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EDITORIAL

Welcome to the first issue of LABRAD 2026, a non-thematic edition that highlights the diversity and expanding horizons of diagnostic medicine across the Departments of Pathology and Radiology. Last year marked a significant milestone as the Department of Pathology and Laboratory Medicine celebrated 40 years of excellence at Aga Khan University, underscoring our enduring commitment to quality, innovation and service.

This issue brings together a thoughtfully selected range of articles that reflect the needs and realities of modern laboratory and imaging practice. A featured contribution focuses on ALK-rearranged lung cancer, highlighting advances in diagnostic integration using immunohistochemistry, FISH, and next-generation sequencing. Complementary molecular pathology content further emphasizes the expanding role of clinically actionable biomarkers and precision oncology in lung cancer management. Emerging areas such as the application of artificial intelligence in laboratory practice are explored alongside updates on myeloid NGS panels, instructive case-based discussions in hematology, and clinically relevant insights from microbiology and HPV genotyping. The Radiology Update presents modern, evidence-based approaches to pelvimetry, underscoring the importance of selective imaging and standardized reporting in contemporary clinical care.

The Happenings and Polaroid sections highlight academic engagement and departmental achievements, including participation in national and international conferences and key institutional milestones. “The Best of the Past” offers a reflective perspective on professional growth and leadership within radiology practice. We hope this issue contributes to meaningful learning, promotes critical reflection, and inspires continued engagement within our community.

Dr Syed Bilal Hashmi
Associate Editor
Chemical Pathology

ALK-Rearranged Lung Cancer:
Advances in Detection and Diagnostic Integration.

Dr Fatima Safdar and Dr Sarosh Moeen Histopathology

Background:

Lung cancer remains the leading cause of cancer-related mortality worldwide, with non–small cell lung cancer (NSCLC) representing nearly 80 percent of all cases. Among these, anaplastic lymphoma kinase (ALK) rearrangements constitute a clinically actionable driver alteration, typically occurring in younger patients, non-smokers, and those with adenocarcinoma histology.

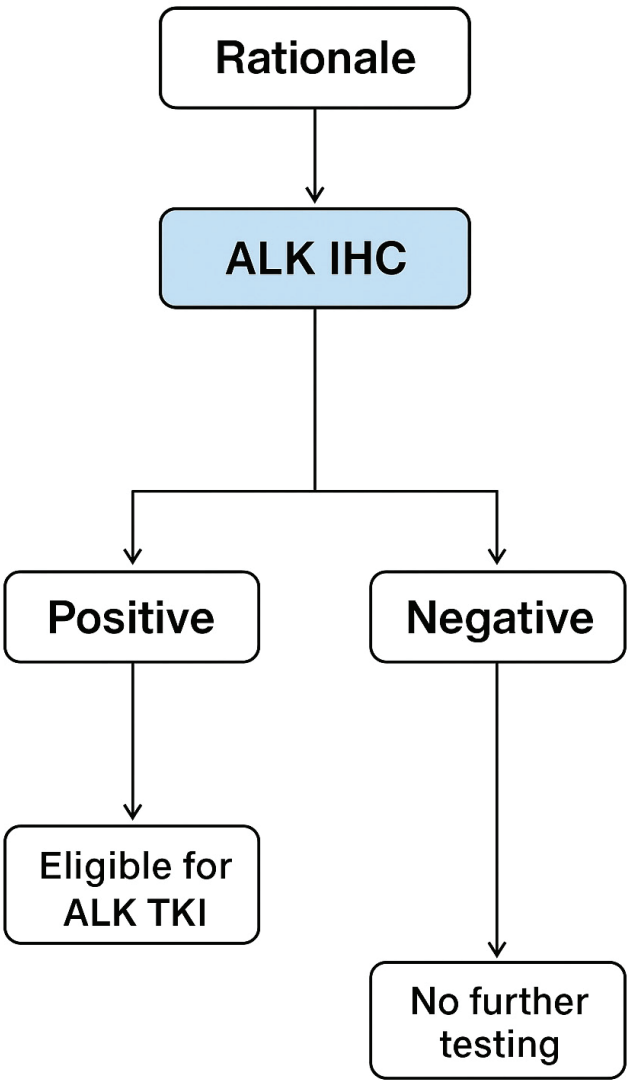
The most common translocation, the EML4–ALK fusion, results from a chromosomal inversion on 2p that creates a constitutively active chimeric oncoprotein. Numerous additional variants and fusion partners have since been identified, highlighting the biological heterogeneity of ALK-driven disease. The introduction of ALK tyrosine kinase inhibitors (TKIs) has transformed outcomes for these patients, with target therapy demonstrating superior efficacy compared with conventional chemotherapy.

Diagnostic Approaches for ALK Rearrangement:

Accurate detection of ALK rearrangements is therefore essential. The Vysis ALK Break-Apart FISH assay historically served as the reference standard, but its technical demands and interpretive variability have prompted broader adoption of alternative platforms. High-sensitivity ALK immunohistochemistry (IHC), particularly the Ventana D5F3 assay, offers a rapid, cost-effective, and reliable screening method. Meanwhile, next-generation sequencing (NGS) provides comprehensive genomic profiling capable of detecting ALK fusions alongside co-occurring driver events, though validation and accessibility constraints remain.

