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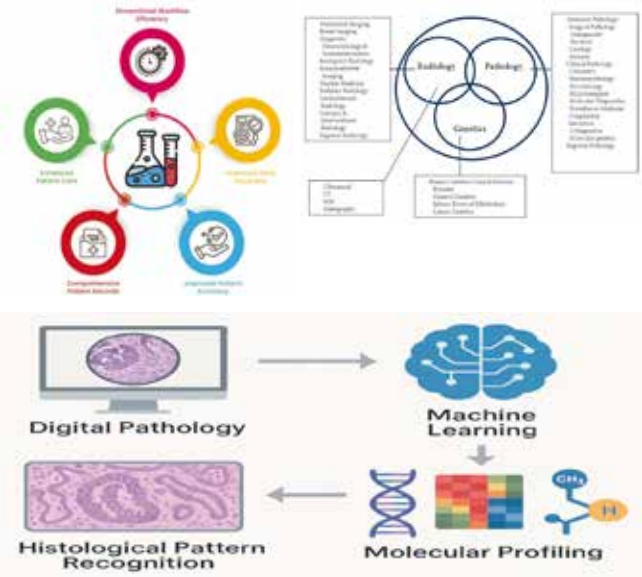
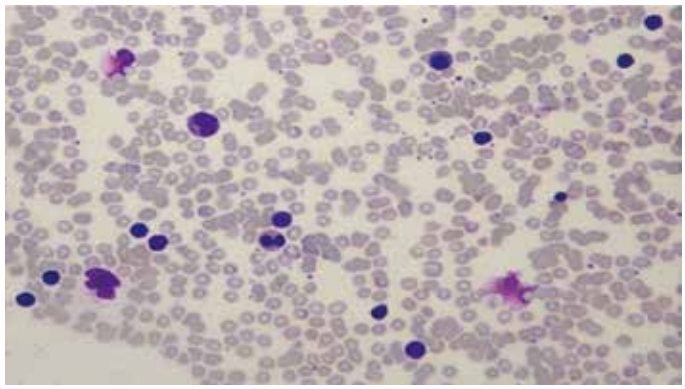
Molecular Integration in Diagnostic Medicine



Integrating Molecular Testing in Diagnosing Rare Childhood Infections



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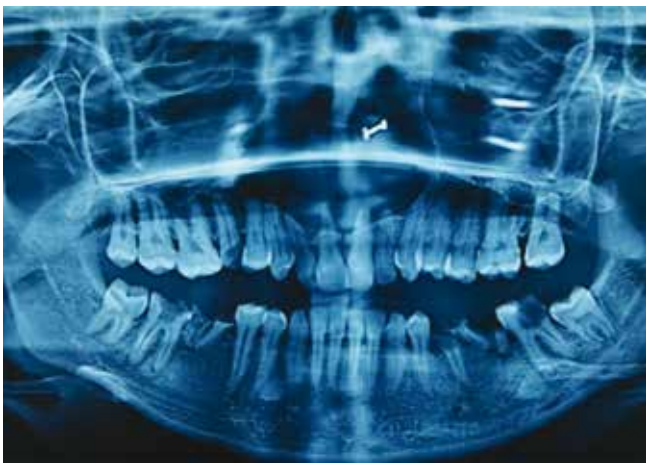

The Breast Imaging Team

آغا خان یونیورسٹی ہسپتال، کراچی

The Aga Khan University Hospital, Karachi

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EDITORIAL

Embracing the Power of Integrated Diagnostics

In today's rapidly evolving healthcare landscape, "Integrated Diagnostics" is no longer a distant concept — it is fast becoming the new standard of excellence.

This shift is not just about technology; it's about teamwork. Pathologists, radiologists, clinicians, and IT professionals must collaborate to deliver faster and more accurate diagnoses. In a system challenged by increasing demand and limited resources, integration offers a path to greater efficiency, fewer errors, and earlier, more informed decision-making.

This edition features insightful articles on molecular integration in diagnostics, including discussions on rare childhood infections and multiple myeloma. It also emphasizes the importance of radiology-pathology correlation, particularly in bone pathology, with a focused look at odontogenic cysts.

Let us commit to shaping a future where diagnostics is driven not by isolated insight but by collaborative intelligence - where molecular data, pathology, and imaging come together in harmony.

Together, we diagnose better.

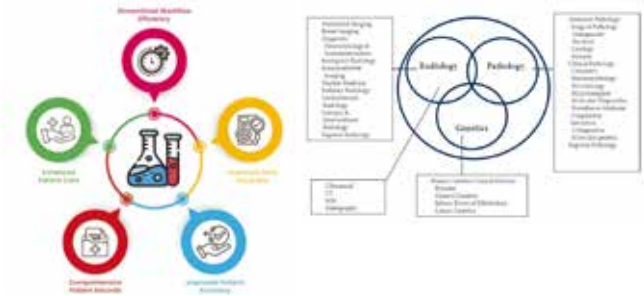
Warm regards,
Sidra Arshad

Molecular Integration in Diagnostic Medicine:
The Convergence of Laboratory and Imaging Disciplines Toward Precision Healthcare

Dr Zeeshan Ansar, Anum Ujala and Nazneen Islam, Molecular Pathology

1. Introduction

The growing field of molecular diagnostics has become a cornerstone of modern medical diagnostics, offering precise insights into disease mechanisms at the genetic and molecular level. Its integration with core diagnostic specialties—including Hematopathology, Histopathology, Chemical Pathology, Microbiology, and Radiology—has not only improved diagnostic accuracy but also enhanced patient stratification and therapeutic planning. This manuscript explores the status of this molecular integration, real-world applications, interdisciplinary challenges, and the transformative potential for future precision medicine and unified diagnostic ecosystems medical diagnostics has historically been compartmentalized, with laboratory disciplines and radiological imaging operating in parallel but separate silos. The rise of molecular diagnostics, however, has begun to dissolve these boundaries, offering a molecular lens through which all diagnostic data—morphological, biochemical, microbiological, and radiological—can be interpreted. The increasing demand for personalized and predictive medicine has accelerated the need for integrated, multidimensional diagnostic frameworks that combine phenotypic, genotypic, and imaging data.



Benefits of Diagnostic Integration

2. Discipline-Specific Molecular Integratio

2.1 Hematopathology:

Hematopathology is a unique field that encompasses both anatomic and clinical pathology while also using molecular technology and flow cytometric analysis. Along with flow cytometry, hematopathologists utilize various other tools and technologies—such as histology, immunohistochemistry, cytogenetics, DNA sequencing, and other molecular analysis—to make diagnoses and predict responses to therapy.

Applications: Molecular diagnostics plays a vital role in diagnosing leukemias, lymphomas, and myeloproliferative neoplasms. Techniques like FISH, RT-PCR, and NGS detect chromosomal translocations (e.g., BCR-ABL1, PML-RARA), gene mutations (JAK2, CALR, MPL), and MRD (Minimal Residual Disease) markers.

Table 1. Integrated Hematopathology and Molecular Markers

Hematologic Malignancy / Disorder	Key Molecular Markers	Diagnostic Role	Prognostic Value	Therapeutic Implication
Chronic Myeloid Leukemia (CML)	BCR-ABL1 (t(9;22), Philadelphia chromosome)	Confirms diagnosis	High prognostic value for disease monitoring	TKIs (e.g., imatinib, dasatinib)
Acute Promyelocytic Leukemia (APL)	PML-RARA (t(15;17)	Pathognomonic for APL	Predicts excellent prognosis with treatment	ATRA + arsenic trioxide
Acute Myeloid Leukemia (AML)	FLT3, NPM1, CEBPA, IDH1/2, RUNX1, TP53	Refines diagnosis and classification	Risk stratification based on mutation combinations	FLT3 inhibitors, IDH inhibitors
Acute Lymphoblastic Leukemia (ALL)	BCR-ABL1, ETV6-RUNX1, TCF3-PBX1, IKZF1 deletions	Molecular subtyping	Prognostic relevance varies by subtype	Targeted therapies (e.g., TKI in Ph+ ALL)
Myelodysplastic Syndromes (MDS)	SF3B1, TET2, ASXL1, TP53, DNMT3A	Aids in clonal identification	Mutation burden correlates with risk stratification	Clinical trials, potential for targeted agents
Myeloproliferative Neoplasms (MPNs)	JAK2 V617F, CALR, MPL	Confirms diagnosis	Type of mutation influences prognosis	JAK inhibitors (e.g., ruxolitinib)
Chronic Lymphocytic Leukemia (CLL)	IGHV mutation status, TP53, del(17p), NOTCH1, SF3B1	Defines risk and treatment stratification	TP53/del(17p) linked to poor prognosis	BTK inhibitors, BCL-2 inhibitors
Non-Hodgkin Lymphoma (NHL)	MYC, BCL2, BCL6 rearrangements	Classification (e.g., double/triple hit lymphomas)	Aggressive course with MYC/BCL2/BCL6 co-rearrangements	Intensive chemotherapy, targeted therapies
Multiple Myeloma	t(4;14), t(11;14), del(17p), gain(1q), RAS, BRAF	Stratifies molecular subtypes	Cytogenetics determine risk category	Proteasome inhibitors, immunomodulators, targeted Rx
Hairy Cell Leukemia	BRAF V600E	Pathognomonic mutation	Favourable prognosis with targeted therapy	BRAF inhibitors (e.g., vemurafenib)
T-cell Neoplasms	STAT3, RHOA, IDH2, TET2	Emerging diagnostic and prognostic utility	Under research	Potential for targeted/ epigenetic therapies

2.2 Histopathology:

Histopathology, the microscopic examination of tissues to diagnose and study disease, has been revolutionized by the integration of molecular techniques, offering unprecedented insights into disease mechanisms and enabling more precise and personalized diagnostic and therapeutic strategies. Applications: Molecular pathology augments traditional histology by offering mutation analysis, gene expression profiling, and epigenetic markers.

