in endemic areas and an early differentiation may help clinicians in better management. Complete blood counts (CBC) are usually the first lab test done in any febrile patient. So, it's imperative to evaluate if it can help in differentiating the two conditions. This study aims to compare the hematological parameters and morphological findings of complete blood counts to distinguish between dengue and malaria.

A cross sectional retrospective study was conduct at section of hematology in the month of September 2022. In this period, there was an outbreak of dengue and malaria in Karachi. Hematological parameters of two groups were compared. Confirmed cases of dengue (n= 201) were included based on positive NS1 antigen in the first group while second group had confirmed cases of malaria (n=135). The diagnosis of malaria parasite was made on finding of parasites on thick and thin film stained with respectively with Field and Leishman stains of EDTA-blood sample in light microscopy. EDTA blood samples were also analyzed to determine the CBC using Sysmex XN – 1000 analyzers. Hematological parameters including RBC count, hemoglobin, hematocrit (HCT), platelets count, WBC count, neutrophils percent and lymphocytes percent and morphology by microscopy were compared between the two groups. Then data was analyzed on SPSS software version 19. Median and interquartile range (IQR) was used for non-parametric data. Comparison was done by Mann-Whitney and a p-value of <0.05 was the threshold for significance.

Demography:

A total 336 patients were included in this study. Median age of patient was 28.9 years and 65 percent were male. There were more male (63 percent) in dengue and in malaria (68 percent). Out of 336 patients, 201 patients had confirmed dengue infection and 135 had malaria infection 97 percent vivax and three percent falciparum). No co-infection of dengue and malaria was not observed in study.

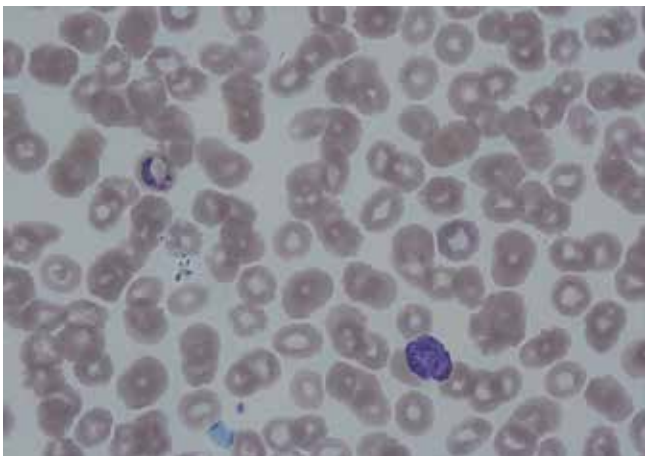
Hematological parameters comparison:

The following parameters were significantly higher in dengue infection as compared to patients with malaria: hemoglobin, HCT, RBC, platelets, and absolute lymphocyte count (ALC). (P-value less than <0.05). The following parameters were significantly lower in dengue as compared to malaria infection: WBC, absolute neutrophil count, neutrophil to lymphocyte ratio, and platelet to lymphocyte ratio (P-value <0.05). Reactive

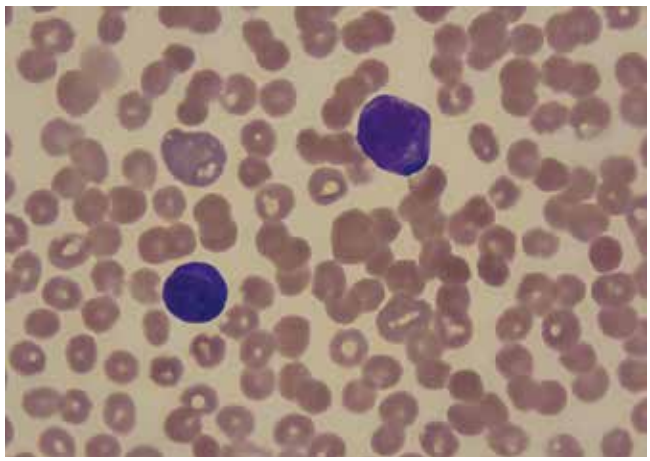
lymphocytes were observed in 95.5 percent of dengue patients. (p-value <0.05). Left shift neutrophils were not significantly different in both infections (p-value >0.05).

Conclusion:

Low hemoglobin, thrombocytopenia and lymphocytopenia had higher odds ratio for malaria than for dengue. Reactive lymphocytes were more frequent in dengue as compared to malaria. This study concluded that several hematological parameters could differentiate dengue fever from malaria.



P. Vivax Gametocytes and trophozoites



Reactive lymphocytes

Coronary CT for Calcium Scoring

Dr Anam Khan
Department of Radiology

Coronary heart disease (CHD) is a major global cause of death, responsible for 16 percent of total deaths in 2019. Atherosclerosis is a key factor in CHD. Vascular calcification strongly correlates with atherosclerosis (both calcified and noncalcified) and predicts cardiovascular morbidity and mortality. CT imaging can detect calcium deposits, leading to the development of coronary artery calcium (CAC) scoring.

Coronary artery calcium (CAC) scoring is a powerful tool for stratifying cardiovascular risk, predicting patient outcomes, and guiding preventive therapy. Currently, several methods to calculate the CAC score exist, and each has its own set of advantages and disadvantages. Agatston CAC scoring is the most extensively used method.

The CAC score acquisition protocol involves the use of computed tomography (CT) imaging to visualize the coronary arteries and quantify the amount of calcium present in them. The CAC score is calculated by multiplying the area of calcification by a density factor derived from the peak Hounsfield unit (HU) within that area. The total score is obtained by summing up all individual scores from different coronary artery segments.

CAC scoring is currently recommended for use in asymptomatic individuals to predict the risk of developing cardiovascular diseases and the disease-specific mortality. Incorporating the CAC score into existing algorithms and guidelines improves cardiovascular risk assessment and enhances clinical decision-making.

CAC scoring can aid in decision making for primary prevention interventions such as statin therapy. The American College of Cardiology/ American Heart Association guidelines recommend

Interpretation of CAC Score Results		
Visual Score	**Absolute CAC Score (Agatston Method)**	**Clinical Interpretation**
None	0*	Very low risk of future coronary events
Mild	1–100	Low risk of future coronary events; low probability of myocardial ischemia
Moderate	101–400	Increased risk of future coronary events; consider reclassifying the individual as being at high risk
Severe	>400	Increased probability of myocardial ischemia
*CAC scores must be interpreted after the patient's clinical risk factors are incorporated. A score of 0 does not imply zero risk.		

statin therapy for individuals with a 10-year risk of developing atherosclerotic cardiovascular disease (ASCVD) greater than or equal to 7.5 percent. However, there are limitations to using traditional risk factors alone to predict ASCVD risk. Incorporating CAC scores into ASCVD risk prediction models can improve accuracy and identify individuals who may benefit from statin therapy despite having low traditional risk factor scores. The progression of CAC scores on follow-up images has been linked with an increased risk of myocardial infarction (MI). A study found that individuals with a baseline CAC score greater than or equal to 100 who had a CAC score progression of greater than or equal to 15 percent per year had a significantly higher risk of MI compared to those with stable or decreasing CAC scores. This suggests that monitoring CAC score progression can aid in identifying individuals who may benefit from more aggressive preventive therapy.

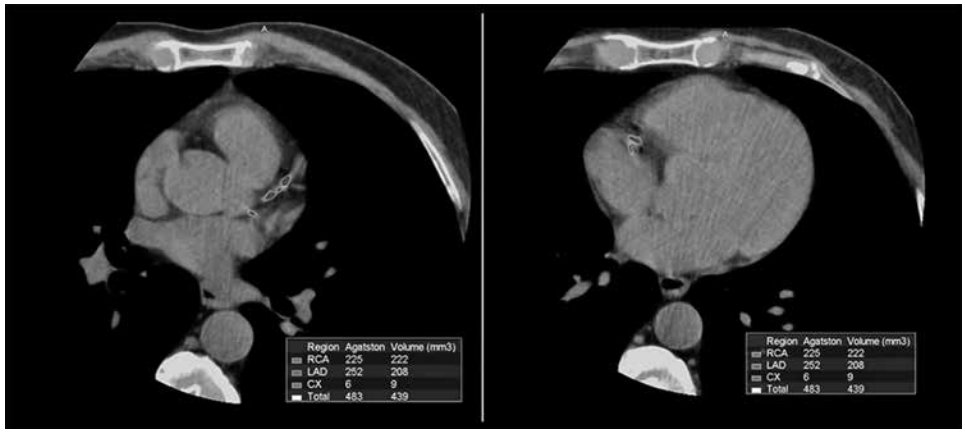


Figure 1: CAC score is 483, sever-increased probability of myocardial ischemia.