Rationale:
The guideline from College of American Pathologists (CAP), International Association for the Study of Lung Cancer (IASLC) and Association for Molecular Pathology (AMP) supports molecular testing (EGFR, ALK, etc.) in lung cancer. The guideline recognizes IHC (with validated assays) and FISH or other molecular methods as acceptable modalities for ALK rearrangement testing depending on laboratory expertise, tissue availability, and testing context.

Thus, in many centers, ALK IHC (particularly D5F3) is accepted as a stand-alone diagnostic test for therapeutic decision-making.



Feature	FISH	IHC (D5F3/5A4)	RT-PCR	NGS
Detects	DNA-level rearrangement	ALK protein overexpression	Fusion transcripts	DNA/RNA fusion events
Clinical Role	Historical gold standard; companion diagnostic	Widely used screening test	Limited use; research/ specialized labs	Emerging comprehensive genomic profiling tool
Sensitivity	High but variable with small inversions	90–100% with optimized protocols	Very high with good RNA	High (depending on panel)
Specificity	High	90–100%	Very high	Very high
Turnaround Time	Slow	Rapid	Moderate	Moderate–long
Advantages	Direct visualization of rearrangement	Cheap, fast, widely available; excellent morphology	Detects specific variants	Detect multiple genomic alterations in one test; tissue-efficient
Limitations	Requires expertise; expensive; cell-count requirements	Pre-analytical variability; artifacts; rare discordant cases	RNA degradation; limited partner detection	Requires validation; cost; bioinformatics needed

Table 2. Practical Considerations.

Parameter	FISH	IHC	RT-PCR	NGS
Works well on small biopsies?	Sometimes limited	Yes	Often limited	Yes
FDA-approved assay?	Yes (Vysis)	Yes (Ventana D5F3 CDx)	No	No (platform dependent)
Detects unknown fusion partners?	Yes	Yes (protein-based)	No	Yes

Strengths:

- High sensitivity and specificity in aggregated analyses across many studies. D5F3 IHC is effective even in small biopsies or cytology specimens, preserving tissue for additional molecular and biomarker testing.
- Regulatory approval and guideline endorsement support its clinical use to guide ALK inhibitor therapy.
- Rapid turnaround time and lower cost compared with FISH or NGS in many settings.

Limitations / Risks:

- Inter-clone variability exists use of non-D5F3 clones (e.g. ALK1, 5A4) may yield inconsistent results depending on staining method.
- False positives or negatives may occur, particularly if pre-analytic conditions (fixation, tissue quality) or staining protocols are suboptimal.
- Some studies report lower sensitivity or specificity depending on the comparator method and case mix, emphasizing the need for rigorous quality control and use of validated assays.

Below is one of the cases showing strong diffuse cytoplasmic positivity for ALKD5F3 performed in our laboratory.

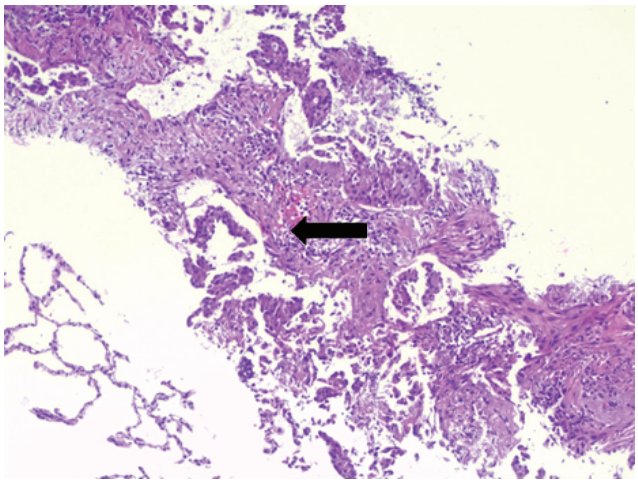


Figure 1a: Showing lung parenchyma exhibiting Adenocarcinoma with signet ring cell features marked by arrow (H&E, X20 magnification).

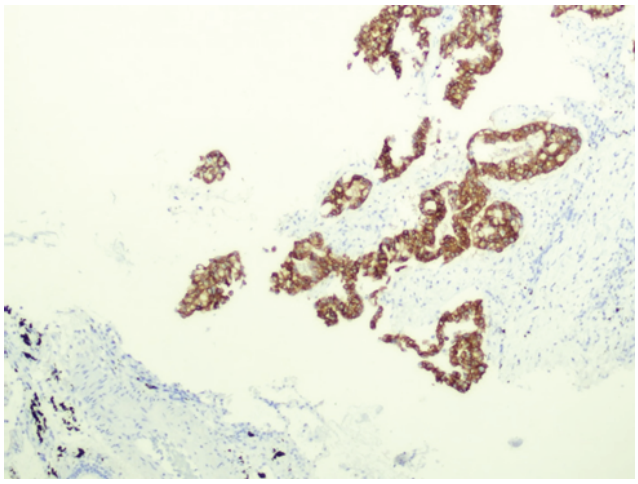


Figure 1b: ALK D5F3 immunohistochemical stain shows strong, diffuse cytoplasmic granular positivity in >90% of tumor cells.