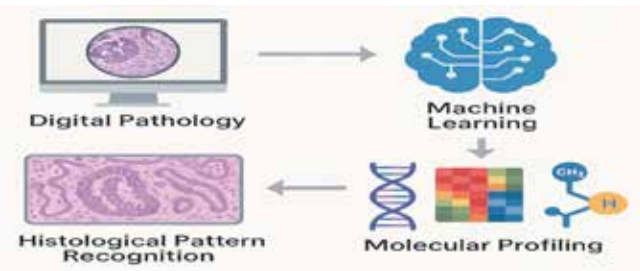
Table 2. Integrated Histopathology, IHC, and Molecular Markers

Tumor Type / Pathology	Key Histopathological Features	IHC Markers	Molecular Markers	Clinical Significance
Gliomas (CNS Tumors)	Diffuse infiltration, necrosis, mitoses	GFAP, IDH1-R132H, ATRX, p53	IDH1/2, TERT, 1p/19q codeletion, MGMT	Molecular subtyping (astrocytoma vs. oligodendroglioma), treatment response (e.g., TMZ)
Colorectal Adenocarcinoma	Glandular formations, dirty necrosis	CK20+, CDX2+, MMR proteins (MLH1, MSH2, etc.)	KRAS, NRAS, BRAF, MSI	Predicts response to EGFR inhibitors and immunotherapy

Tumor Type / Pathology	Key Histopathological Features	IHC Markers	Molecular Markers	Clinical Significance
Breast Carcinoma	Ductal/lobular patterns, nuclear atypia	ER, PR, HER2, Ki-67	PIK3CA, BRCA1/2, HER2 amplification	Defines subtypes (luminal, HER2+, TNBC), guides hormonal and targeted therapy
Lung Adenocarcinoma	Glandular/acinar, papillary structures	TTF-1+, Napsin A+, CK7+	EGFR, ALK, KRAS, ROS1, MET, BRAF	Targeted therapy selection (e.g., EGFR TKIs, ALK inhibitors)
Prostate Adenocarcinoma	Small acini, perineural invasion	PSA+, PSAP+, NKX3.1+, AMACR+	TMPRSS2-ERG fusion, PTEN loss	Molecular markers assist in prognosis and potential targeted therapies
Endometrial Carcinoma	Glandular crowding, nuclear atypia	ER, PR, p53, MMR proteins	PTEN, PIK3CA, ARID1A, MSI	Prognostic classification (Type I vs. Type II), MSI linked to immunotherapy response
Lymphomas (DLBCL, FL, etc.)	Sheets of large lymphoid cells, follicular patterns	CD20, BCL2, BCL6, Ki-67, MUM1, CD10	MYC, BCL2, BCL6 rearrangements	IHC + FISH distinguish aggressive subtypes; impact on therapy and prognosis
Melanoma	Atypical melanocytes, pagetoid spread	S100, HMB-45, Melan-A, SOX10	BRAF V600E, NRAS, KIT	BRAF mutation guides use of BRAF/MEK inhibitors
GIST (Gastrointestinal Stromal Tumor)	Spindle or epithelioid cells	DOG1+, CD117+, CD34+	KIT, PDGFRA mutations	Predictive of response to imatinib and other TKIs
Thyroid Carcinoma (Papillary)	Nuclear grooves, Orphan Annie nuclei	TTF-1+, thyroglobulin+, BRAF V600E (by IHC)	BRAF, RET/PTC, NTRK, RAS	Affects prognosis and eligibility for targeted therapies
Ewing Sarcoma	Small round blue cell tumor	CD99+, FLI1+	EWSR1-FLI1 translocation	Diagnostic confirmation by molecular analysis (FISH, RT-PCR)
Soft Tissue Sarcomas (e.g., Synovial)	Biphasic/spindle cell patterns	TLE1+, EMA+, Cytokeratin+	SYT-SSX fusion (t(X;18))	Confirms diagnosis and informs prognosis

Technologies: Immunohistochemistry (IHC) with molecular confirmation (e.g., IDH1/2 in gliomas, TP53 in breast cancer), FISH for gene rearrangements (ALK, ROS1), and sequencing panels for actionable mutations.

Integration with AI: Digital pathology and machine learning allow histological pattern recognition aligned with molecular profiles.



Example: Identification of EGFR, ALK, or KRAS mutations in lung adenocarcinoma for targeted therapy.

2.3 Chemical Pathology

Applications: Biochemical parameters are now frequently correlated with molecular profiles.

Pharmacogenetics is a growing area, identifying SNPs that influence drug metabolism (e.g., CYP450 polymorphisms).

Emerging Tools: Integration of metabolomics with genomics in metabolic disorders, familial hypercholesterolemia gene testing, and molecular endocrinology (e.g., RET mutations in MEN syndromes).

Molecular Techniques Used in Chemical Pathology

Technique	Purpose	Applications in Chemical Pathology
PCR (Polymerase Chain Reaction)	Amplification of DNA/RNA	Mutation detection (e.g., RET, CYP genes)
qPCR (Real-Time PCR)	Quantification of gene expression	Viral load, gene dosage studies
Sanger Sequencing	Mutation analysis	Single gene disorders
Next-Generation Sequencing (NGS)	Parallel sequencing of multiple genes	Panels for inborn errors, pharmacogenomics cancer genetics
Microarray	SNP and expression profiling	Pharmacogenetic Screening, tumor classification

Example: Molecular confirmation of inborn errors of

metabolism suspected from abnormal biochemical profiles.

2.4 Microbiology

Applications: Molecular microbiology allows rapid pathogen identification, antimicrobial resistance detection, and genotyping for epidemiology.

Technologies: Multiplex PCR panels (e.g., for meningitis, respiratory viruses), whole-genome sequencing (WGS), CRISPR-based detection (e.g., SHERLOCK), and metagenomics.

Key Molecular Techniques in Microbiology

Technique	Purpose	Common Use
PCR (Polymerase Chain Reaction)	Amplifies DNA for pathogen detection	TB, MRSA, viral diagnostics
Real-Time PCR (qPCR)	Quantifies nucleic acid in real-time	Viral load (HIV, HBV), COVID-19
Multiplex PCR	Simultaneous detection of multiple pathogens	Respiratory virus panels, meningitis panels
Next-Generation Sequencing (NGS)	High-throughput sequencing of microbial genomes	Pathogen discovery, outbreak tracking, AMR gene surveillance
DNA Microarray	Detects multiple gene targets in parallel	Detection of resistance genes or microbial

Impact: Faster turnaround times than culture, higher sensitivity, and better outbreak management.

Example: Detection of Mycobacterium tuberculosis and rifampin resistance using GeneXpert.

2.5 Integration of Constitutional Cytogenetics with Radiological Findings

Constitutional cytogenetic blood analysis, which examines chromosomes for abnormalities, is often integrated with radiological findings to provide a more comprehensive picture of a patient's condition. This integration can help and confirm suspected diagnoses, identify the cause of specific clinical presentations, and guide genetic counselling and management. For example, a cytogenetic abnormality like Down syndrome, identified through blood analysis, may be correlated with characteristic features seen on ultrasound or other imaging modalities.

3. Future Directions

3.1 Unified Molecular Diagnostic Platforms

Integrated platforms combining molecular, biochemical, and imaging data within a single ecosystem using AI-assisted interpretation (e.g., molecular tumor boards).

3.2 Liquid Biopsy and Non-Invasive Diagnostics

Detection of circulating tumor DNA (ctDNA), microRNAs, and exosomes for real-time monitoring and early cancer detection.

3.3 Artificial Intelligence and Big Data

Deep learning models will enable pattern recognition from integrated datasets (radiology + pathology + genomics), enabling predictive diagnostics.

3.4 Personalized Preventive Medicine

Risk prediction based on polygenic risk scores, microbiome profiling, and environmental exposures—customized screening and intervention plans.

3.5 Training and Education

Cross-disciplinary training in molecular diagnostics for all specialties is essential to drive integration.

4. Conclusion

The integration of molecular diagnostics across core diagnostic disciplines is ushering in a new era of precision medicine. As we move toward holistic, systems-based diagnostics, the convergence of hematopathology, histopathology, chemical pathology, microbiology, and radiology will redefine how diseases are diagnosed, monitored, and treated. Future efforts must focus on breaking disciplinary silos, standardizing practices, and leveraging AI and big data to achieve truly integrated diagnostics for individualized patient care.

Diagnosis of Odontogenic Cysts:
Integrated Approach; Radiology-Pathology Correlation

Dr Taha Nafees, Dr Alka Rani and Dr Madiha Bilal Qureshi, Histopathology

A cyst is a cavity lined by epithelium and contains fluid or semi fluid. Odontogenic cysts are those cysts that are derived from the remnants of tooth forming tissues. Odontogenic cysts are classified into inflammatory and developmental types. The most common inflammatory odontogenic cyst is Radicular cyst whereas the most common developmental odontogenic cyst is Dentigerous cyst, followed by Odontogenic keratocyst.

Most of the odontogenic cysts radiologically appear as well defined, unilocular radiolucent swellings (Figure 1). These general non-specific radiologic features are not very helpful to diagnose the cyst type. However, the association with the type and part of the tooth seen on radiology is very important in diagnosis. Information regarding the vitality of the involved tooth and its spatial relationship with the cyst is helpful in this respect as well.