In conclusion, Coronary Artery Calcium Scoring (CAC) has emerged as a powerful tool for stratifying cardiovascular risk, predicting patient outcomes, and guiding preventive therapy. The Agatston score is the most extensively used method for CAC scoring. With ongoing research and advancements in technology, CAC scoring is likely to continue to play an important role in primary prevention interventions such as statin therapy.

Screening for Breast Cancer

Drs Shaista Afzal and Imrana Masroor
Department of Radiology

The aim of breast cancer (BC) screening is early cancer detection to enhance the probability of successful treatment. However, it's important to note that early detection is not helpful in the absence of effective therapy. Of note is the fact that therapeutic outcomes are improved when the patients present with early-stage disease. The limitations and potential harms of screening methods and therapies justifies the adoption of management options that minimize / mitigate the associated risks.

Multiple RCTs conducted at the end of 20th century

have reported the efficacy of screening mammography and their meta-analysis suggested 23 percent mortality reduction in screened women aged 50 to 69 years.

The screen detected lesions are smaller without any metastatic disease hence likely reduces the need for more morbid therapy like mastectomy, axillary lymph node dissection and chemotherapy. As per the study by Barth et al, screen detected breast cancer patients were more likely to undergo breast conservation surgery (56 vs 32 percent) and less likely to be treated with chemotherapy (28 percent vs 56 percent)

The cost of breast cancer management is often not considered by the policy developers. The women diagnosed with breast cancer may be supporting parents, raising children and or active in the workforce and the morbidity and mortality associated with this condition leads to societal cost and psychosocial and economic consequences.

Determining the Risk of Breast Cancer:

The highest benefit of screening is for those who are most likely to acquire breast cancer and for whom earlier therapy reduces mortality. Determining a person's risk of developing breast cancer is crucial because it can be used to recommend the type and frequency of screenings as well as to decide whether referrals for genetic testing, consideration of chemoprevention, and/or prophylactic surgery, are required. The lifetime risk for developing breast cancer is reported as average (less than 15 percent), moderate (roughly 15 percent to 20 percent), or high (more than 20 percent).

Most women can be classified based just on their histories, but for some, risk assessment models are available. Based on a patient's history, the important variables used to assign a risk category include e.g., personal or family history of breast, ovarian, tubal, or peritoneal cancer, family history of BRCA one or two mutation, mammographic breast density, high risk lesions diagnosed of prior breast biopsies, early age at menarche or late menopause, age at first live birth, personal history of chest radiotherapy at age 10 to 30 years etc.

Age related screening approach for women at average risk:

Age is an important factor in women with average risk i.e., less than 15 percent lifetime time risk as the incidence of breast cancer is quite low under 40 years of age and the mammographic sensitivity and specificity is also age dependent and is higher in older women. It's important to consider the benefits/costs, inconvenience, and possibility of overtreatment due to screening in the younger age group.

Multiple expert groups have come up with age related recommendations for initiation of screening for breast cancer. These societies and expert include government sponsored like US Preventive Services Task Force, National Health Service, United Kingdom, Medical societies like American College of Radiology (ACR) and Society of Breast Imaging (SBI) and National Comprehensive Cancer Network etc.

Under the age of 40 years, there are no screening recommendation for average risk women. At age 40 to 49 years, United States expert groups like ACR, SBI promote initiation of breast cancer screening at 40 years while some also suggest shared decision making so that the women are aware of the benefits and harms. However, the European guidelines propose the commencement of screening at the age of 45 years.

At age 50 to 74 years, screening mammography is recommended by all major US and international groups, however they differ on the frequency of the screening. At age 75 and older screening mammography should be provided screening if they have a minimum life expectancy of 10 years.

For average risk women, the screening mammography can be performed every one to two years depending upon the guidelines they follow. Limited data is available on the optimal frequency of performing screening however more harms and associated cost are reported with annual screening than with screening every two years and the difference in absolute benefits between the two is negligible. While some results indicate that some women (e.g., premenopausal) may benefit from annual screening, this advantage must be balanced against the higher risk of false-positive mammographic findings and overdiagnosis.

Moderate risk women: The screening approach recommended for moderate risk women remain same as for average risk, however some practitioners do recommend it earlier if the first degree relative was diagnosed with cancer in premenopausal period. Data for supplemental imaging is also inconclusive in such women.

High risk women: having greater than 20 percent lifetime risk of breast cancer requires high risk

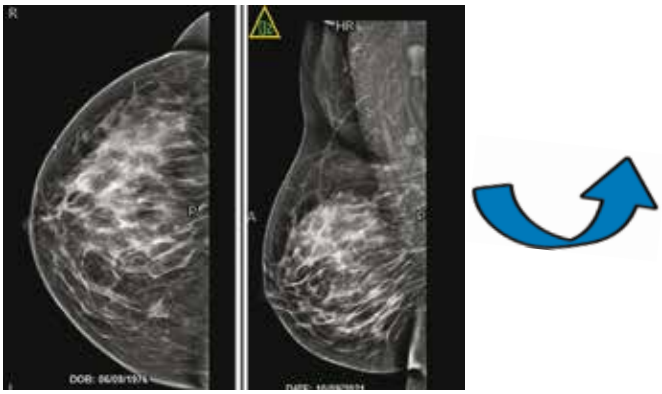
i.e., intensified screening protocols with enhanced modalities and at greater frequency. They are likely to undergo genetic testing and be considered for risk reducing treatment like chemoprevention and prophylactic surgery.

Imaging Modalities for screening:

These include mammography, other radiologic procedures, such as ultrasound and magnetic resonance imaging (MRI), are used as supplemental method to further analyze mammographic results or to screen women who are at high risk to develop breast cancer. It is debatable whether clinician breast examination (CBE) or patient breast self-examination (BSE) is effective as a supplement to mammography. However, should not be the only screening approach. There is a need to develop awareness of women regarding breast cancer symptoms and signs, and they should be urged to report any issues they may have.

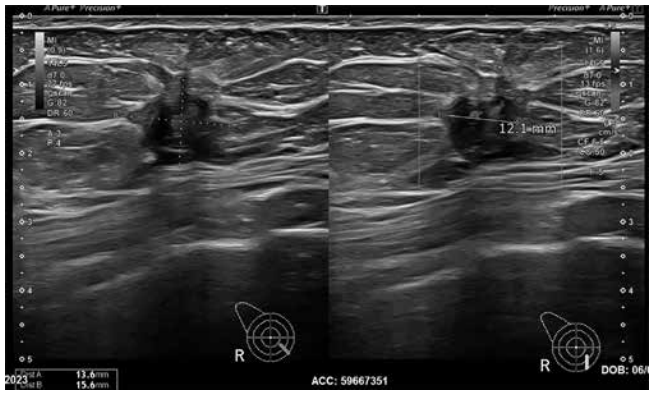
Mammography:

Mammography is the main screening method for women at average risk for breast cancer. It can be performed as 2D digital, 3D digital breast tomosynthesis, or as film screen technique. It is best



Screening Mammography of right breast showed an irregular lesion in the inner half in CC view (arrow). Ultrasound of right breast confirmed the presence of a spiculated suspicious lesion in right lower inner quadrant (curved arrow). On histopathology the lesion diagnosed as Invasive breast carcinoma, grade 1.
cribriform and focal tubular patterns, grade I,

studied, and multiple randomized control trials have demonstrated the reduction of breast cancer mortality associated with its usage for breast cancer screening. But it's crucial to understand that mammography may miss up to 20 percent of underlying breast cancers, even under ideal circumstances. In women with dense breast, Digital mammography or DBT are preferred because of better sensitivity and specificity of these techniques.