Clinically Actionable Lung Cancer Biomarkers and Corresponding Targeted Therapies in Precision Oncology

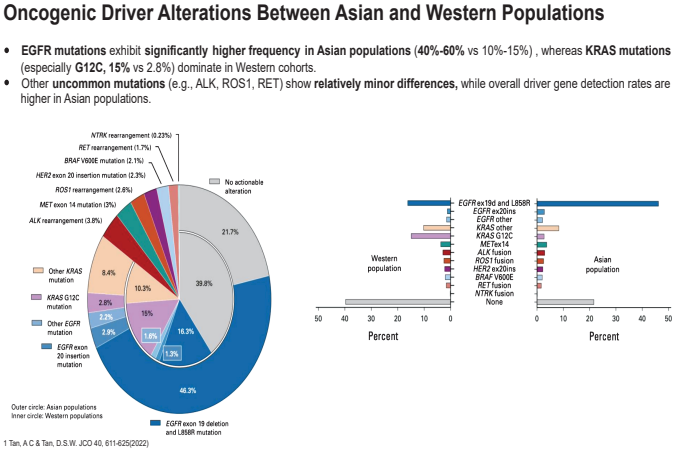
Muneeba Sharif, Nida Naz, Irma Aijaz, Nazneen Islam, Dr. Zeeshan Ansar Molecular Pathology

Introduction

Lung cancer remains the leading cause of cancer-related mortality globally. Over the past decade, major progress in molecular profiling has reshaped the classification and treatment of NSCLC. The discovery of oncogenic driver alterations—such as EGFR mutations, ALK and ROS1 rearrangements, BRAF V600E, MET exon 14 skipping, KRAS G12C, RET fusions, NTRK fusions, and ERBB2 (HER2) alterations—has led to the development of highly effective targeted therapies. These biomarkers not only refine diagnosis but also allow for individualized treatment selection, improving response rates and survival outcomes.

Precision oncology relies on accurate biomarker detection using validated diagnostic modalities, including tissue-based NGS, targeted PCR assays, and circulating tumor DNA (ctDNA) analysis.

Comprehensive molecular testing at initial diagnosis of advanced NSCLC is now strongly recommended by all major guidelines.



Clinically Actionable Biomarkers in Lung Cancer

1. EGFR Mutations
EGFR mutations are among the most common actionable alterations in NSCLC, particularly in adenocarcinoma. Classic sensitizing mutations—exon 19 deletions and exon 21 L858R—respond to EGFR tyrosine kinase inhibitors (TKIs) such as osimertinib, which is the first-line standard of care. Resistance mechanisms, including MET amplification or secondary EGFR mutations, highlight the need for sequential testing at progression.
Targeted Therapies: Osimertinib (first-line), afatinib, erlotinib, gefitinib; emerging therapies for resistance pathways.

2. ALK Rearrangements
ALK rearrangements occur in ~three–five percent of NSCLC, typically in younger, non-smoking patients. Detection via IHC, FISH, or NGS enables use of ALK inhibitors such as alectinib, brigatinib, and lorlatinib, which show high response rates and excellent CNS penetration.
Targeted Therapies: Alectinib (first-line), brigatinib, lorlatinib.

3. ROS1 Fusions
ROS1 rearrangements (one–two percent of NSCLC) are actionable drivers associated with dramatic responses to specific TKIs. Diagnostic testing is commonly performed using FISH, IHC as a screening tool, or NGS for fusion identification.
Targeted Therapies: Crizotinib, entrectinib; lorlatinib in select settings.

4. BRAF V600E
The BRAF V600E mutation defines a distinct molecular subtype of NSCLC. Combination therapy with BRAF and MEK inhibitors has significantly improved outcomes.
Targeted Therapies: Dabrafenib + trametinib.

5. MET Exon 14 Skipping Alterations
MET exon 14 skipping mutations (~three percent of NSCLC) lead to impaired MET degradation and oncogenic activation. Dedicated RNA-based NGS improves detection due to splice-site variability.
Targeted Therapies: Capmatinib, tepotinib.

6. RET Fusions
RET rearrangements occur in one–two percent of NSCLC and respond favorably to selective RET inhibitors.
Targeted Therapies: Selpercatinib, pralsetinib.

7. NTRK Fusions
NTRK fusions are rare but highly actionable across tumor types. Immunohistochemistry or NGS fusion panels are preferred for diagnosis.
Targeted Therapies: Larotrectinib, entrectinib.

8. KRAS G12C
KRAS mutations are the most common driver in NSCLC, with KRAS G12C representing a therapeutically targetable variant. The advent of KRAS G12C inhibitors marks a critical advancement in treatment.
Targeted Therapies: Sotorasib, adagrasib.