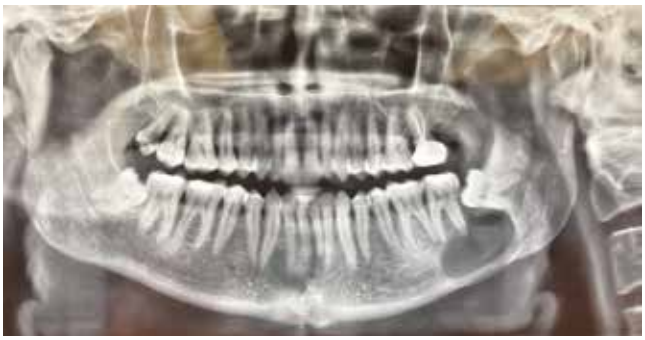


Figure 1: A well-defined unilocular radiolucent cyst associated with the root of lower left 2nd molar

Radicular cyst is most commonly associated with non-vital tooth and involves the root part of tooth (Figure 2), whereas Dentigerous cyst encircles the crown and is associated with unerupted tooth (Figure 3). Both of these cysts show similar histologic features and are distinguished on the basis of radiolographic findings only. Hence provision of preoperative Orthopantomogram (OPG) X-ray is necessary to distinguish between the two. With the presence of pre-operative OPG, most of the odontogenic lesions are easily classified.

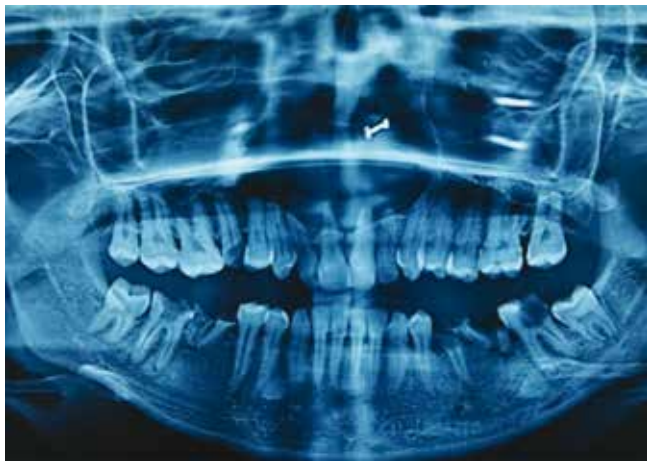


Figure 2: A well-defined unilocular anterior maxillary cyst associated with the roots of multiple upper midline teeth; Radicular Cyst

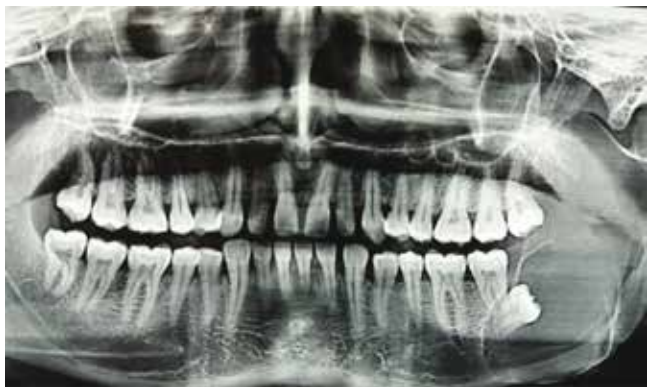


Figure 3: A well-defined unilocular lower mandibular cyst associated with the crown of left third molar unerupted tooth; Dentigerous Cyst

Other odontogenic cysts like Odontogenic keratocyst and Orthokeratinized Odontogenic cyst may have similar radiologic findings. Moreover, Unicystic ameloblastoma has cystic change and shows identical radiological features. Specific histologic features in odontogenic cysts/ lesions determine the exact type of cyst/lesion. The histologic features provide diagnosis that leads to appropriate treatment and management. Hence biopsy of the cyst along with preoperative OPG remains essential to classify such lesions.

Radiologic Correlation in Bone Pathology Diagnosis - Essential Aspect

Fatima Safdar, Anwar Ahmed, Madiha Bilal Qureshi

Bone lesions are broadly divided into primary benign and malignant tumors, metastatic tumors, inflammation (osteomyelitis), and pseudo-tumors. The role of conventional radiography in the initial evaluation of bone lesions is highly significant, even in the era of advanced imaging technologies. Plain X-ray (AP/Lateral views) gives substantial information about the lesion. Correct diagnosis of bone pathology requires complete clinical details including patient's age, clinical symptoms, site, radiological characteristics of lesion (location, focality, density, zone of transition, cortical break, periosteal reaction and type of matrix) and histologic evaluation. Moreover, follow-up or management of the lesions is also assessed by radiological characteristics.

Bone tumors commonly involve young patients with typical history of pain, swelling or fracture. The location of the lesion within bone is extremely important since certain tumors have potential for origin in certain parts of the bone (epiphysis, metaphysis and diaphysis; cortex, medulla and surface). Giant cell tumor of bone commonly occurs after the growth plate fusion typically arises at the meta-epiphyseal region, Ewing sarcoma involves the diaphyseal region and osteosarcoma involves the metadiaphysis of long bones. (Figure 1). Lesions with narrow zone of transition and defined margins are usually benign on histology (Figure 2) and those with wide zone of transition, cortical break and periosteal reaction are likely to be malignant (Figure 3). Osteoid matrix and chondroid matrix can also be determined by skillful radiological expertise that helps to determine the origin of tumor.

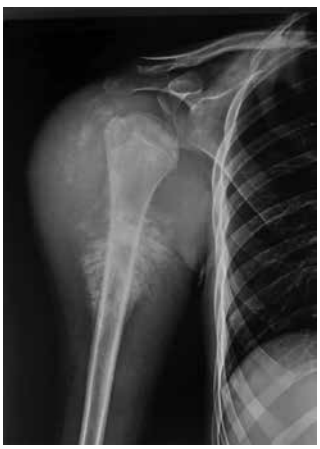


Figure 1: Aggressive mixed density lesion involving metadiapyseal region of right humerus; Osteosarcoma

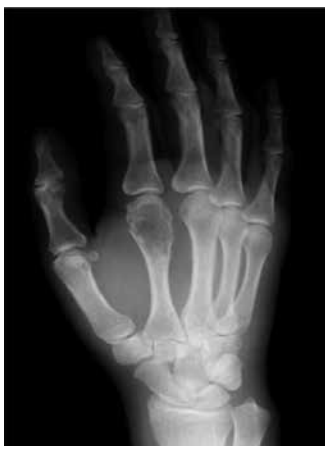


Figure 2: Well-defined lytic lesion involving medullary region of right second metacarpal bone; Enchondroma

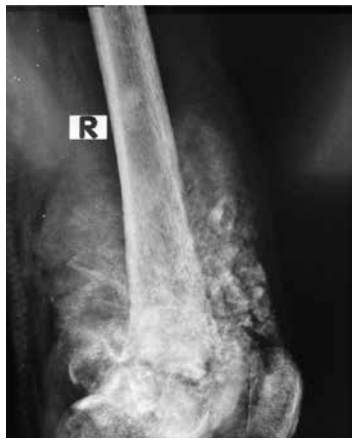


Figure 3: Cortical break and aggressive periosteal reaction with prominent osteoid matrix; Osteosarcoma mmmamatrmatrixmatrixmetadiapyseal region of right humerus

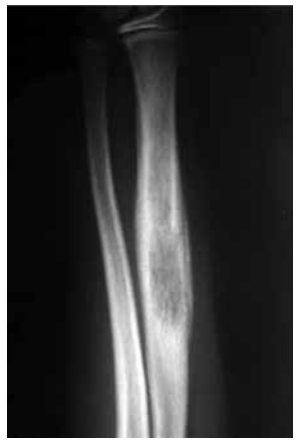


Figure 4: Ill-defined diaphyseal lesion with periosteal reaction; Chronic Osteomyelitis

Osteomyelitis is a bone infection caused predominantly by microbes, especially bacteria. Imaging characteristics of osteomyelitis and Ewing sarcoma may be identical: Ill-defined diaphyseal lesion with periosteal reaction that may be of lamellated type (Figure 4) and to be treated appropriately, they must be differentiated from each other. Histology remains the gold standard in such cases. Ewing sarcoma shows sheets and large aggregates of small round blue cells whereas osteomyelitis shows plasma cells within the bony trabeculae and dead bone.

The radiographic correlation is often overlooked, despite it being a crucial adjunct for the precise identification of orthopedic pathologies. To show why this association is not just an intriguing addition to the surgical pathology of orthopedic lesions, but a crucial step in the diagnostic process, it must be performed. It is essential to get a diagnosis for the patient with symptoms as soon as possible, although diagnosis delays are seen due to several reasons. A multidisciplinary team conference with the orthopaedic surgeon, radiologist, pathologist involved in the diagnosis and oncologist engaged in the patient's care should be held for all patients with malignant bone tumors.

Integrated Diagnostics in Microbiology:
A Comprehensive Approach to Tuberculosis Diagnosis

Mohammad Zeeshan, Microbiology

Tuberculosis (TB) remains one of the leading infectious disease killers worldwide, disproportionately affecting low- and middle-income countries (LMICs), including Pakistan. According to the World Health Organization (WHO), Pakistan ranks among the top eight countries with the highest TB burden. Despite advancements in diagnostic technologies, delayed and incomplete diagnosis continues to hinder TB control efforts. This underscores the critical need for a harmonized, integrated diagnostic approach —one that leverages the strengths of radiology, microbiology, and molecular diagnostics to achieve rapid and accurate diagnosis.