Other Imaging modalities:

These include ultrasound, MRI and newer techniques like contrast enhanced mammography. However, there is lack of evidence with regards to their use in women at average risk of breast cancer. These are useful as supplemental screening methods. Ultrasound screening for women at average risk is not advised. The effectiveness of ultrasound as a screening method to lower breast cancer mortality in women at average risk, including those with dense breasts, has not been studied. However, in the US several states require that patients be informed that ultrasonography may be used as a supplement to mammography in women with increased breast density.

According to supplemental screening MRI guidelines by the American cancer Society, screening MRI is not advised for women with average risk. High-risk patients with a lifetime risk of >20 percent are typically screened using MRI in conjunction with mammography.

Status of breast cancer screening in low and middle-income countries (LMIC):

Several research trials have been conducted in LMIC to determine the best ways of implementation of population-based screening for breast cancer and for these various combinations of clinical/self-breast examination and screening mammography were tried. However, there is a need for more research in the context of LMIC. There are many barriers to its achievement like non-inclusion in public health agenda, and lack of awareness and apathy among policy makers regarding the cost effectiveness of BC screening. The other barriers are related to the population like presence of stigma and unawareness of women regarding BC and lack of adopting to proper screening behaviors such as conducting breast self-examination.

Implementation of population-level programmes for BC screening and early detection, as well as strategies to raise women's knowledge of BC, can be crucial in reducing the growing burden of BC in LMICs. The use of more supplemental techniques, such ultrasonography, which is more suited to LMIC populations, as well as the use of social media to raise awareness and improve screening compliance are crucial additions to the overall agenda of LMICs in prevention of BC.

Conclusion:

The recommendation for breast cancer screening aims to maximize proven benefits, which comprises of breast cancer mortality reduction due to regular screening and better treatment options for those with breast cancer diagnosis. It's important that the pros and cons of breast cancer screening especially with respect to mammography are shared to assist women in making an informed decision.

Unveiling the Dangers of Primary Amoebic Encephalitis." The Brain-Eating Amoeba: A Comprehensive Guide

Mohammad Zeeshan
Microbiology

Naegleria fowleri is a rare but potentially deadly amoeba that can cause a severe brain infection called primary amebic meningoencephalitis (PAM). This amoeba is commonly found in warm freshwater environments such as lakes, rivers, and hot springs. While Naegleria fowleri infections are relatively rare worldwide, they have been reported in various countries, including Pakistan.

In Pakistan, Naegleria fowleri has been recognized as a public health concern due to several cases of PAM reported in the past. The amoeba thrives in warm water temperatures, and during the summer months when temperatures rise, the risk of infection increases. The primary mode of infection is through the entry of contaminated water into the nose, typically while swimming, diving, or engaging in other water-related activities.

The diagnosis of Naegleria fowleri primarily relies on a combination of clinical symptoms, patient history, and laboratory tests.

However, it's important to note that medical advancements and new diagnostic modalities may have emerged since then. Following are some conventional diagnostic methods and potential advancements that were being explored at that time.

Clinical Presentation: The initial symptoms of PAM can be like those of bacterial or viral meningitis, including headache, fever, nausea, vomiting, and neck stiffness. However, the infection progresses rapidly, leading to seizures, hallucinations, altered mental status, and coma. A rapid onset of symptoms, particularly in individuals with recent exposure to freshwater bodies, can raise suspicion for Naegleria fowleri infection.

Patient History: Obtaining information about recent freshwater exposure is crucial, as Naegleria fowleri is commonly found in warm freshwater environments, such as lakes, hot springs, and poorly maintained swimming pools.

Laboratory Tests: Traditionally, the definitive diagnosis of Naegleria fowleri infection has relied on the examination of cerebrospinal fluid (CSF) obtained through a lumbar puncture.

Direct visualization in Cerebrospinal fluid: The diagnosis of Naegleria fowleri infection can be made by microscopic examination of fresh, unfrozen, unrefrigerated cerebrospinal fluid (CSF). A wet mount of freshly centrifuged CSF sediment might demonstrate actively moving trophozoites. Naegleria fowleri (15-30 µm trophozoite) moves rapidly (~1

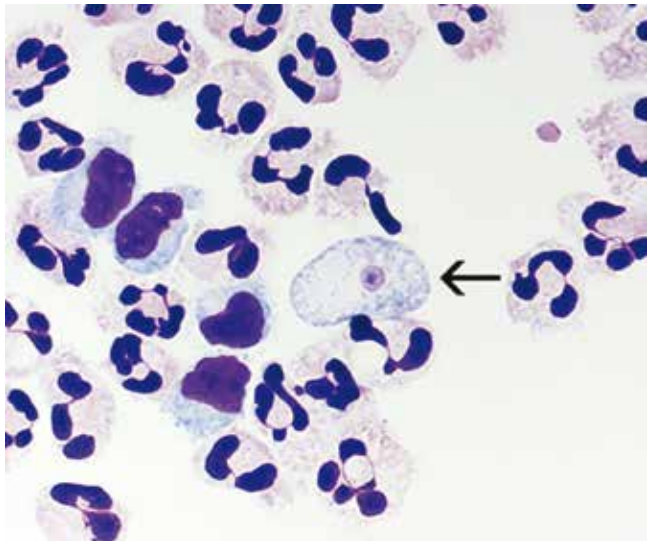
µm/s) using eruptive pseudopods and moves sinuously in a generally linear forward direction. The use of staining techniques, such as Wright-Giemsa or trichrome staining, hematoxylin, and eosin (H&E), periodic acid-Schiff (PAS), trichrome, Giemsa, can aid in the identification of the amoeba.

CSF Detail report:
The typical CSF findings associated with Naegleria fowleri infection:

- High Red Blood Cell count
- Elevated White Blood Cell Count
- Lymphocytic Predominance
- Elevated Protein Levels
- Normal Glucose Levels:

Potential advancements in diagnosing Naegleria fowleri may include:

Molecular Techniques:
Specific molecular tools can amplify DNA from the amebae in CSF or tissue to specifically identify if the amebae are present. Recently, next-generation sequencing has also been used in the diagnosis of a PAM case.
Serological Assays: Developing serological tests to detect specific antibodies against Naegleria fowleri in patient blood samples could provide an alternative diagnostic method. However, research in this area is ongoing.



Giemsa-Wright Stain: A cytospin of fixed CSF showing a *Naegleria fowleri* trophozoite (arrow) stained with Giemsa-wright amidst polymorphonuclear leukocyte and a few lymphocyte. Within the trophozoite, the nucleus and nucleolus can be seen. Magnification: 1000 x (courtesy: https://www.cdc.gov/parasites/naegleria/naegleria-fowleri-media.html)

Importance of Surveillance for Antifungal Drug Resistance: The Story of C. Auris

Dr Joveria Farooqi
Microbiology

Historically, fungal infections have been considered opportunistic, and occurring in a very select group of patients. However, in recent years with improving life expectancy, broad spectrum antibiotics and more hospital-based interventions, invasive fungal infections have become more common. Hence, the use of antifungal agents has also increased. The antifungal agents were primarily used empirically, but importance of targeted therapy based on susceptibility profile has become essential in the face of emerging resistance.

Since 2005, the Department of Pathology and Laboratory Medicine of Aga Khan University, realising how important the surveillance of resistance in *Candida* species was, embarked upon periodic antifungal resistance surveys as a research priority. Over ten years, two surveys showed the emergence and increase in resistance of *Candida glabrata, C. tropicalis* and other species against fluconazole. Hence, in 2013, the clinical laboratory introduced antifungal susceptibility testing routinely for *Candida* species isolated from sterile samples.

Just one year later, in late 2014, an unidentified *Candida* species, with a specific appearance and

consistently resistant to fluconazole, sometimes also voriconazole, was noticed to be occurring in clusters of patients with candidemia and other types of invasive candidiasis. Had we not been performing routine antifungal susceptibilities, we would not have been able to characterise this strain. Later on, this *Candida* species was confirmed as *Candida auris*, now recognised as the first fungal superbug, capable of multidrug resistance and causing hospital outbreaks, even possibly a One Health concern.

Candida auris is now a critical priority fungal pathogen recognised by World Health Organisation in their list of priority fungal pathogens. In a span of five years, 2014-2019, this yeast had spread to more than 30 countries, most reporting outbreaks (1). The emergence of C. auris has elicited a drive by WHO to track antifungal resistance in all *Candida* species and aiming to start an antifungal arm of the Global Antimicrobial Resistance and Use Surveillance System (GLASS).