9. HER2 (ERBB2) Mutations
HER2 exon 20 insertions define a subset of NSCLC responsive to targeted antibody–drug conjugates.
Targeted Therapies: Trastuzumab deruxtecan.

Diagnostic Approaches and Integration into Clinical Practice

Comprehensive Genomic Profiling
NGS-based multigene panels are now preferred for simultaneous detection of point mutations, indels, copy number alterations, and gene fusions. Both DNA and RNA sequencing enhance fusion detection accuracy.

FISH and IHC
FISH remains a gold standard for ALK and ROS1 fusion confirmation. IHC is a reliable screening tool for ALK and ROS1 and is widely used due to accessibility and rapid turnaround.

Liquid Biopsy
Circulating tumor DNA analysis plays a critical role when tissue is insufficient or inaccessible. It is increasingly used for both initial profiling and detection of resistance mechanisms at progression.

Guideline Recommendations
International guidelines (CAP/IASLC/AMP, NCCN, ESMO) endorse routine testing for all actionable biomarkers in all patients with advanced non-squamous NSCLC and select squamous cases with clinical risk factors.

Implications for Precision Oncology

Targeted therapies have reshaped lung cancer therapeutics by achieving higher response rates, longer progression-free survival, and improved quality of life compared with conventional chemotherapy. Biomarker-driven treatment has become essential for individualized care, and ongoing discovery of resistance pathways and emerging biomarkers continues to refine treatment selection. Incorporating comprehensive molecular profiling into clinical workflows ensures optimal therapeutic decisions and supports enrollment in clinical trials exploring next-generation inhibitors, combination therapies, and immunotherapy synergies.

Conclusion

Precision oncology has revolutionized lung cancer management through the identification of clinically actionable biomarkers and the availability of corresponding targeted therapies. Accurate and timely molecular testing—including NGS, FISH, IHC, and liquid biopsy—forms the foundation for personalized treatment strategies. As targeted therapies expand and molecular insights deepen, biomarker-driven care will continue to transform outcomes for patients with lung cancer.

Adoption of Artificial Intelligence Technologies to Strengthen Laboratory Quality and Diagnostic Precision

Sonana Riaz, Nazneen Islam Dr. Zeeshan Ansar Molecular Pathology Section

Introduction

Artificial intelligence (AI) is rapidly transforming healthcare, and laboratory medicine is one of the fields where its impact is most evident. Laboratories in tertiary-care hospitals in Pakistan face rising pressures such as high sample loads, seasonal infectious disease surges, strict turnaround time requirements, and increasing expectations for accuracy and reliability. Integrating AI into laboratory operations offers a practical way to improve diagnostic precision, streamline workflows, and maintain high-quality standards. This article explains how AI can be realistically incorporated into laboratory systems in Pakistan.

AI for Modern Laboratory Systems in Pakistan

Large hospital-based laboratories routinely process thousands of samples each day, especially during periods of high disease burden such as dengue or influenza seasons. Maintaining precision and consistency under these conditions is challenging. Human fatigue, manual processes, and high workload can contribute to delays and errors. AI helps address these issues by automating repetitive tasks, supporting the interpretation of complex diagnostic data, and improving overall workflow efficiency. Rather than replacing professionals, AI enhances their diagnostic capabilities and strengthens decision-making accuracy. Recent studies show that AI systems can reduce result variability and improve detection of abnormal findings in high-volume clinical settings.

Practical Applications for Laboratory Systems

AI-Assisted Data Handling and Reporting

Many laboratories still rely on manual data entry and verification, which increases the risk of transcription errors and slows down reporting. AI systems can automatically extract patient information, check for inconsistencies, and generate standardized reports. These systems improve accuracy, reduce reporting delays, and free laboratory staff to focus on more complex tasks. Recent work demonstrates that AI-supported reporting can significantly reduce clerical error rates and improve turnaround time.

AI-Based Interpretation of Routine and Specialized Tests AI applications are increasingly valuable in hematology, chemistry, microbiology, and molecular diagnostics. In hematology, AI can detect abnormal cells, analyze morphology, and prioritize slides requiring immediate review. This is particularly useful

during infection surges when CBC requests sharply increase. In clinical chemistry, AI can identify abnormal result patterns and detect potential calibration drift or instrument malfunction. In microbiology, image-based AI tools can analyze culture plates, characterize colonies, and support early identification of resistant organisms—crucial for countries facing rising antimicrobial resistance. Molecular diagnostics also benefit through AI-assisted interpretation of PCR curves and genetic variant detection. Literature from 2021–2024 consistently shows that AI improves diagnostic accuracy and reduces review time across these specialties.

AI for Integrated Diagnostics

Modern patient care increasingly depends on synthesizing laboratory data, radiology findings, and clinical information. AI facilitates this integration by correlating test results with imaging data and highlighting potential inconsistencies. This helps clinicians interpret complex cases more efficiently and supports multidisciplinary decision-making. Studies show that AI-driven integrated diagnostics improves clinical accuracy and reduce missed correlations in complex disease presentations.