The Rationale for Integrated Diagnostics in TB:

TB is a multisystemic disease with a wide spectrum of presentations. Relying on a single diagnostic modality may lead to underdiagnosis or delayed treatment initiation. Integrated diagnostics refer to the coordinated application of radiological imaging, conventional microbiological methods, and molecular technologies to improve diagnostic accuracy and clinical decision-making. This model is especially crucial in LMICs where resources are limited, and the burden of TB—particularly multidrug-resistant TB (MDR-TB)—is high.

An integrated diagnostic workflow accelerates detection, reduces diagnostic errors, and ensures that appropriate treatment can be initiated promptly. It also facilitates monitoring and surveillance, both critical for public health planning and resource allocation.

Radiology: The First Line of Suspicion:

Radiological imaging, particularly **chest X-rays (CXR)**, plays a pivotal role in the early identification of suspected pulmonary TB. While not confirmatory, imaging findings such as upper lobe infiltrates, cavitary lesions, and lymphadenopathy often serve as early indicators prompting microbiological testing.

Computed Tomography (CT) adds value in detecting atypical or extrapulmonary TB, revealing subtle parenchymal changes, mediastinal lymphadenopathy, and miliary patterns not easily seen on CXR. In paediatric TB or HIV-associated TB, where microbiological yield is often low, radiologic evidence is invaluable.

However, the lack of specificity and operator-dependent interpretation limits the standalone utility of radiology. Therefore, imaging findings must be interpreted alongside microbiological and molecular data.

Conventional Microbiological Methods: The Foundation

1. **Acid-Fast Bacilli (AFB) Smear Microscopy**

Smear microscopy remains widely used in LMICs due to its low cost and rapid turnaround. However, its sensitivity is limited, particularly in paucibacillary cases or HIV co-infection. It also does not differentiate Mycobacterium tuberculosis from non-tuberculous mycobacteria (NTM), nor does it provide drug susceptibility information.

2. **Culture and Drug Susceptibility Testing (DST)**

Mycobacterial culture remains the gold standard for diagnosis and offers the benefit of DST. Solid media (Löwenstein-Jensen) and liquid culture systems (e.g., MGIT 960) allow for species identification and assessment of resistance to first line and second-line drugs.

However, culture is time-consuming, requiring two to eight weeks for results, which delays treatment decisions. In the current era of drug resistance, conventional DST remains essential, especially in MDR-TB and XDR-TB evaluation.

Molecular Diagnostics: Rapid and Transformative

Recent years have seen the introduction of nucleic acid amplification tests (NAATs), revolutionizing TB diagnostics, especially in LMICs.

1. **Xpert MTB/RIF and Xpert Ultra**

These cartridge-based tests detect M. Tuberculosis DNA and rifampicin resistance directly from sputum in under two hours. Recommended by WHO as the initial diagnostic test for TB, Xpert offers high sensitivity (particularly in smear-negative cases) and rapid detection of rifampicin resistance, a key MDR-TB marker.

2. **Line Probe Assays (LPAs)**

These genotypic tests detect mutations associated with resistance to first- and second-line drugs.

LPAs are essential for MDR and pre-XDR/XDR-TB diagnosis and allow for faster treatment regimen selection, crucial for patient outcomes.

3. **Whole Genome Sequencing (WGS)**

Though not yet routine in LMICs due to cost and infrastructure needs, WGS offers unparalleled insights into drug resistance, transmission patterns, and strain diversity.

Why Integration Matters for LMICs Like Pakistan

In LMICs, challenges such as poor infrastructure, limited human resources, and constrained diagnostic capacity often lead to fragmented and inefficient diagnostic pathways. The delay between suspicion and confirmation allows for disease progression and ongoing transmission.

An integrated diagnostic strategy ensures that:

- Radiology triggers early suspicion and guides sample collection.
- Microbiology confirms infection and guides antimicrobial sensitivity.
- Molecular diagnostics enable rapid detection and resistance profiling.

Such synergy reduces diagnostic delay, improves resource utilization, and facilitates early, appropriate treatment initiation—a cornerstone of TB control.

Strategic Implementation: The Way Forward

For effective implementation in Pakistan, we recommend:

Capacity building across radiology and microbiology departments with integrated training programs.

Infrastructure upgrades to ensure the availability of both conventional and molecular testing platforms in peripheral and tertiary centers.

Interdisciplinary collaboration between radiologists, microbiologists, pulmonologists, and general practitioners for holistic patient management.

Policy advocacy to support universal access to integrated TB diagnostics as part of national TB control programs.

Conclusion

Tuberculosis remains a diagnostic challenge, particularly in LMICs like Pakistan. By combining radiologic assessment, traditional microbiology, and modern molecular methods in a unified diagnostic pathway, we can significantly enhance the accuracy and timeliness of TB diagnosis. Such integration is not only scientifically sound but also ethically essential—to ensure that every patient receives the right diagnosis and timely care.

Integrated Diagnostics in Multiple Myeloma

Dr Faiza Jamal, Dr Umer Naeem Effendi and Dr Syed Bilal Hashmi, Clinical Chemistry

Multiple myeloma (MM), a malignancy of plasma cells, poses significant diagnostic and therapeutic challenges due to its complex presentation. Unlike many cancers diagnosable through a single modality, MM demands an integrated diagnostic strategy that synthesizes biochemical, hematologic, radiologic, and molecular findings to ensure accurate diagnosis, risk assessment, and disease monitoring.

Biochemical testing forms the initial step in MM diagnosis. Serum protein electrophoresis (SPEP) identifies monoclonal protein spikes, while immunofixation electrophoresis (IFE) pinpoints the specific immunoglobulin subtype. The serum free light chain assay (FLCP) is particularly useful in detecting non-secretory or oligosecretory myeloma. These tests collectively offer an essential overview of disease burden and guide early diagnostic decision-making.

Hematologic evaluations add critical depth. Bone marrow aspiration and biopsy confirm plasma cell infiltration, a key diagnostic marker when plasma cells exceed 10% of marrow content. Flow cytometry further identifies characteristic surface markers (e.g., CD38, CD138, CD56) and enables the detection of minimal residual disease (MRD), an important measure of treatment response and relapse risk. Radiologic assessments, particularly skeletal surveys, detect MM-associated lytic bone lesions. Although conventional X-rays remain common, advanced imaging modalities like MRI and PET-CT offer superior sensitivity for staging and monitoring skeletal involvement.

Molecular diagnostics provide additional insights into disease behavior. Cytogenetic techniques such as fluorescence in situ hybridization (FISH) detect high-risk chromosomal abnormalities (e.g., t(4;14), del(17p13)), which inform prognosis and treatment intensity. Next-generation sequencing (NGS) identifies somatic mutations (e.g., TP53, KRAS),

facilitating personalized therapy by targeting specific molecular alterations.

The integration of these diagnostic tools creates a holistic understanding of MM. This synergy not only improves diagnostic accuracy and risk stratification but also allows for personalized treatment planning and precise disease monitoring. For example, MRD tracking through flow cytometry or NGS can help evaluate the depth of response and guide timely treatment modifications.

In summary, integrated diagnostics represent a paradigm shift in the management of multiple myeloma. By combining data from diverse testing modalities, clinicians can deliver more personalizedand effective care—ultimately improving outcomes for patients facing this complex disease.

Sweat and DNA: A Unified Approach to Diagnosing Cystic Fibrosis

Dr Syed Bilal Hashmi, Ms Tahira Parveen, Dr Zeeshan Ansar and Ms Kanwal Tariq, Clinical Chemistry

Cystic fibrosis (CF) is a widespread autosomal recessive genetic disorder that predominantly impacts multiple organ systems, including the respiratory tract, gastrointestinal system, and reproductive organs. This condition arises due to mutations in the CFTR (cystic fibrosis transmembrane conductance regulator) gene, which disrupts the normal function of the chloride ion channels, resulting in impaired ion transport across epithelial surfaces. Given the progressive and debilitating nature of CF, early and accurate diagnosis is critically important. Timely identification enables early treatment, reducing complications and improving quality of life and long-term outcomes.

Sweat chloride analysis is currently the most reliable and widely accepted method for diagnosing Cystic fibrosis. Sweat, the biofluid analyzed in this test, contains sodium (Na^+) and potassium (K^+) as the primary cations, along with trace amounts of ammonium (NH_4^+). The anionic composition is predominantly chloride (Cl^-), balanced by smaller contributions from lactate and bicarbonate (HCO_3^-). Because these electrolytes influence the overall ionic composition of sweat, measuring its conductivity provides an indirect estimate of total electrolyte levels, typically reported in millimoles per liter (mmol/L). To induce sweat for diagnostic testing, the pilocarpine iontophoresis method is employed. In this procedure, pilocarpine nitrate, a cholinergic agonist, is delivered transdermally into the eccrine sweat glands via iontophoresis. A low-intensity electrical current, generated by a battery-powered device, facilitates the migration of pilocarpine through the aqueous pathways of the sweat ducts. This is achieved using two electrodes affixed to the patient's limb—typically the forearm or thigh—ensuring localized sweat production for subsequent collection and analysis.

Figure 1: Sweat Chloride testing procedure

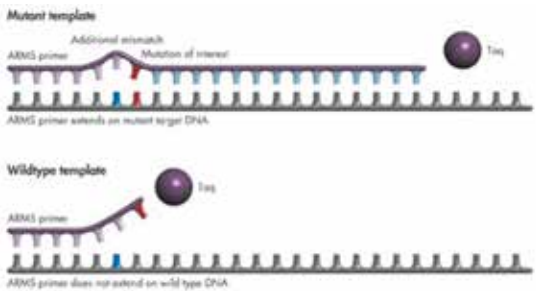


Figure 2: Principle of ARMS PCR

Given the genetic diversity across populations, it is essential to conduct further research to identify the most common CFTR variants in the Pakistani population. Current diagnostic protocols primarily target globally prevalent mutations like ΔF508, which may not capture region-specific variants. Establishing a local mutation spectrum will enhance the accuracy of genetic screening, enable better diagnostic coverage, and inform population-specific treatment strategies.