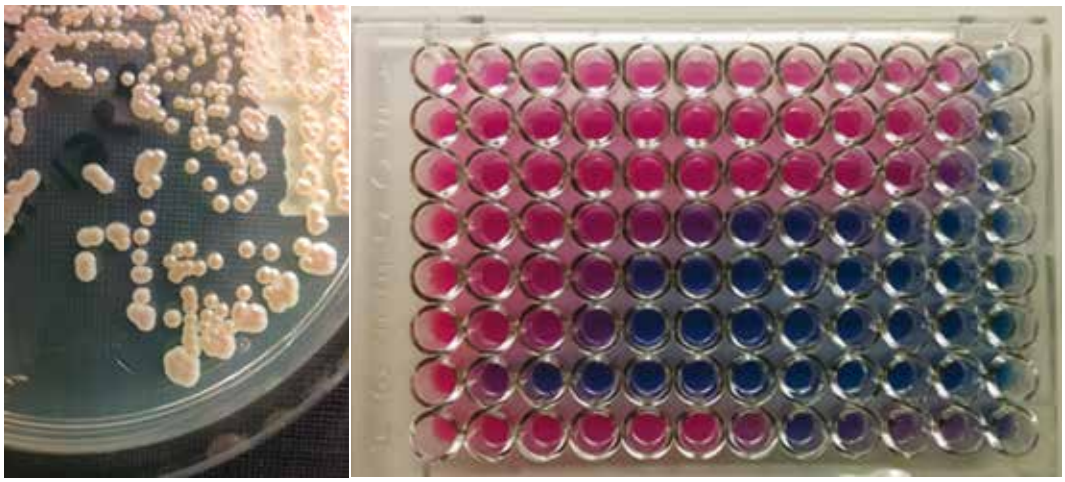


Figure: *Left*- Colonies of C. auris on BiGGY (Bismuth Sulfite Glucose Glycine Yeast) Agar. *Right*- antifungal susceptibility test by colorimetric broth microdilution method using YeastOne Sensititre® plate. Pink wells show growth while blue wells show inhibition of growth. This is an echinocandin resistant yeast showing extremely high MICs to all three echinocandins in the first three rows.

The impact of this surveillance on public health is obvious: identifying rising resistance in certain species will guide empiric antifungal treatment guidelines, provide data for development of better diagnostics and newer antifungals, and also inform prevention and control strategies by identifying populations, interventions, and regions at highest risk. Environmental surveillance led to detection of not only *Candida auris* in various environment niches including food sources, but other yeasts that have also been reported as uncommon fungal pathogens. Surveillance of pathogens and drug resistance is essential to track changing epidemiology, emerging pathogens, and resistance; this must remain a priority for public health.

Chronic Pulmonary Aspergillosis Diagnosis; role of Laboratory and Radiology in Improving case Detection

Drs Kauser Jabeen and Muhammad Irfan
Microbiology

Chronic pulmonary aspergillosis (CPA) is one of the common pulmonary disease caused by Aspergillus species. It has a variable clinical spectrum; most common entity is chronic cavitary pulmonary aspergillosis. Other forms are single aspergilloma (fungal ball), Aspergillus nodule, chronic fibrosing pulmonary aspergillosis and subacute invasive pulmonary aspergillosis. In contrast

to invasive pulmonary aspergillosis that affects immunocompromised patients, CPA mainly occurs in immunocompetent patients with existing or prior lung diseases. Pakistan has a huge burden of CPA with most cases occurring as a sequlae of tuberculosis (TB) and bronchiectasis. Aspergillus flavus and Aspergillus fumigatus are common species in CPA cases in Pakistan. Although CPA is associated with high morbidity and mortality, awareness and diagnostic capacity of CPA is limited in Pakistan. Improving diagnosis of CPA will lead to early case detection and prompt treatment leading to decreased complications and mortality.

Diagnosis of CPA

A combination of clinical, radiological and laboratory criteria is used for CPA diagnosis. Additionally, some important alternative diagnosis such as active TB is also excluded.

Clinical features: includes more than three months of hemoptysis, chest tightness, cough, shortness of breath, fatigue and weight loss.

Radiological features: chest imaging is also an important toll to diagnose CPA (Figure 1). CT chest is better than chest radiograph for CPA diagnosis. The typical radiological feature of CPA are presence of:

- more than one fungal balls, which are a complex mass of fungal hyphae mixed with extracellular matrix in an existing cavity in the lung or pleura or an ectatic bronchus. Single aspergilloma is the presence of a single fungal ball within a localized area with minimum symptoms
- pulmonary nodules. The size and number of these nodules can be variable
- multiple cavities is a very important feature of CPA. These cavities can be of variable size and may expand during the course of infection along with pericavitary fibrosis or infiltrates
- pleural thickening due to fibrosis of pleura and presence of extrapleural fat. It is a common finding in CPA

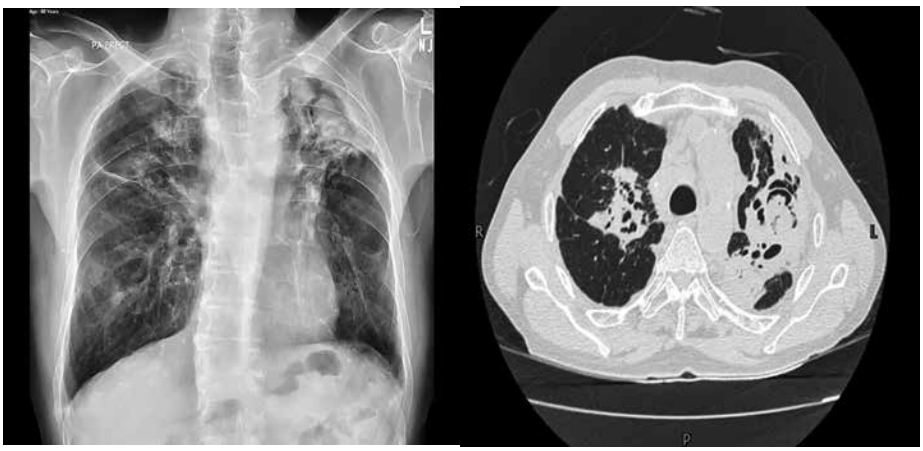


Figure 1: Chest radiograph and CT scan of a patient with chronic cavitary pulmonary aspergillosis. Panel A shows loss of lung volume, pleural thickening and fibrocavitary changes in the upper zone of left lung. Air crescent sign is also visible in this area. Fibrocavitary changes are also seen in the upper zone of right lung. Panel B shows pleural thickening, fibrocavitary changes and multiple aspergilloma in left lung and fibrocavitary changes in right lung.

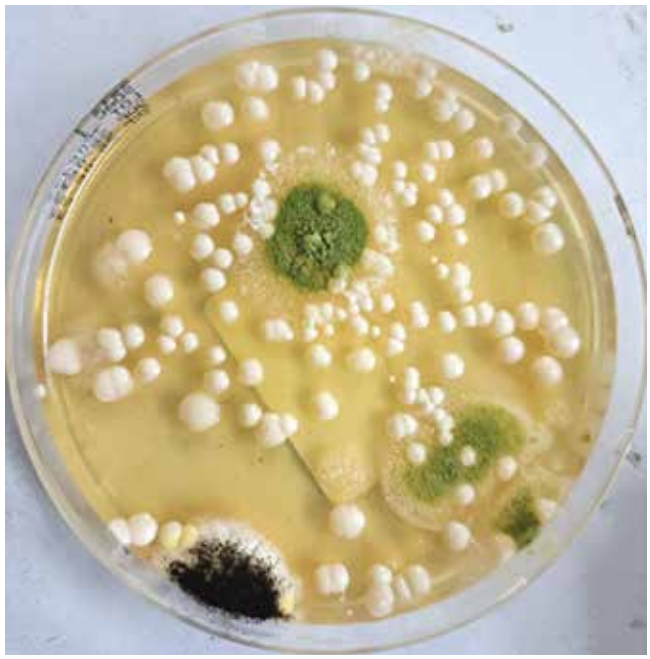


Figure 2: High volume culture of sputum showing colonies of Aspergillus flavus and Aspergillus niger