AI for Quality Control and Equipment Monitoring

Quality control is central to laboratory operations, and AI enhances this by detecting subtle shifts in QC trends and predicting equipment failures before they affect patient results. AI systems can recognize reagent degradation, identify performance anomalies, and forecast maintenance needs. These capabilities reduce downtime and help maintain continuous workflow reliability. Research indicates that predictive AI models can reduce instrument failure incidents and improve QC stability in clinical laboratories.

AI-Enhanced Laboratory Information Management Systems (LIMS)

AI integrated within LIMS platforms can track samples in real time, predict workflow delays, manage reagent inventory more effectively, and route urgent cases for faster processing. Enhanced LIMS systems also support digital reporting, create automated performance dashboards, and assist in maintaining accreditation standards. Recent advancements show that AI-enabled LIMS improve workflow visibility and reduce processing bottlenecks in high-volume labs.

Challenges and Practical Solutions

AI adoption can face challenges such as cost constraints, staff hesitation, data security concerns, and system integration issues. These can be managed by deploying modular AI tools that do not require major infrastructure changes, offering targeted training to build confidence, implementing strict cybersecurity controls, and coordinating closely across departments to ensure compatibility with existing systems. Evidence from multiple healthcare settings shows that phased implementation significantly improves adoption success.

Conclusion

AI provides an important opportunity to strengthen laboratory systems in Pakistan's leading healthcare institutions. It enhances accuracy, improves efficiency, supports research, and enables laboratory professionals to handle complex diagnostic challenges more effectively. With careful planning, structured implementation, staff training, and cross-department collaboration, AI can modernize the diagnostic workflow, reduce turnaround times, and elevate the overall quality of patient care. As global evidence continues to demonstrate the value of AI in laboratory medicine, adopting these tools thoughtfully can play a transformative role in the future of diagnostic services.

Modern Standards of Pelvimetry in Contemporary Obstetrics; Radiology Update

Dr Hina Pathan and Dr Anam Khan Radiology

Pelvimetry has long played a role in evaluating the maternal pelvis for safe vaginal delivery. Historically performed using conventional radiographs, its utility was limited by poor predictive value and fetal radiation exposure. With modern imaging advancements, the approach to pelvimetry has evolved,

becoming more focused, safer, and more clinically meaningful, especially in selected obstetric scenarios. This update focuses on the essential role of radiology in applying these standards. Its purpose is not to predict delivery success, but to provide accurate anatomic detail that supports individualized obstetric decision-making. Radiologists must therefore report standardized objective measurements, while mode-of-delivery decisions remain the obstetrician's responsibility.

Why Pelvimetry Practices Have Changed

Routine pelvimetry in uncomplicated pregnancies has largely been abandoned. Several key factors contribute to this shift:

- Static bony measurements have limited ability to predict labor outcomes, given that labor involves complex interactions between fetal size, molding, uterine contractility, and maternal pelvic soft tissues.
- Maternal pelvis demonstrates dynamic adaptability, including widening of the pubic symphysis and slight sacroiliac rotation; supine imaging does not capture these physiological changes.
- X-ray pelvimetry exposes the fetus to ionizing radiation, without evidence of improved maternal or neonatal outcomes. A Cochrane review found no benefit and reported increased cesarean rates in women assessed with radiographic pelvimetry.
- Clinical assessment during labor remains the gold standard for diagnosing cephalopelvic disproportion.

  Despite this, pelvimetry retains clinical value when pelvic anatomy may influence delivery planning.

Current Indications for Pelvimetry:

Today, pelvimetry is advised only in circumstances where additional anatomical insight directly supports obstetric decision making:

- Breech presentation with an aim for vaginal breech trial
- History of pelvic fractures, orthopedic deformity, or spinal pathology
- Skeletal dysplasia or suspected pelvic abnormalities
- Masses affecting birth canal (e.g., large fibroids, adnexal masses)
- Prior complex pelvic surgeries

In these contexts, imaging quantifies pelvic diameters and identifies structural or soft-tissue constraints that cannot be assessed accurately by clinical examination alone.

Modern Imaging Modalities

MRI Pelvimetry

MRI is now the preferred method for pelvimetry due to its key advantages like no radiation exposure, superior soft-tissue visualization, high accuracy in measuring the inlet, mid-pelvis, and outlet and helpful for assessing factors beyond bone (e.g., masses, sacrococcygeal angle, soft-tissue narrowing).
MRI is frequently used internationally for vaginal breech evaluation and is now recognized as a valuable tool in individualized delivery planning.
Key MRI Measurements:
1.Obstetric conjugate (inlet)
2.Interspinous diameter (most predictive of difficult vaginal delivery)
3.AP diameter of mid-pelvis
4.Transverse and AP outlet diameters
5.Sacrococcygeal angle / sacral curvature

Low-Dose CT Pelvimetry

CT provides excellent assessment of bony architecture but is limited by fetal radiation exposure.
Ultra-low-dose protocols reduce this risk, yet CT is reserved for cases in which MRI is contraindicated (implants, pacemakers, severe claustrophobia) or is unavailable and urgent evaluation is required.