Integrated Breast Biopsy and Predictive Marker Testing: A New Era in Streamlined Cancer Diagnostics

Zeba Anwar/ Dr Tamana Asghari, Histopathology

In today's artificial intelligence era everything is getting on a single click, for healthcare setup, accuracy and efficiency in cancer diagnostics are more critical than ever and sometimes it becomes challenging. Recognizing this need, we are proud to introduce an **integrated test that merges breast biopsy procedures with predictive marker analysis**—a pioneering step toward streamlined breast cancer management.

This innovation not only streamlines processes but also completely reimagines the diagnostic process for both physicians and patients.

Previously, breast core biopsies and predictive marker testing (e.g., ER, PR, HER2) were performed in separate steps—biopsy first, followed by a second request for immunohistochemistry. This often results in delays, duplicate processing and higher costs.

Our integrated test combines:

- **Tissue biopsy (breast core)** for histological diagnosis.
- **Simultaneous processing for predictive markers** (ER, PR and HER2) from the same specimen block.

This integrated approach ensures that by the time a diagnosis of invasive breast carcinoma is made, key therapeutic markers are already available, eventually reducing the time to treatment planning.

Why Integration Matters?

Improved Workflow

- Eliminates the need for, re-sectioning, and re-requesting IHC, saving pathology team time and effort.

Cost Efficiency

- For patient we have offered approximately 24.45% cost reduction

Better Patient Experience

- Reduces patient anxiety by shortening the diagnostic timeline and avoiding repeated procedures.

Enhanced Multidisciplinary Coordination

- Tumor boards receive a complete diagnostic picture upfront, supporting faster treatment decisions.

Technical Highlights

- **Sample Type**: Core needle biopsy should be preserved in formalin.
- **Assays**:
  - o **H&E** (Hematoxylin and Eosin)
  - o **ER/PR** via IHC
  - o **HER2** via IHC

  Platform: Fully integrated with our LIS (Laboratory integrated System).
  Validation: Performed in accordance with CAP and ASCO/CAP guidelines for predictive markers.

A Transformative Workflow

Before Integration:	After Integration:
Day 1: Biopsy gross Day2 : Biopsy Process and Distributed H&E	Day 1: Biopsy gross Day2: Biopsy Process and Distributed H&E
Day3-5 :Work up and diagnosis reported	Day 3-8: Work up and diagnosis including Predictive marker Process and Reported.
Day-6-10: Predictive markers requested separately	
Day 11-18: Predictive marker Process and Reported	
Total 18 days to decide Management of Cancer	Total 08 days to decide Management of Cancer
This saves almost 10 days—a vital difference in oncology care	

☑ **Future Outlook: Foundation for Precision Oncology**

This integrated approach lays the groundwork for next-generation diagnostics, where biopsy specimens can be simultaneously profiled for:

- **Genomic panels**
- **PD-L1 expression**
- **Tumor mutational burden (TMB)**
- **BRCA/HRD status**

As precision oncology evolves, **integrated testing platforms** will become the standard, not the exception.

Conclusion

The fusion of breast biopsy and predictive marker analysis into a single diagnostic workflow represents a significant leap in modern pathology. By bringing speed, precision, and patient-cantered care to the forefront, our integrated test is not just a technical improvement—it's a **clinical paradigm shift**. Whether you're a clinician, a laboratory leader, or a healthcare administrator, adopting integrated diagnostics is the path forward in delivering timely, personalized cancer care.

Unmasking the Hook Effect in Prolactinomas:
A Diagnostic Trap Requiring an Integrated Approach

Dr. Muhammad Umer Naeem Effendi and Ms. Noureen Niazali, Clinical Chemistry

In the intricate landscape of endocrine diagnostics, few phenomena illustrate the necessity for interdisciplinary collaboration as vividly as the hook effect—a deceptive analytical artifact most famously encountered in patients with giant prolactin-secreting pituitary adenomas (prolactinomas). This effect poses a significant risk of underdiagnosis if not recognized and corrected, emphasizing the need for integrated diagnostics in clinical practice.

Understanding the Hook Effect

The hook effect, also referred to as the prozone phenomenon, occurs in non-competitive (sandwich) immunoassays—a common method used for measuring hormone levels such as serum prolactin. In these assays, accurate quantification relies on the simultaneous binding of the analyte to a capture antibody and a labeled detection antibody.

However, when prolactin levels are extremely high, they can saturate both antibodies independently, preventing the formation of the antibody-analyte-antibody "sandwich" complex required for signal generation. The result is a falsely low or inappropriately normal prolactin concentration, despite the patient harboring a large, hormone-secreting pituitary tumor.

This paradoxical result is most likely to occur in patients with giant prolactinomas, in whom true prolactin levels may exceed the upper detection limit of the assay, sometimes by several orders of magnitude.

Clinical Clues: When to Suspect the Hook Effect

The hook effect is a well-known phenomenon where misleading results can deceive even seasoned clinicians—unless clinical, radiological, and biochemical findings are carefully cross-checked. Key indicators include:

- Clinical signs and symptoms of hyperprolactinemia: galactorrhea, amenorrhea, decreased libido, infertility, or visual disturbances.
- Neuroimaging (typically MRI) showing a large sellar/suprasellar mass, consistent with a macroadenoma or giant pituitary tumor.
- Serum prolactin levels that are only mildly elevated or even within the normal range contradicting the imaging and clinical picture.

Such discordance should immediately prompt consideration of the hook effect. Failure to do so may result in a misdiagnosis, with the lesion potentially mistaken for a non-functioning macroadenoma or other pituitary pathology.

Laboratory Strategy: Simple Dilution, Significant Insight

Fortunately, the hook effect is both predictable and preventable—as long as laboratory professionals and clinicians remain vigilant. The solution is straightforward yet essential:
dilute the sample (commonly at ratios of 1:10, 1:100, or higher) and repeat the prolactin assay. This dilution brings the analyte concentration within the assay's linear range, revealing the true prolactin level, which is often significantly higher than initially reported. Some guidelines even recommend routine dilution of prolactin samples in the presence of large pituitary masses to avoid missing hyperprolactinemia due to this effect.

Conclusion

In the era of precision medicine, the hook effect serves as a powerful reminder that advanced diagnostics require more than sophisticated tools—they require collaboration, context, and clinical awareness. Recognizing this immunoassay pitfall not only prevents diagnostic errors but also reinforces the principle that integrated diagnostics is not an option—it's a necessity.

Hook effect in a nutshell

Analytical Modification

Diluting the sample brings values into the assay's linear range, revealing true prolactin levels.

Consider Imaging

Imaging can provide additional clinical context to avoid misdiagnosis.

Clinical Suspicion

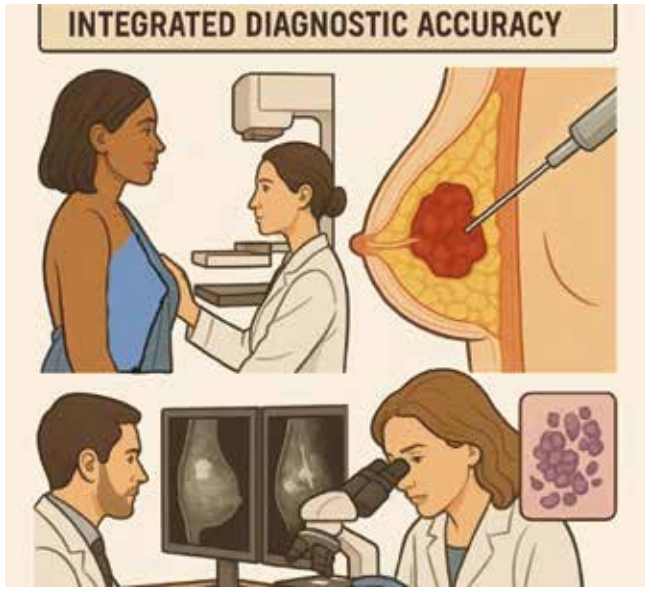
Clinical signs can guide the interpretation of lab results.

Radiology-Pathology Correlation: A Cornerstone of Integrated Diagnostic Accuracy

Dr Anam Khan, Dr Hina Pathan and Dr. Sadaf Asjad, Radiology

In modern healthcare, the true strength of diagnosis lies not in isolated interpretations, but in integration. This integrated approach is increasingly recognized as essential to high-quality patient care. It involves not only collecting data from various diagnostic modalities but also fostering collaboration across specialties. Among the most critical of these interdepartmental relationships is the partnership between radiology and pathology—particularly in the evaluation of oncologic and breast imaging findings.

Radiology-pathology concordance refers to the alignment between imaging findings and histopathologic results. At its core, this concordance represents a post-biopsy diagnostic dialogue. In breast imaging, this relationship is especially significant. When a lesion categorized as BI-RADS 4 (suspicious) or BI-RADS 5 (highly suggestive of malignancy) on mammography or ultrasound undergoes core needle biopsy, the expectation, guided by the American College of Radiology (ACR) BI-RADS® Atlas, is that pathology will confirm the radiologic suspicion. If a benign pathology is reported for such suspicious imaging, the case must be meticulously reviewed for discordance. This is a critical scenario, as highlighted by studies like Youk et al., where discordant results necessitate careful management, often involving repeat biopsy, surgical excision, or additional



imaging, to rule out sampling error or an undetected malignancy. The ACR BI-RADS Atlas provides the standardized framework and management recommendations that underpin these decisions, ensuring consistent and evidence-based practice.