Microbiological evidence: The most helpful for CPA diagnosis is the presence of Aspergillus specific IgG in the presence of above clinical and radiological findings. This test has been recently introduced in the Aga Khan University laboratory. IgG antibodies to both Aspergillus flavus and Aspergillus fumigatus are reported. As significant geographical variations may exist in the cut-off for positivity, the Aga Khan University laboratory has developed and is reporting results on locally relevant cut-offs (Table 1). Detection of Aspergillus spp. in the sputum, tracheal aspirate

Table 1: Interpretation of Aspergillus flavus and Aspergillus fumigatus specific IgG results

Aspergillus IgG	Level (mg/L)	Interpretation
Aspergillus fumigatus IgG	<20	Negative
Aspergillus fumigatus IgG	≥20	Positive
Aspergillus flavus IgG	<30	Negative
Aspergillus flavus IgG	≥30	Positive

or bronchoalveolar lavage (BAL) by microscopy, culture or PCR is also a reliable diagnostic tool. On microscopy hyphae of Aspergillus spp. typically appear as septate with branching at acute angle. High volume cultures of respiratory specimens are now preferred over conventional culture due to its higher yield and are regularly performed at the Aga Khan University laboratory (Figure 2). Although galactomannan detection in BAL is useful for CPA diagnosis, serum galactomannan has a limited role and should not be requested. It is also important to rule out TB infection by Xpert MTB/Rif or TB culture.

Conclusion:

CPA diagnosis could be made by consistent clinical symptoms for more than three months duration, corresponding radiological findings and positive serum Aspergillus specific IgG. In settings where serum Aspergillus specific IgG is not available or if serum Aspergillus specific IgG is negative in patient with strong clinical suspicion high volume culture of respiratory specimens should be performed. TB should also be excluded in high TB endemic areas.

Molecular Screening for Non BCR ABL1 Myeloproliferative Neoplasms

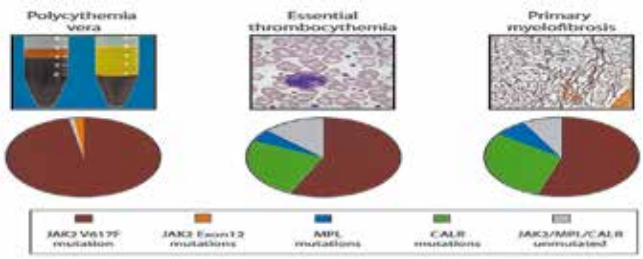
Ms Samra Naz, Anum Ujala and Dr Zeeshan Ansar Ahmed
Molecular Pathology

Introduction

Molecular screening investigations for the common MPN phenotype driver mutations (JAK2, CALR, MPL), usually performed on peripheral-blood DNA, are shown in Table I. These assays will identify a mutation in almost all patients with polycythaemia vera (PV) and 85–90 percent with essential thrombocythemia (ET) and primary myelofibrosis (PMF). Single-target assays may be employed sequentially but multiplex assays, typically using Multiple Ligation Probe Amplification (MLPA), with several targets in parallel and are more cost-effective. Either approach is acceptable if laboratory turnaround times and assay sensitivity are satisfactory, usually referred to as JAK2 V617F, and five percent VAF for JAK2 exon 12, CALR exon 9 or MPL exon 10 variants]. The use of broad myeloid NGS panels to screen cases with suspected MPN is unlikely to be cost-effective, but if larger panels are used, we recommend that the initial analysis and report should be limited to common MPN driver mutations (Table I). Universal reporting of mutant allele burden on diagnostic samples is not essential, although this should be considered were prognostically useful, e.g., suspected progression of PV to post-PV myelofibrosis (MF), or where demonstration of molecular response will be relevant. In patients with low-level JAK2 V617F and MPN phenotype, screening for CALR and MPL mutations should be carried out as these mutations may coexist. JAK2 V617F and CALR mutations may also coexist with BCR–ABL1, with such cases usually being identified following the persistence of thrombocytosis or other MPN features despite achievement of a good molecular response to tyrosine kinase inhibitor therapy for CML. Specific CALR mutations (type 1, 52-bp deletion; type 2, 5-bp insertion; type 1-like, and type 2-like)25 have prognostic significance in PMF and should be reported routinely.

Table I. Peripheral blood screening targets in suspected myeloproliferative neoplasms (MPN).

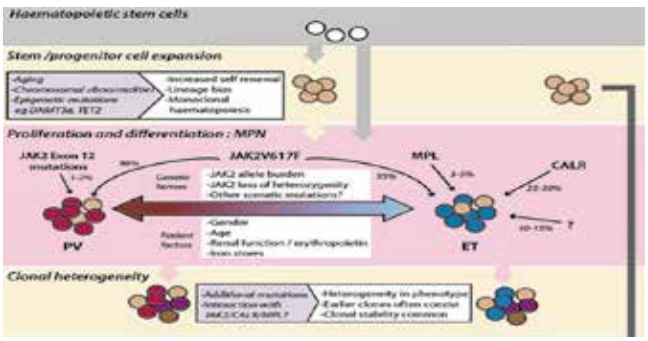
Presentation	Variant	Frequency
Erythrocytosis	*JAK2* V617F	96–97% PV
	JAK2 exon 12 mutations*	~3% PV
Thrombocytosis	*JAK2* V617F	50–60% ET
	CALR exon 9 mutation	25–30% ET
	MPL exon 10 mutation	3–11% ET
	BCR–ABL1 fusion	To exclude CML
Suspected primary myelofibrosis	*JAK2* V617F	50–60% PMF
	CALR exon 9 mutation	15–35% PMF
	MPL exon 10 mutation	6–9% PMF
Suspected chronic myeloid leukaemia	*BCR–ABL1* fusion	100% CML



Clinical context must be considered prior to performing screening assays. In patients with erythrocytosis or thrombocytosis, molecular screening investigations (Table I) are recommended in those with persistently and significantly elevated counts (haematocrit >0_52 l/l in males or >0_48 l/l in females; platelet count ≥450 9 109/l),3,4 after exclusion of secondary causes or where abnormalities are out of keeping with any possible secondary cause. Exclusion of BCR–ABL1 is important for all patients with thrombocytosis lacking a JAK2, CALR or MPL mutation or with atypical features (e.g., basophilia, left-shifted granulocytes, small hypo lobated megakaryocytes). JAK2 V617F is also found in healthy individuals, at increasing prevalence with older age ('clonal haematopoiesis', CH). Although CH is associated with increased risk of developing cardiovascular disease, there is no prospective evidence to guide management of most patients with normal or near-normal blood counts who harbour JAK2 V617F but do not fulfil diagnostic criteria for MPN, even if there are also abnormalities on bone marrow histology.

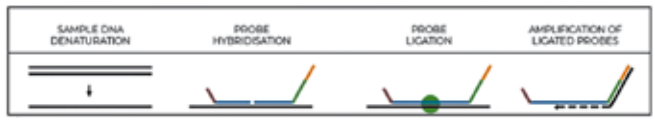
Importance of molecular markers in MPNs

The World Health Organization (WHO) diagnostic criteria were revised in 2017 to incorporate molecular markers such as JAK2V617F, JAK2 Exon 12, and MPL mutations as new major diagnostic criteria for MPNs. Screening for CALR mutations is now also part of the diagnostic workup of suspected ET or MF in many centres internationally and further revisions to WHO criteria are expected. In addition, a probe specific for the D816V mutation in the KIT gene (4q12) is present. This is the most common KIT mutation and is present in >90 percent of patients with systemic mastocytosis



(SM). Consequently, the presence of this mutation is considered a diagnostic criterion of SM according to the WHO classification.

The principle of MLPA is based on the amplification of up to 60 probes that each detect a specific DNA sequence of approximately 60 nt in length. The MLPA reaction results in a set of unique PCR amplicons between 64- 500 nt in length that are separated by capillary electrophoresis. After initial denaturation of the sample DNA, a mixture of MLPA probes is added to the sample. In general, each MLPA probe consists of two oligonucleotides that must hybridise to directly adjacent target sequences in order to be ligated into a single probe. During the subsequent PCR reaction, all ligated probes are amplified simultaneously using the same PCR primer pair, resulting in a set of unique PCR amplicons. One PCR primer is fluorescently labelled, enabling the amplification products to be visualised during fragment separation on a capillary electrophoresis instrument ABI 3500 Genetic Analyzer. Fragment separation yields a sample-specific electropherogram on Coffalyser.Net software used for analysis of MLPA experiments.