Low-Dose Stereoradiography (SRI):

Low-dose stereoradiography, utilizes bi-planar slot-scanning X-rays to generate 3D models of the bony pelvis. SRI offers the advantage of extremely low radiation exposure (significantly lower than low-dose CT) while maintaining high accuracy for bony measurements, making it a compelling alternative when MRI is contraindicated or logistically unfeasible

X-ray Pelvimetry

Conventional radiographic pelvimetry is no longer recommended in modern obstetrics due to limited clinical value, increased fetal radiation exposure, availability of safer, more accurate alternatives and standardized Reporting for Clinicians.

Standardized Reporting for Clinicians

To ensure clarity and usefulness for obstetric teams, radiology reports should include:

1. Pelvic Inlet:
a. Obstetric conjugate
b. Transverse diameter
2. Mid-Pelvis:
   a. Interspinous diameter
   b. AP mid-pelvis diameter
3.Pelvic Outlet:
   a. AP and transverse outlet diameters
4. Additional Findings:
5. Shape of sacrum (e.g., flat vs. well-curved)
6. Presence of masses, fibroids, bony anomalies

Conclusion:

Modern pelvimetry has shifted from routine application to a targeted, evidence-based tool within obstetric practice. MRI has emerged as the safest and most reproducible modality, enabling comprehensive assessment without radiation exposure. When used selectively in appropriate clinical scenarios, pelvimetry enhances obstetric planning, particularly for breech delivery and complex pelvic anatomy. Close collaboration between radiologists and obstetricians ensures that imaging findings are integrated thoughtfully into individualized delivery planning, supporting safe, patient-centered obstetric care.

2nd International Allied Health Conference 2025 – Active Participation by AKUH Clinical Laboratory Team.

Mashhooda Irfan Quality Assurance, Clinical Laboratory Medicine

The 2nd International Allied Health Conference, organized by the Office of Allied Health, was held on October 14, 2025, at Aga Khan University under the theme “Three Shifts: Hospital to Community, Treatment to Prevention, and Analog to Digital.”

The event featured pre- and post-conference symposia, workshops, and a scientific program chaired by Dr. Aysha Habib Khan, Professor, Department of Pathology and Laboratory Medicine. The Clinical Laboratory team played an active role across the Scientific, Organizing, and Workshop Committees, with Ms. Mashhooda Irfan, Dr. Yousra Sarfaraz, Ms. Nazneen Islam, and Ms. Mahwish Saleem contributing significantly. Ms. Mashhooda Irfan also served as Co-chair of the Scientific Committee.

Two symposia were organized by the Clinical Chemistry Section: “From Detection to Care: Shaping the Future of Newborn Screening in Pakistan through Allied Health” and “From Hospital to Community: The Expanding Role of Point of Care Testing,” both featuring national and international experts.

The AKUH Clinical Laboratory team submitted four workshops to the Workshop Committee—all of which were accepted, successfully conducted, and well attended by participants from within and outside Aga Khan University Hospital.

Details of the workshops:

- External Quality Assurance: Enhancing Laboratory Quality through Proficiency Testing – led by Ms. Lubna Khaleeq along with sectional QC Coordinators
- Mastering Phlebotomy: Hands-on Skills for Safe and Effective Blood Collection

Conducted by the Phlebotomy Team in collaboration with Ms. Nazneen Islam

- Risk Mitigation in Clinical Laboratory through Individualized Quality Control Plan (IQCP) - Conducted by the Clinical Microbiology Section along with the QA group
- Clinical/Quality Audit: A Tool for Continuous Improvement
  Conducted by the Quality Assurance Group, led by Ms. Saba Sohail

The scientific sessions featured speakers from national and international institutions. The first session, “Hospital to Community,” was moderated by Ms. Mashhooda Irfan, with Dr. Sumaira Farooqi and Dr. Masood ul Hasan serving as chair and co-chair.

An E-poster competition, efficiently managed by Dr. Yousra Sarfaraz and Ms. Mahwish Saleem, concluded the event.

Overall, the conference was a highly rewarding experience, highlighting the active engagement and leadership of the AKUH Clinical Laboratory Team in advancing allied health practices.

Inaugural Session

Organizing Committee

Scientific Committee

Workshop Committee

Chair & Co-chair: Hospital to Community

Moderator, Hospital to Community Session

Hospital to Community Session

Chair & Co-chair and Moderator – Treatment to Prevention session

Clinical Utility and Diagnostic Application of a Hotspot Gene Next-Generation Sequencing Panel in Myeloid Neoplasms

Samina Ghani, Nazneen Islam and Dr Zeeshan Ansar Molecular Pathology

Introduction

Myeloid neoplasms are a heterogeneous group of clonal disorders arising from hematopoietic stem or progenitor cells, including acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), myeloproliferative neoplasms (MPN), and overlap syndromes. Traditional diagnostic methods—morphology, cytochemistry, flow cytometry, and cytogenetics—remain central; however, the integration of molecular profiling has revolutionized disease classification, prognostication, and therapy selection.