Radiology-pathology concordance is more than a diagnostic checkpoint—it serves as a mechanism for quality assurance and integrated clinical

reasoning. In practice, concordance is assessed through structured correlation workflows, often in the setting of multidisciplinary tumour boards. These forums facilitate collaborative decision-making and promote diagnostic alignment, especially in cases with indeterminate or borderline findings. A College of American Pathologists (CAP) Q-Probes study by Idowu et al. empirically demonstrated that active participation by pathologists in correlating findings and attending interdepartmental multidisciplinary conferences significantly improves overall correlation rates, underscoring the vital role of these collaborative environments in enhancing diagnostic accuracy.

This integrative approach is even more critical in resource-limited settings, where repeated testing may not be feasible or accessible. Ensuring concordance from the outset significantly reduces the risk of delayed or missed diagnoses, thereby reinforcing confidence in the healthcare system and optimizing patient outcomes by providing the correct diagnosis efficiently.

One cannot deny the role of Artificial intelligence in this era, as evolving new techniques further underscore the importance of integration. Radiology and pathology both utilize artificial intelligence and machine learning. As the datasets on which these tools are trained become more robust, they are expected to yield excellent results in the future. Accurate radiologic-pathologic correlation will not only improve patient care but will also support the development of authentic, AI-driven diagnostic support tools.

Ultimately, radiology-pathology correlation is not just about agreement; it represents a shared diagnostic responsibility. This ongoing, often silent, yet profoundly impactful dialogue ensures that no specialty functions in isolation, and that patient care remains accurate, collaborative, and aligned with the highest standards and best practices in modern medicine.

Integrated Diagnostic Assessment of von Willebrand Factor Antigen and Activity Correlation

Yumna Tariq and Bushra Rizvi, Coagulation

Introduction

Von Willebrand Disease (VWD) is an inherited bleeding disorder caused by a deficiency or dysfunction of Von Willebrand Factor (VWF), which stabilizes Factor VIII (FVIII) and supports platelet adhesion to blood vessel walls. It is typically screened using prolonged APTT, and low to normal levels of VWF antigen (VWF: Ag), Ristocetin cofactor activity (RCOF), and FVIII levels. At the Main Lab of AKUH Karachi, some patient samples showed abnormal VWF: Ag and RCOF results that did not align with any of the three recognized VWD types. To investigate these discrepancies, the Coagulation section conducted a correlation study between VWF: Ag and RCOF.

In December 2024, a total of 122 samples were analyzed as part of a bleeding disorder investigation, focusing on correlation of VWF: Ag and RCOF. Out of 122 patient samples, 78 samples were specifically charged for VWF: Ag, 7 for RCOF, and 37 for both.

Data:

VWAF Antigen Analysis: Normal levels: VWFAg: 50-158 percent

Sample Category	Number of Samples
Normal levels	87
Low levels	17
<2%	11
Total Samples	115

RCOF Analysis: Normal levels: RCOF: 58-172 percent

Sample Category	Number of Samples
Normal levels	26
Low levels	13
<10%	5
Total Samples	44

Out of 122 samples with initially low VWF: Ag and RCOF results, 32 were re-evaluated using Factor VIII (Normal FVIII: 50-149 percent) and APTT (Normal APTT: 22.9-34.5 sec) to aid in result correlation. Of 32, only two samples remained discrepant and were subsequently re-tested using fresh specimens for confirmation, as detailed below:

Table 1: Sample 1

	Initial sample	Fresh sample
VWF Ag	27%	61%
RCOF	<10%	23%
FVIII	47%	>200
APTT	>170 sec	117 sec

Table 2: Sample 2

	Initial sample	Fresh sample
VWF Ag	32%	43%
RCOF	12%	16%
FVIII	68%	32%
APTT	29sec	31 sec

The concise interpretation of the findings from **Sample 1** and **Sample 2**, highlighting the significance of the re-analysis are:

Sample 1 – Suggestive of Pre-Analytical Error

- **Initial vs. Fresh Sample:**
  - ➢ **VWF Ag** increased from **27 percent to 61 percent**
  - ➢ **RCOF** increased from **<10 percent to 23 percent**
  - ➢ **FVIII** increased from **47 percent to >200 percent**
  - ➢ **APTT** improved from **>170 sec to 117 sec**

Sample 2 – Suggestive of a True Clinical Deficiency

- **Initial vs. Fresh Sample:**
  - ➢ **VWF Ag** remained low (32 percent → 43 percent)
  - ➢ **RCOF** remained low (12 percent → 16 percent)
  - ➢ **FVIII** decreased (68 percent → 32 percent)
  - ➢ **APTT** remained within normal range (29 sec → 31 sec)

Summary

- **Sample 1**: Likely falsely low results, which persistently remained discrepant.
  Resolution steps:
  - ➢ Repeat testing under controlled conditions (fasting, rest, no acute illness), if not performed earlier may help clarify VWD subtype or
  - ➢ Consider VWF multimer analysis and VWF: CB (collagen binding) may help or
  - ➢ Ensure to document the timing of any treatment or transfusion administered, as this will be crucial for accurately interpreting the results.
- **Sample 2**: Likely represents a true low VWF-related condition; further clinical and genetic evaluation was warranted.

This integrated correlation study demonstrated that relying solely on VWFAg and RCOF results for screening can sometimes lead to difficulties in interpretation and diagnosis. Therefore, for effective screening of Von Willebrand disease APTT, FVIII, VWFAg, and RCOF should all be requested together to ensure a comprehensive evaluation.

Metachromatic Clues: A Diagnostic Challenge of Systemic Mastocytosis in a Patient with Bone Marrow Fibrosis

Dr Maria Owais and Dr Amna Qadri, Hematology

Case Details:

A 39-year-old married male from Quetta presented in September 2024 with complaints of severe back pain, intermittent undocumented fever and headache. CT scan of the abdomen and pelvis revealed hepatomegaly and multiple small lytic lesions involving the spine and pelvic bones (largest lesion measuring 11.0 mm in the right iliac bone).

His laboratory investigations for the workup of plasma cell neoplasm including serum protein electrophoresis, immunofixation and free light chain ratio revealed no monoclonal gammopathy. Bone marrow biopsy and a separate bone biopsy from the left iliac blade were taken to evaluate the lytic lesions further.

Histopathological analysis of the core of bone trephine and bone biopsy from the left iliac blade revealed marrow elements with clusters of histiocytic infiltration along with sheets of atypical cells with granular densely eosinophilic cytoplasm (Fig: A) and dense fibrosis (Fig: B). Immunohistochemical stains for plasma cells (CD138), epithelial cells (CKAE1/AE3) and Langerhans cells (CD1a) were negative. CD20 and CD3 were also negative in sheets of eosinophilic cells, while CD68 highlighted the admixed population of histiocytes (Fig: C). Hence

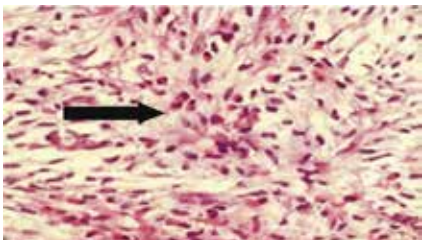


Fig. A: Bone biopsy section exhibiting sheets of atypical cells with granular densely eosinophilic cytoplasm (highlighted by black arrow)

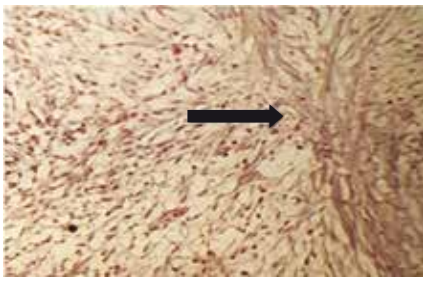


Fig. B: Bone Trephine section showing dense fibrosis (Grade: MF-2); highlighted by black arrow

a diagnosis of Myelofibrosis was favored.

As part of the further evaluation for Myelofibrosis, molecular testing—including JAK2 V617F and an extended MPN panel (MPL W515K/L, KIT, and CALR frameshift mutations)—was conducted. The results identified a KIT p.D816V (c.2447A>T) mutation in exon 17 of the C-KIT gene, with no other mutations detected, raising suspicion for an underlying Mastocytosis.

The bone marrow and bone biopsy from left iliac blade was reviewed, and CD117 immunohistochemical staining was performed, revealing positivity in clusters of atypical cells, confirming them as mast cells [Fig: D]. Further special staining showed strong metachromatic positivity for mast cell granules with toluidine blue, which further corroborated the diagnosis [Fig: E].

Fig. C: CD68 stain showing increased histiocytes (highlighted by black arrow)

Discussion: Systemic Mastocytosis (SM) is a rare and heterogeneous group of clonal mast cell disorders classified under Myeloproliferative Neoplasms (MPNs). It is characterized by the abnormal accumulation of neoplastic mast cells in extracutaneous organs, most commonly the bone marrow, liver, spleen, lymph nodes, and gastrointestinal tract. 95% of the cases are driven by an activating mutation in the KIT gene, most frequently the KIT D816V mutation. This mutation leads to constitutive activation of the KIT receptor tyrosine kinase, promoting mast cell survival, proliferation, and accumulation.