Concluding remarks and future perspectives

Our understanding of the genomic landscape of MPNs has leapt forward over the last decade. JAK2 mutation testing allows diagnosis of 99% of patients with PV, and combined testing for JAK2, MPL, and CALR mutations captures 85–90 percent of patients with ET and MF. The causative genetic lesions in 10–15 percent of ET and MF cases remains unknown at this stage and this subset may include patients with nonclonal myeloproliferation due to reactive causes, germline genetic aberrations, other myeloid disorders, or as-yet unidentified somatic mutations within the exome or genome. We are beginning to understand the prognostic impact of coexisting mutations in epigenetic or spliceosome regulators in MPNs, but whether this should affect treatment decisions remains to be tested in future clinical trials. Discovery of mutations in JAK2 and epigenetic regulators has led to the successful use of JAK inhibitors in MF and ongoing trials with multiple other novel agents, and the recent identification of mutant CALR has the potential for the development of a tumor- and genotype-specific targeted therapy.

Soft Tissue Sarcoma: Patient Centred Approach for Functional Outcome

Drs Madiha Bilal Qureshi, Tamana Asghari and Nasir ud Din
Histopathology

Sarcoma is a ***heterogeneous group*** of malignant tumors that arises from the connective tissue in any organ or anatomic location of the body. Approximately 80 percent of sarcomas originate from soft tissue and the rest from bone. The annual incidence is about 50 cases per one million population, however frequency increases in children. Around 10 percent of the patients have detectable metastatic disease at diagnosis of the primary tumor. Most common metastatic site is lung. About one-third of soft tissue sarcoma patients die from tumor-related disease, mostly from lung metastasis. Hence, their diagnosis and management are a challenging task.

Nearly 65 percent of soft tissue sarcomas are histologically diagnosed as Undifferentiated Pleomorphic Sarcoma, Liposarcoma, Leiomyosarcoma, Myxofibrosarcoma, Synovial Sarcoma or Malignant Peripheral Nerve Sheath tumor and majority are highly malignant. Management of such tumors requires a multidisciplinary team (surgeon, radiologist, histopathologist, medical and radiation oncologist), evidence-based practice and patient centered approach for functional outcome and quality of life.

A thorough clinical history, physical examination and imaging before pre-operative biopsy are necessary. All superficial soft tissue lesions with > 5 cm size and deep-seated lesions are more likely to be sarcoma. These patients should specifically be referred to a specialized tumor centre for diagnostic biopsy and expert pathology followed by multimodality treatment. Biopsy in this regard is mandatory to prove malignancy and determine histologic type, subtype and provisional grade of the tumor.

Trucut core biopsies taken through a single tract is a general recommendation for most limb and superficial trunk masses. If two or three good adequate cores are taken from varied tumor areas, the accuracy of diagnosis, grading and prognostication is high. In cases where trucut biopsy is not possible or non-diagnostic, incisional biopsy with minimal extension into adjacent soft tissue planes can be done. Excisional biopsy should be avoided specifically for >2cm lesions to prevent risk of contamination of surrounding tissue. Frozen sections and Fine needle aspiration cytology are not a general recommendation.

Correct histologic diagnosis is essential and a histopathologist with sarcoma expertise can ascertain accuracy of diagnosis. After assessment of size, histologic diagnosis with tumor grade and metastatic work up, a multidisciplinary team can plan the most effective treatment for the patient. The treatment plan ensures minimization of recurrence and preservation of patient's function and quality of life. Surgical resection is the primary therapeutic and potentially curative treatment in most soft tissue sarcoma cases. The role of adjuvant chemoradiation may vary depending upon individual patient and improves the risk of local and distant recurrence.

The total number of operations and local recurrence rate for primary tumors not referred to a specialized tumor centre or in patients referred after surgery is higher than patients before surgery. Primary unplanned resections and positive margin surgeries are associated with increased recurrence and decreased five-year recurrence free survival rate. Therefore, soft tissue sarcoma must be treated at a specialized tumor centre and should be referred before surgery.

The Aga Khan University Hospital has a specialized team to deal with soft tissue sarcomas including expert representatives in all areas. Utilization of expert knowledge and practice results in the most effective patient care.

AKUH Musculoskeletal Tumors Multidisciplinary Team

Above from left to right: Dr. Masood Umer (Sarcoma Specialist Surgeon), Dr. Nasir Ud Din (Expert Orthopedics Histopathologist), Dr. Bilal Mazhar Qureshi (Radiation Oncologist), Dr. Dawar Khan (Radiologist)
Below from left to right: Dr. Sadaf Altaf (Pediatric Oncologist), Dr. Mehwish Shahzadi (Medical Oncologist), Dr. Kiran Hilal (Radiologist)

Precision Therapy Unleashed: Unlocking Personalized Medicine with Therapeutic Drug Monitoring

Dr Muhammad Umer Naeem Effendi and Ms Tahira Parveen
Clinical Chemistry

Therapeutic drug monitoring (TDM) plays a vital role in personalized medicine, allowing healthcare professionals to optimize drug therapy based on individual patient characteristics. By measuring drug concentrations in a patient's blood or other bodily fluids, TDM provides valuable insights into drug efficacy, toxicity, and dose adjustments. This monitoring approach ensures that patients receive the right drug at the right dose, tailored to their specific needs.

Precision therapy, also known as personalized medicine, aims to deliver optimal treatment outcomes by considering individual variations in drug response. TDM aligns perfectly with this concept, as it enables clinicians to individualize drug therapy based on real-time patient data. Rather than relying solely on population-based dosing guidelines, TDM allows for the adjustment of drug dosages according to a patient's specific metabolism, genetic factors, drug-drug interactions, and other relevant variables.

One example of TDM's impact on precision therapy is seen in the field of oncology. Chemotherapy drugs

have a narrow therapeutic index, meaning that a small deviation from the optimal drug concentration can lead to either insufficient therapeutic effect or severe toxicity. By utilizing TDM, clinicians can precisely monitor the drug levels in a patient's body and make timely adjustments to the dosage, ensuring the optimal balance between efficacy and safety. Studies have demonstrated that TDM-guided chemotherapy dosing can significantly improve treatment outcomes, minimize side effects, and enhance patient quality of life.

Another area where TDM proves invaluable is in the management of psychiatric disorders. Drugs used to treat mental health conditions often exhibit substantial inter-individual variability in response due to factors such as genetic variations and drug interactions. Through TDM, clinicians can evaluate drug concentrations in a patient's system and fine-tune medication regimens accordingly. This personalized approach helps achieve therapeutic drug levels more consistently, enhancing treatment efficacy and reducing the risk of adverse effects.

In conclusion, therapeutic drug monitoring serves as a crucial tool in the advancement of precision therapy. By enabling clinicians to monitor drug concentrations and tailor treatment regimens based on individual patient characteristics, TDM enhances treatment outcomes, minimizes side effects, and improves patient satisfaction. As personalized medicine continues to evolve, the integration of TDM into clinical practice will play a pivotal role in optimizing drug therapy and moving towards a more precise and individualized approach to patient care.

THE BEST OF THE PAST

#Pathologists #Followtheirlead #changeagents

Interviewee: Dr. Zubair Ahmed
Interview Recorded by Dr Qurratulain Chundriger

Dr. Zubair Ahmad is one of the most senior faculty members in the Section of Histopathology. After completing his MBBS degree from Sindh Medical College (now known as Jinnah Sindh Medical University) Karachi in 1990, he joined the Section of Histopathology at AKUH as a trainee in 1996. Since then, he has been an integral part of the team and has been working with us as a full Professor. Dr. Zubair Ahmad is leaving us to join Sultan Qaboos Comprehensive Cancer Care and Research Center in the Sultanate of Oman. Here is a brief insight on his journey at AKUH.