The advent of next-generation sequencing (NGS) allows simultaneous interrogation of multiple clinically relevant genes in a single assay. A 58-gene myeloid panel provides comprehensive coverage of recurrently mutated genes involved in signalling pathways, epigenetic regulation, transcription, and RNA splicing. Such panels enhance the precision of diagnosis, enable risk stratification, and guide targeted therapy decisions.

Myeloid Panel (58 genes)

ABL1	BRAF	CEBPA	ETV6	HRAS	KDM6A	NPM1	PTEN	SMC1A	TP53
ANKRD26	CALR	CSF3R	EZH2	IDH1	KIT	NRAS	PTPN11	SMC3	U2AF1
ASXL1	CBL	CUX1	FLT3	IDH2	KMT2A	PDGFRA	RAD21	SRSF2	WT1
ATRX	CBLB	DDX41	GATA1	IKZF1	KRAS	PHF6	RUNX1	STAG1	ZRSR2
BCOR	CBLC	DNMT3A	GATA2	JAK2	MPL	PIGA	SETBP1	STAG2	
BCORL1	CDKN2A	ETNK1	GNAS	JAK3	NF1	PPM1D	SF3B1	TET2	

Note: Gene marked in green full covers the coding sequence

Diagnostic Application

The 58-gene myeloid NGS panel detects single nucleotide variants (SNVs), small insertions/deletions (indels), and selected copy number changes across key driver genes including FLT3, NPM1, CEBPA, DNMT3A, TET2, ASXL1, RUNX1, TP53, IDH1/2, JAK2, CALR, and MPL, among others.

1. Refinement of Diagnosis

- AML: Identification of mutations such as NPM1 or CEBPA double mutations confirms AML even in the absence of defining cytogenetic abnormalities.
- MDS and MDS/MPN overlap: The presence of SF3B1, SRSF2, U2AF1, or ZRSR2 mutations supports clonal myeloid disease and assists in distinguishing reactive cytopenias from neoplastic disorders.
- MPN: Detection of JAK2, CALR, or MPL mutations provides molecular confirmation of a clonal process per WHO fifth edition criteria.

2. Detection of Co-mutations and Clonal Architecture

NGS enables simultaneous identification of multiple mutations, revealing the clonal hierarchy and evolution of disease. Co-occurring mutations (e.g., DNMT3A, ASXL1, and TET2) can indicate early clonal haematopoiesis, whereas FLT3 or RAS pathway mutations often mark disease progression.

Clinical Utility

1. Prognostic Stratification

The European LeukemiaNet (ELN 2022) and WHO 5th Edition (2022) classifications integrate molecular findings into risk categories.

- Favourable risk: NPM1, CEBPA (biallelic)
- Adverse risk: TP53, RUNX1, ASXL1, FLT3-ITD (high ratio)

The ability to identify such mutations in a single assay supports evidence-based prognostication and treatment selection.

2. Therapeutic Decision-Making

Certain mutations directly influence therapy:

- FLT3 mutations → eligibility for FLT3 inhibitors (e.g., midostaurin, gilteritinib).
- IDH1/2 mutations → potential for IDH inhibitors (ivosidenib, enasidenib).
- TP53 mutations → associated with hypomethylating agent resistance and guide toward novel therapies or clinical trials.

NGS results also aid in determining suitability for hematopoietic stem cell transplantation (HSCT) and in monitoring measurable residual disease (MRD) using mutation tracking.

3. Distinguishing Germline from Somatic Variants

Comprehensive gene panels can identify mutations suggestive of germline predisposition syndromes (e.g., DDX41, RUNX1, GATA2), prompting appropriate genetic counselling and family screening.

4. Monitoring Disease Progression and Relapse

Serial NGS testing provides insight into clonal evolution—for example, acquisition of RAS pathway mutations at relapse or persistence of DNMT3A mutations as preleukemic clones. This information supports dynamic therapeutic adjustment.

Technical and Practical Considerations

The 58-gene NGS panel typically requires 50–200 ng of DNA from bone marrow or peripheral blood. Turnaround time ranges from 10–14 days, depending on laboratory workflow.

Analytical sensitivity often reaches two–five percent variant allele frequency (VAF), allowing detection of minor clones.

Interpretation follows AMP/ASCO/CAP guidelines, classifying variants into pathogenic, likely pathogenic, and variants of uncertain significance (VUS), with correlation to morphology, cytogenetics, and clinical context essential for accurate reporting.

Educational and Clinical Impact
For physicians, understanding NGS results is key to integrated diagnostics. Awareness of clinically actionable mutations and their therapeutic correlations enhances patient management.
The comprehensive approach of a 58-gene panel reduces diagnostic uncertainty, supports personalized therapy, and facilitates enrolment in mutation-specific clinical trials.
Continuous education on molecular variant interpretation and updates to classification systems (WHO 2022, ICC 2022, ELN 2022) is crucial to ensure appropriate use of molecular data in daily hematology practice.