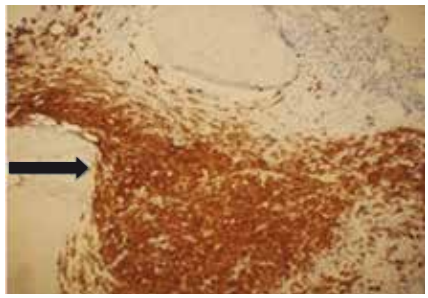


Fig. D: CD117 stain showing positivity in sheets of mast cells

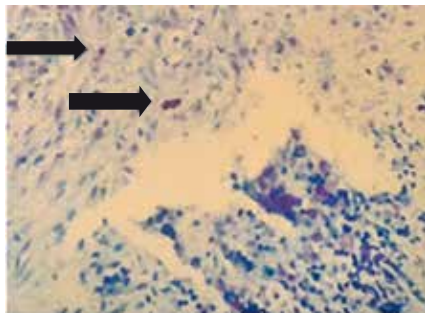


Fig. E: Toluidine Blue stain; showed strong metachromatic positivity for mast cell granules

Diagnosis relies on a combination of histopathological findings, immunophenotyping, and molecular analysis as outlined in the World Health Organization (WHO) classification. The detection of multifocal dense infiltrates of mast cells in the bone marrow, aberrant expression of CD25 and/or CD2, presence of KIT mutations, and positive special stains such as toluidine blue are critical diagnostic elements.
This case report highlights the diagnostic journey of a middle-aged patient who presented with non-specific systemic symptoms and skeletal pain and was ultimately diagnosed with Systemic Mastocytosis using an integrated approach, taking radiological, histopathological and molecular findings onboard. The case highlights the importance of a multidisciplinary diagnostic approach in patients with unexplained skeletal lesions and systemic symptoms.

Integrating Molecular Testing in Diagnosing Rare Childhood Infections: A Case of Congenital Neutropenia

Dr Sana Hassan, Hematology

Congenital neutropenia is a rare yet life-threatening condition that often manifests early on in life with recurrent infections. Prompt diagnosis is essential to initiate appropriate management and prevent complications, including leukemic transformation due to acquisition of secondary mutations such as RUNX1

and many others. We recently encountered a rare but classic presentation of congenital neutropenia in our hospital AKUH.

A 5-month-old baby boy presented to the emergency room with cough, chest congestion, and a maculopapular rash on face and neck. The child had a history of multiple hospital admissions in the last 3 months of age, initially for bronchopneumonia, later for suspected meningitis and recurrent fevers. Family and birth history were unremarkable.

Initial CBC revealed Hb: 11.3 g/dL, WBC: 8.32 × 10^9/L with ANC: <0.05 × 10^9/L, ALC: 6.6 x 10^9/L and Plt: 458 x 10^9/L (Figure 1). Bone marrow biopsy showed maturation arrest at the promyelocyte stage.

The diagnostic clue came from persistent neutropenia and propensity to infections on multiple occasions with a normal total WBC count. The Bone marrow examination showed arrest at the promyelocyte stage and raised suspicion for congenital neutropenia. For further testing the child's Next Generation Sequence testing, were sent to Turkey that identified a pathogenic

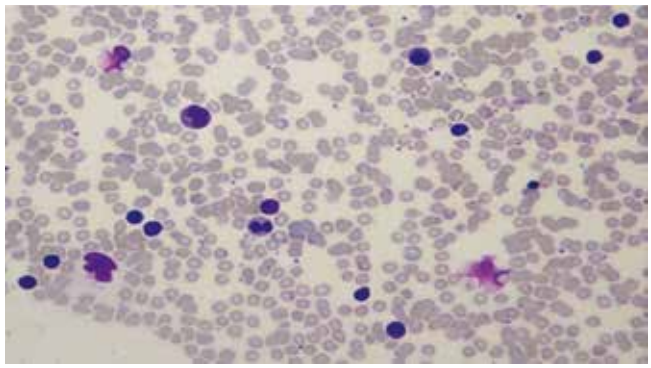


Figure 1: Aspirate showing arrest at the promyelocyte stage.

ELANE mutation, further confirming the diagnosis.

Persistent neutropenia with recurrent infections in infancy should prompt evaluation for congenital neutropenia. The Bone marrow examination revealed maturation arrest, and integrated molecular testing for *ELANE* mutation through NGS confirmed the diagnosis. Early detection is crucial, as it can be lifesaving by guiding G-CSF therapy and enabling timely stem cell transplantation in cases resistant to treatment.

An Undifferentiated Neoplasm-A case

Dr Hajrah Syndeed Pal, Hematology

Introduction: Integrated reporting exemplifies the collaborative efforts of multiple departments, all working together for the benefit of patient care. The pathology department often serves as a hub for such integration, combining diverse findings into a unified, comprehensive report. In this case report, we present a neoplasm that underwent extensive evaluation and multidisciplinary review by consultants from various specialties.

Case details: A 57-year-old male presented with painless right testicular swelling for three-four months. There was no history of cryptorchidism, abnormal sexual development, trauma in the inguino-scrotal region, or primary tumor in other sites. On examination the testis was firm and painful on palpation. The overlying skin was without any visible changes. There was no associated weight loss, hepatosplenomegaly or palpable lymphadenopathy. Testicular biopsy performed outside AKUH and reported with the possibility of mast cell sarcoma.

Workup for the case: A complete workup of the patient was performed at AKUH inclusive of baseline testing. CBC showed bicytopenia and leucoerythroblastic blood picture. Bone marrow trephine biopsy and cytogenetic studies were proceeded as well.

Bone marrow aspirate showed infiltration with large atypical mononuclear cells showing hyperchromatic nuclei, condensed chromatin and moderate basophilic cytoplasm (as shown in Fig: 1). Bone trephine showed altered marrow architecture, presence of necrosis and

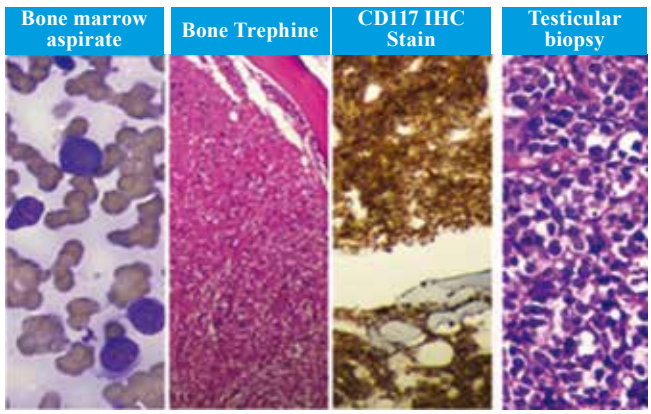


Fig. 1: Bone marrow aspirate, trephine biopsy with CD117 IHC stain and testicular biopsy

infiltration with large atypical mononuclear cells (as shown in Fig: 1).

Review of testicular block showed effacement of testicular parenchyma by back-to-back closely packed nests and aggregates of neoplastic cells which are mildly pleomorphic, have hyperchromatic nuclei, inconspicuous nucleoli and moderate to abundant eosinophilic cytoplasm (as shown in Fig: 1).

Immuno-histochemical panel performed on bone marrow and testicular block included SALL-4, CD117, OCT3/4, CD30, Synaptophysin, Chromogranin-A, CD56, LCA, CD20, CD3, DOG-1, Desmin, CD34, TDT, MUM1, PAX5, MPO, Ki-67, Cytokeratin CAM5.2, CD138, PLAP, HMB-45, Melan-A, Glypican-3.Out of all these stains, CD117 was diffuse positive, Synaptophysin patchy positive and Cytokeratin CAM 5.2 focal positive (as shown in Fig: 1).
The cytogenetic studies showed 47, XY, del (3) (p26), del (5) (q31) x 2, -10, add (20) (q13.3), +21 [20] (as shown in Fig:2).

Conclusion:
The case was reviewed in the multidisciplinary team (MDT) meeting and concluded to represent a malignant undifferentiated neoplasm, based on the correlation of the available investigative findings.

Fig. 2: Karyogram

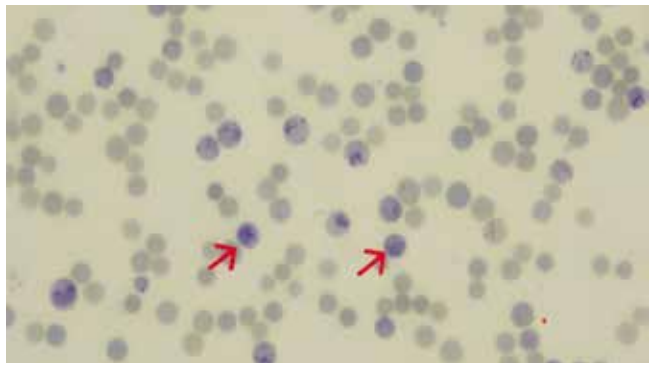
Discussion:
A malignant undifferentiated neoplasm, often called an undifferentiated cancer, is a tumor in which the cells lack the typical characteristics of the tissue they originated from, making it difficult to determine their origin. These tumors are usually aggressive and high-grade and pose diagnostic as well as therapeutic challenge.
Treatment options vary depending on the location and type of undifferentiated neoplasm, but may include surgery, radiation, and chemotherapy. Additionally, immunotherapies have been proven to have better outcomes in some studies. In patients with undifferentiated neoplasms, treatment response varies and depends upon the combination offered. Those who don't respond to induction chemotherapy generally have a poor outcome.

Stories in a Glance

Reticulocytes: Significance and Differentiation from RBC Inclusions

Dr Rimsha Imran, Hematology

Reticulocytes are immature red cells released from bone marrow that contain remnant cytoplasmic RNA and organelles like mitochondria and ribosomes. They mature in circulation over one-two days. The reticulocyte count determines bone marrow erythropoietic activity in response to anemia. Reticulocytosis is seen in conditions like hemolytic anemias, hemorrhage, post-splenectomy and during recovery from anemia. They are visualized using supravital stains like methylene blue. On a blood film, reticulocytes (as highlighted by red arrows in the below figure) appear as pale blue containing dark blue reticular or granular material. It's imperative to



distinguish them from RBC inclusions like Howell-Jolly bodies, Heinz bodies, Pappenheimer bodies, and HbH inclusions during manual counting.