1. **Considering your entire time as a Histopathologist at Aga Khan University, can you recall a time (any 'AHA' moment) when you felt most excited about your involvement in the organization?**

It has been sustained excitement. No single "aha" moment. Over 26 and a half years, I have always

felt very excited and thankful about my involvement with AKU especially with the Section of Histopathology, an exciting and dynamic place which has redefined histopathology in Pakistan and upgraded the practice of Histopathology to international standards. It continues to keep abreast of the latest advancements and protocols in reporting and has a leading national role in providing diagnostic and prognostic information about malignant tumors. Over the years, it has maintained its leading role as the best center for Histopathology in the country and those trained here (including myself) are serving nationally and internationally as consultant histopathologists in

leading centers around the world.

2. What was your inspiration to become a histopathologist? Please briefly share your initial phase of journey i.e., from medical graduate to consultant.

Two demonstrators in Med school in year three of MBBS who took amazing tutorials on systemic pathology (Dr. Ghous and Dr. Tanveer) totally fascinated me with their enthusiasm and passion. I am forever grateful to them. However, I still had no real clue about the practice of histopathology when I joined the residency at AKU. Initial years as a resident were extremely difficult. We were a small but close-knit group of residents who always helped each other and, in the process, became lifelong friends. We worked really hard and used to relax after six in the evening by going to cafeteria for tea and samosas. Sometimes we used to go outside. We helped each other in cases, in gross and on calls. There were no medical officers in those days, so we had to do calls in addition to carrying the daily workload. Also in those days, only histopathology residents used to do FNAs and so we were also on FNA call regularly. Sometimes our workload seemed like hell. But we survived with each other 's support and our determination, hard work and commitment. As we progressed, things became gradually better, but that hard work and perseverance has remained with me throughout and helped me immensely in my career.

3. Share your one moment when you felt most proud as a histopathologist.

There is no one moment when I felt most proud. I always feel proud to be a histopathologist, since histopathologists play an absolutely crucial role in diagnosis and subsequent management of severely ill patients. This is most true for malignant tumors. By providing accurate, timely diagnosis as well as reliable grading, staging and critical prognostic/ predictive information, histopathologists can play a major role in the management of patients with malignant tumors.

4. As one of the senior histopathologists of the country, please share your experience of development of Histopathology field in Pakistan.

I believe histopathology has developed by leaps and bounds in Pakistan. When I started my residency back in 1996, there were few centers, and the practice was quite old-fashioned. Now there are multiple centers throughout the country providing top class histopathology services. All these centers have faculty and consultants with diverse subspeciality interests, well trained technologists, good infrastructure and ancillary aids which are critically important in modern histopathology practice. However, I believe there are some areas which need attention. These include investment in training of technologists and incorporation of molecular pathology in routine histopathology practice. Training residents, consultants and technologists in basics of molecular pathology is important. Digital and remote pathology including whole slide imaging and incorporation of artificial intelligence in histopathology are also essential in the coming years. Adoption of these can help in democratization of care, while histopathologists can designate more time for difficult and challenging cases

5. Any advice for young histopathologists?

Hard work is absolutely essential. Histopathology is a vast and dynamic field, and it is advancing at a frenetic pace. There are no shortcuts. You need to read and acquire knowledge and then learn to apply the knowledge by seeing as many cases as you can. Now there are so many sources which can be accessed from the Internet such as Pathology Outlines and many many more which the residents know much better than me. Decide on two or three subspeciality interests in the later years of your training and focus on those. As more and more ancillary aids are available, always keep in mind that the gold standard for reaching a differential in difficult cases is still the routine H&E stain. You do additional workup after reaching a differential diagnosis on routine H&E slides. I advise residents and trainees and the young consultants to develop themselves through conferences, meetings, workshops etc. in molecular pathology and artificial intelligence so that they can use these tools in practicing top notch histopathologists in the coming era.
And please be active in research. There is so much archived material here (the Section of Histopathology at AKU is, in my opinion, a national treasure) that it's a shame if we don't publish. The material is more than what you have in the biggest centers in the US. So please utilize this treasure while you can.

THE BEST OF THE PAST

Radiologist # Bodyimaging #Followtheirlead

Interviewee: Dr. Wasim Memon
Interview recorded by Dr. Shayan Anwar

1. **Considering your entire time as Body Imaging Consultant (diagnostic + intervention) 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?**

I can recall the time when I was back from UK after doing my observer ship in oncology and chest imaging. I translocated CT workstation from CT room and started reporting on the console directly, just like we do on PACS now a days, as at that time we were reporting on hard films and there was no PACS available.
Initially everyone resisted but ultimately, they were convinced that reporting on console is much superior to films.

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

Graduated from SMC in 1992.
During my house job I cleared Federal commission and joined as medical officer in NICH, did two years pediatric anesthesia (as was only vacant position in anesthesia) and ultimately transferred to radiology department and did my FCPS-I and in 1996 joined here in AKUH as resident. Went back to rejoin NICH after completion of my residency, resigned from government job, joined back as instructor here again in 2001, since then I am serving AKUH.

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?**

Empathy and honesty, I think these are two important and basic things for anyone in our profession, without

these you can't fulfill the oath you took as a doctor.

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

I think, things are changing, people are now aware of importance of sub-specialty as world is moving towards super-specialty, I am glad we have started body imaging fellowship here in AKU. Also, RSP has started body imaging fellowship in other parts of country. I think it's just a start, very much optimistic about the future of sub-specialty in radiology in next 10 years.

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

Work hard, be honest and empathic. Try to select any subspecialty of your interest and excel to make your institution and country proud.

HAPPENINGS IN PATHOLOGY

Unraveling the AKU Department of Pathology and Laboratory Medicine's Online Universe - a Report by the Departmental PathCom Committee

Ms. Noorshah Somani, Drs Sibtain Ahmed, Joveria Farooqi, Asghar Nasir, Hasan Hayat, Sarosh Moeen, and Ms. Shamsha Punjwani
Department of Pathology and Laboratory Medicine, AKU

The Aga Khan University (AKU) Department of Pathology and Laboratory Medicine is at the forefront of cutting-edge research, diagnostic services, and education in the field. The department's website **(https://www.aku.edu/mcpk/pathology/Pages/home.aspx)** serves as a comprehensive resource hub, providing a wealth of information on various aspects of pathology and lab medicine. Visitors can access details about the department's faculty, research projects, specialized services, and educational programs. The website also offers a repository of publications, allowing healthcare professionals and researchers to stay updated with the latest breakthroughs and scientific advancements.

Specifically the webpage, showcases four key components that represent the ongoing activities: Achievement Unlocked, Happenings, Diagnostic Services, and Scholarly Activities. These components offer detailed information on various aspects such as Continuing Medical Education (CME) events, upcoming departmental events, audits and accreditations, current developments within the department, a collection of memories from senior faculty members, a monthly departmental blog for sharing diverse perspectives. Test in focus videos highlight specialized tests performed in our state of the art laboratories with updates from our highly experienced and expert faculty members.

To further engage with the medical community, patients, and the public, the department maintains an active presence on Twitter. The Twitter handle, **@pathlabcomms**, serves as a platform for sharing timely updates, research findings, educational resources, and events related to pathology and lab medicine. It fosters meaningful conversations, facilitates knowledge exchange, and strengthens professional networks within the field.

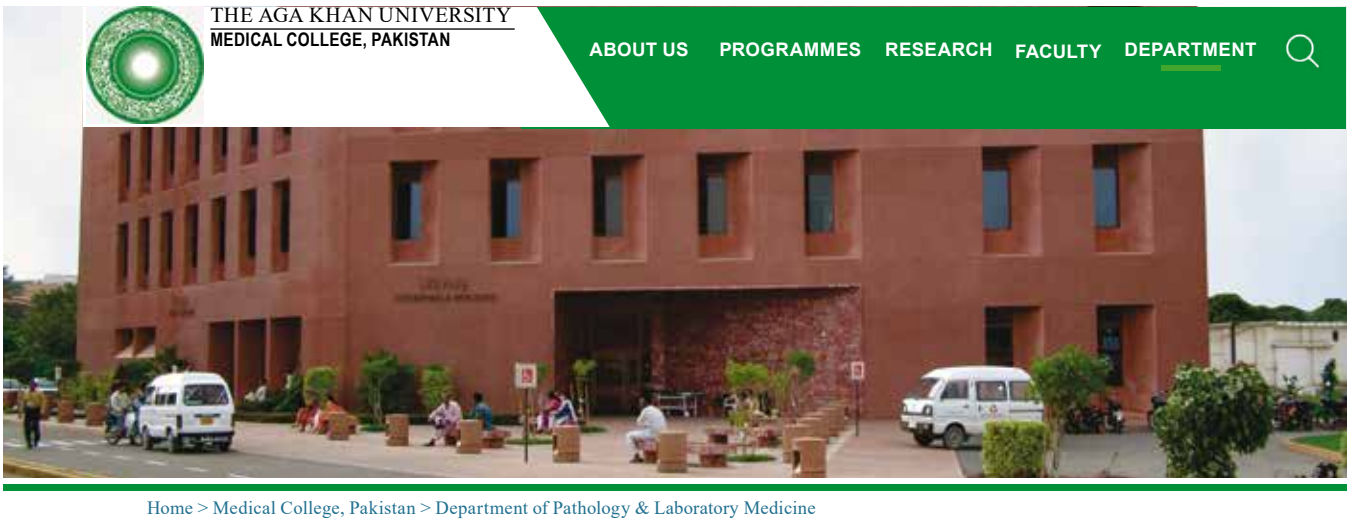


Figure 01: Website homepage

Figure 02: Twitter handle

Polaroid

Radiology

Exciting News! Aga Khan University Hospital, PK, is undergoing an incredible transformation from PACS (picture archiving and communication system) to Enterprise Imaging for its Radiology Services. Enterprise Imaging is a cutting-edge solution known for revolutionizing healthcare image management. ✨

Dr Farhat Abbas, the Interim CEO of AKUH, along with Dr Zafar Sajjad (chair radiology) and Dr Nadeem Ahmad (ex-chair radiology), recently met with Mustafa Hamdi, the Regional SM Middle East at Agfa Healthcare, and their team to kickstart this exciting project. 🏢💻

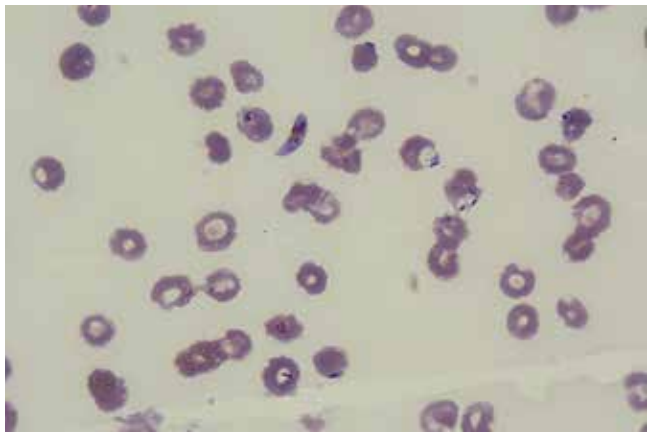
Chemistry

The urea breath test (UBT) is significant for diagnosing Helicobacter pylori infections, a common cause of gastric ulcers and other digestive disorders. The picture above shows a technologist conducting UBT on a patient. If the patient has a Helicobacter pylori infection, the bacterium will produce an enzyme called urease, which breaks down the urea into carbon dioxide, which is exhaled through the breath, collected in an airbag and analyzed using infrared spectroscopy.

The Urine toxicology test is performed for testing drug of abuse (Amphetamine, Alcohol, Barbiturate, Benzodiazepine, Cannabinoids, Cocaine, Opioids). The picture above shows the process of testing, including oxidant test, done by dip strip method to evaluate sample integrity (adulterant detection), then screening test is performed by enzyme-multiplied immunoassay technique. To ensure accuracy and eliminate the possibility of false positive results, it is essential to conduct a reflex confirmatory test following a positive initial test. The AKU is dedicated to enhancing patient care and, therefore, has validated the recently acquired LC-MS/MS technique, depicted in the accompanying image, for the detection of abused drugs and their derivatives/metabolites, if present.

Hematology

Haematology resident analyzing hemoglobin variant studies for haemoglobinopathies



Haematology resident is screening malarial parasite on microscopic examination of peripheral blood smear.

Histopathology

We have an experienced faculty conducting complexed cases of lymphoma received in our section of histopathology
Dr Shahid pervez and Arsalan Ahmed conducting Lymphoma difficult case conference.

Twice a week, the team of breast and gynecological pathologists sits together to discuss difficult cases. The team includes Dr. Naila Kayani, Dr. Romana Idrees, Dr. Qurratulain Chundriger, Dr. Saba Anjum and Dr. Ummiya Tahir. This activity has also proved to be of value for the residents, as they learn from the most senior experts in this subject in Pakistan

The section of Histopathology bade farewell to one of the most senior faculty members, Dr. Zubair Ahmad, who left AKU after a tenure of more than 30 years. From left to right the picture shows Dr. Arsalan Ahmed (section head Histopathology), Dr. Afia Zafar (microbiology), Dr. Zubair Ahmad, Dr. Salman Adil (Hematology), Dr. Erum Khan (Chair), Dr. Seema Khan (Microbiology), Dr. Fazal Hameed Khan (associate dean for faculty) and Ms. Shamsha Panjwani (admin).

Microbiology

Rapid method for the detection of Chlamydia trachomatis and Neisseria gonorrhea in urine specimen through multiplex PCR.

Molecular Pathology

Clinical Lab

آغا خان یونیورسٹی ہسپتال
The Aga Khan University Hospital

Congratulations!

The Aga Khan University Hospital's 300th Lab Collection Point

Global Medical Laboratory Professional's Week Celebrations

Our Department of Pathology and Laboratory Medicine celebrated Global Medical Laboratory Professional's Week to raise awareness about laboratory's impact on patient's diagnosis and care and to appreciate the team behind it.

TESTS IN FOCUS

Therapeutic Drug Monitoring Information for Clinicians

Drug	Sample type	Time of Sample Collection	Therapeutic Ranges		
Valproic Acid		Trough	50 - 100 µg/ml		
Tegretol		Trough	4-12 µg/ml		
Phenytoin		Trough	10-20 ug/ml		
Phenobarbital		Trough	10-40 ug/ml		
Gentamicin		Peak testing (exactly 1 h after start of infusion of 3rd dose, or 1st dose in critically ill patients) Trough	**Adults Multiple daily dose** Peak: 4-10 ug/ml Trough: 1-2 ug/ml	**Adult Once daily dose** Trough <1 ug/ml	**Pediatrics trough conc.:** <2ug/ml
Vancomycin		Trough concentrations should be obtained prior to the fourth or fifth dose.	Therapeutic concentration >10 ug/ml	Trough 15-20 ug/ml	
Amikacin		Peak testing (exactly 1 h after start of infusion of 3rd dose, or 1st dose in critically ill patients) Trough	**Amikacin-Once daily dose** Trough = <1.0 ug/ml Peak = 56-64 ug/ml	**Amikacin-Multiple daily dose** Trough = 5-10 ug/ml Peak = 15-30 ug/ml	
Cyclosporin		Trough	**Post renal transplants:** Upto 2 months 200-300 ng/ml 3 months 200 ng/ml 4-12 months 80-180 ng/ml	**Post liver transplants:** ≤ 1 month 350-450 ng/ml 2-6 months 250-350 ng/ml >6 months 170-240 ng/ml	**Post bone marrow transplants:** First 3-4 weeks 200-300 ng/ml Until 3 months 100-200 ng/ml
Tacrolimus		Trough	Target range for trough sample 3-20 ng/ml	Toxic >20 ng/ml	
Methotrexate		Trough or sample collected after 72 hrs of therapy	Non-toxic concentration <0.1 umol/l		
Lithium		Trough (Predose or after 12 hours post dose)	Therapeutic Range: 0.6 - 1.2 mmol/l	Toxic: >2.0mmol/l	
Theophylline		Peak testing (after 2-4 hours post dose) Trough	Therapeutic Range 10-20 ug/ml		
Digoxin		Peak (after 8 hours post dose) Trough	Therapeutic Range 0.6-1.2 ng/ml	Toxic >2.0ng/ml	
Special instruction: Mention drug dosing frequency, route and sample is trough or peak. Mention STAT on the request slip if results are needed shortly.					

- Where trough is predose sample

hospitals.aku.edu/Karachi/clinical-laboratories