HAPPENINGS IN PATHOLOGY

Aga Khan University Shines at HAEMCON 2025

Dr Hareem Alam, Hematology

The 27th Annual Conference of the Pakistan Society of Hematology was held at the Marriott Hotel, Karachi, from April 17th to 20th, 2025 under the supervision of Professor Dr Bushra Moiz [AKU] (Chair Scientific Committee HeamCon 2025) and Dr Saba Jamal [IHHN] (Chair Organizing Committee HeamCon 2025). Five successful pre-conference workshops were conducted, covering topics such as diagnostic challenges in molecular hematopathology, case-based learning and hands-on practice in coagulation, integrated diagnostic hematology, immunohematology in transfusion medicine, and a case-based and hands-on workshop on hemoglobin disorders.

The conference commenced with an inaugural session, moderated by Dr. Sana Brohi (AKU) and Dr. Hareem Alam (AKU). The Ibne Sina Lecture was delivered by Maj. Gen (R) Dr. Pervez Ahmed, who spoke on bone marrow transplantation and cellular therapies in Pakistan. The keynote address was given by Dr. Flora Peyvandi, Professor of Internal Medicine at the University of Milan, Italy. The Lifetime Achievement Award was presented to Maj. Gen (R) Suhaib Ahmed, and a posthumous award was given to Dr. Tahir Shamsi (late). The session concluded with the opening of the exhibition by the conference patrons i.e. Distinguished University Professor Dr Mohammad Khurshid (AKU) and Dr Khalid Zafar Hashmi (SIUT).

Over the following three days, eight scientific sessions were held. Each session featured three free research paper presentations, followed by three invited talks by national and international speakers, and concluded with a panel discussion.

Aga Khan University's significant participation in HAEMCON 2025 included:

- Professor Dr. Bushra Moiz chaired the Scientific Committee of the conference.
- Dr. Shariq Shaikh, Dr. Muhammad Hasan, and Dr. Zeeshan Ansar served as facilitators for the workshops.
- Professor Dr. Salman Adil chaired the session on acute leukemia and bone marrow failure.
- The session on challenges in transfusion was chaired by Professor Dr. Muhammad Khurshid, with the panel discussion led by Dr. Muhammad Hasan.
- Professor Dr. Usman Shaikh chaired the session on myeloproliferative neoplasms, moderated by Dr. Nabiha Saeed.
- Professor Dr. Bushra Moiz co-chaired the

session on advancing hematology.

- Dr. Zeeshan Ansar delivered a talk on molecular screening for alpha and beta thalassemia.
- Professor Dr. Zehra Fadoo spoke on pediatric acute myeloid leukemia.
- Professor Dr. Natasha Ali gave an outstanding talk on transplant viral infections, focusing on CMV.
- Dr. Afsar Ali Mian discussed gene editing technologies for hemoglobinopathies.
- Oral presentations were delivered by Dr. Rimsha Imran (Hematology R-II), Dr. Maria Owais (Hematology R-IV), and Dr. Muhammad Umer Naeem Effendi (Chemical Pathology R-V).
- Posters were presented by Dr. Haleema Urooj (R-V), Dr. Fatima Farhan (R-IV), Dr Muhammad Shayan Ashfaq (R-III), Dr. Wajeeha Iftikhar (R-II), Ms. Anila Zafar (Senior Technologist Hematology) & Muhammad Salman (Charge Technologist Blood Bank).
- Dr. Fatima Farhan (Hematology R-IV) served as the overall moderator of the conference.

The conference concluded with an award ceremony highlighting the achievements of our residents and staff:

- Dr. Muhammad Umer Naeem Effendi (Chemical Pathology R-V) received the first prize in oral presentation for his research paper on the 'Prevalence and clinical utility of anti-β2 Glycoprotein-1 antibodies in Pakistani patients suspected of Antiphospholipid syndrome'. (Co-Authors: Dr Hafsa Majid, Dr Bushra Moiz, Dr Lena Jafri & Dr Aysha Habib Khan)
- The first prize for poster presentation was jointly awarded to Hematology Residents Dr. Haleema Urooj (R-V) [Co-Author: Dr Bushra Moiz], Dr. Fatima Farhan (R-IV) [Co-Author: Dr Sana Brohi], and Dr. Wajeeha Iftikhar (R-II) [Co-Authors: Dr Zehra Fadoo, Dr Bushra Moiz, Dr Salman Kirmani, Dr Ali Hussain & Dr Fizza Akbar].
- A complimentary prize was presented to Ms. Anila Zafar, Senior Technologist Hematology, for her poster on the 'Significance of white cell ratio as biomarker in mosquito borne infections' [Co-Author: Dr Bushra Moiz].

These accomplishments marked a proud and memorable conclusion to the conference for our department.

Connecting with Pathology: Exploring our Facebook Page and Website

Dr Sana Brohi, Hematology

In today's digitally driven healthcare landscape, maintaining a strong online presence is crucial for pathology labs to connect with patients, share services, and establish trust. To strengthen its outreach and accessibility, the Department of Pathology & Laboratory Medicine at AKU has launched a dedicated Facebook page and is in the process of enhancing its existing website.

AKU's Facebook page **(link: https://www.facebook.com/profile.php?id= 61574240963703)** will serve as an effective platform to directly engage with patients, caregivers and students. It will feature high-quality visuals, including images of the facility, advanced equipment, and staff, along with detailed information about available tests to enhance trust and credibility. Consistent updates—such as announcements on test availability, health tips, promotional packages, and patient feedback—will help keep the page dynamic and informative.

The website **(link: https://www.aku.edu/mcpk/pathology/Pages/home.aspx)** for the Department of Pathology and Laboratory Medicine at Aga Khan University serves as a comprehensive resource, showcasing the department's key functions in diagnostics, education, research, and clinical services. It offers detailed information about the various branches of pathology, faculty, and laboratory networks across Pakistan. The site also highlights available pathology services, quality standards, and professional development opportunities, making it valuable for students, healthcare professionals, and patients. Furthermore, the website features regular updates through Lablog posts, a Memory Book, and a "Meet Our Team" section, providing a thorough overview of the department's vibrant academic and clinical landscape.
We kindly request LabRad readers to click on the links below, like our Facebook page, and explore our website.

Links:
Facebook link: https://www.facebook.com/profile.php?id= 61574240963703
Website link: https://www.aku.edu/mcpk/pathology/Pages/home.aspx

Polaroid

Histopathology

Bone and soft tissue departmental consultation conference

Neuropathology departmental consultation conference

Microbiology

Enhancing Tuberculosis Diagnosis through an Integrated Approach: The Role of Radiology, Culture, and Molecular Methods

Hematology

Dr. Komal (Hematology Resident) is reviewing a flow cytometry case and integrating the findings with morphological analysis.

Fatima Matloob (Senior Technologist in Hematology) is discussing the HPLC case with Shahmina Sadaf (Assistant Manager in Hematology) and relating the HPLC results to the CBC indices for better integration of the findings.

Clinical Chemistry

Our integrated pathology approach in action: Charge Technologist Noreen Zeeshan conducts a crucial Sweat Quantification test with the ChloroChek system. This precise analysis is vital for diagnosing conditions like cystic fibrosis, demonstrating our commitment to comprehensive patient care

Molecular Pathology

Molecular Pathology staff and faculty participated in 27th Annual Meeting (Haemcon2025) | Apr 17-20, 2025 | Karachi

Molecular Pathology staff and faculty participated in workshop: "DNA to Data: A practical approach for understanding the Nanopore sequencing through lab work and bioinformatics". April 21-22, 2025.

Molecular Pathology staff and faculty participated in First International Conference on transplantation) | Apr 09-13, 2025, | Karachi

Molecular Pathology staff and faculty participated in Conference: Thalassemia Insights: From Diagnosis to Management -20 May 2025

Radiology

The Breast Imaging Team from Aga Khan University Hospital participated in the joint conference of the National Breast Radiology Society of Pakistan (NBRSP) and the Breast Imaging Radiology Society of Pakistan (BIRSP), held in Karachi in April 2025.
The event brought together radiologists, clinicians, and researchers from across Pakistan to share advances in breast imaging and foster multidisciplinary collaboration. The AKUH team contributed through scientific presentations and educational sessions, reflecting our commitment to clinical excellence, academic leadership, and impactful research in breast health.
From right to left: Dr. Shaista Afzal, Dr. Anam Khan, Dr. Fatima Qaiser, Dr. Imrana Masroor, Dr. Gulnaz Shafqat, and Dr. Hina Pathan

Radiology trainees from Aga Khan University celebrated Dr. Imrana Masroor on receiving the Lifetime Achievement Award at the NBRSP–BIRSP Conference 2025.
The award honors her outstanding contributions to breast imaging, unwavering commitment to academic excellence, and enduring impact as a mentor, educator, and leader in radiology.
From right to left: *Dr. Fatima Qaiser, Dr. Mehreen Rasool, Dr. Anam Khan, Dr, Imrana Masroor, Dr. Rafeah Khan, Dr. Hina Iqbal, Dr. Hina Pathan, and Dr. Afshan Sheikh.*

Pre-Conference Workshop: "Role of AI in Radiology Research and Education"
An interactive hands-on session held at the Radiology Department, Aga Khan University, as part of the NBRSP–BIRSP 2025 pre-conference activities.
The workshop focused on the integration of artificial intelligence in radiology research and education, highlighting practical tools and future directions for academic radiologists

hospitals.aku.edu/Karachi/clinical-laboratories