Conclusion
The integration of 58-gene myeloid NGS panels into routine hematopathology represents a paradigm shift from morphology-based to molecularly driven diagnostics.
Such testing enables precise classification, prognostic refinement, and personalized treatment planning in myeloid neoplasms.
As sequencing becomes increasingly accessible, the role of the clinician evolves from ordering tests to interpreting molecular data within a multidisciplinary framework, ultimately improving patient outcomes.

Plasma Cell Leukemia: An Aggressive Plasma Cell Neoplasm

Dr Zeenat Hadi Hematology

Case presentation
A fifty-eight-year-old female, known case of hypertension presented to outpatient department with a history of ground level fall two weeks back. She complained of backpain after fall which worsened with standing, prolonged sitting or changes in posture. She also had neck pain radiating to right arm up to thumb for three months. Her X-Ray and MRI lumbosacral spine reported degenerative changes in dorsal spine with compression fracture of D12 vertebra and altered marrow signals. While CT head demonstrated multiple metastatic lesions in skull vault with soft tissue involvement in some areas. Bilateral pleural effusion with subpleural lung nodules were identified along with metastatic skeletal deposits on CT CAP. Cytology of pleural fluid was negative for malignancy.

Based on these findings, work up for multiple myeloma was carried out. Bone marrow aspirate showed 50% plasma cells with diffuse infiltration of bone trephine by plasma cells establishing the diagnosis of multiple myeloma. Immunofixation and serum free light chain assay revealed lambda light chain monoclonal gammopathy. She underwent six cycles of VRD regimen (Bortezomib, Lenalidomide, Dexamethasone) of chemotherapy and was kept on maintenance with Dexamethasone and Lenalidomide. The latter was discontinued due to lower limb neuropathy and persistent thrombocytopenia. She had persistent bilateral pleural effusion despite treatment. After 14 months, during a follow-up visit she complained of increase in backpain, generalized weakness, intractable cough and shortness of breath. Her peripheral blood smear was reviewed which revealed normocytic normochromic anaemia with rouleaux formation, thrombocytopenia and 48% plasma cells (as shown in Figure 1) demonstrating transformation to plasma cell leukemia. A plasma cell is a round to ovoid cell with abundant basophilic cytoplasm and a round eccentric nucleus with prominent perinuclear halo. The nucleus has coarse chromatin arranged in “spoke wheel” pattern (as illustrated in Figure 2). The patient was then started on CyBorD chemotherapy but failed to achieve remission. She was then shifted to palliative care.

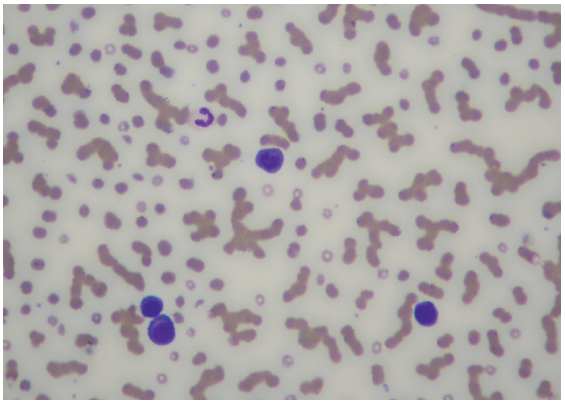


Figure 1

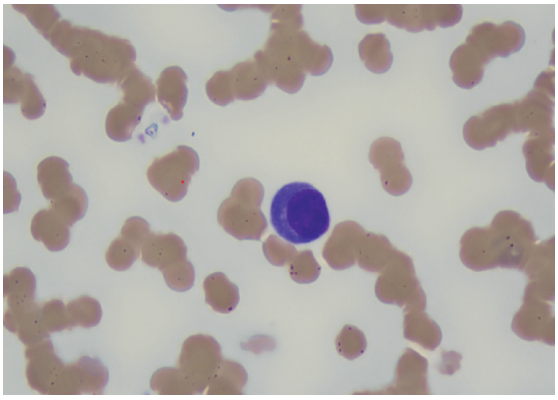


Figure 2

Discussion

Plasma cell leukemia is a rare aggressive disorder of clonal plasma cells. The diagnosis requires presence of ≥five percent plasma cells on peripheral blood smear examination along with fulfilment of diagnostic criteria for multiple myeloma (Table 1). It is classified as primary and secondary plasma cell leukemia based on clinical presentation, as the initial manifestation of disease or later transformation from multiple myeloma respectively. Primary plasma cell leukemia usually presents at a younger age and has a better median overall survival as compared to secondary plasma cell leukemia. However, the overall prognosis is poor.

Table 1: International Myeloma Working Group Revised Diagnostic Criteria For Multiple Myeloma And Related Plasma Cell Disorders.

Disorder	Disease definition

Multiple myeloma	Both criteria must be met:
	• Clonal bone marrow plasma cells ≥10% or biopsy-proven bony or extramedullary plasmacytoma
	• Any one or more of the following myeloma defining events
	  ○ Evidence of end organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically:
	  ▪ Hypercalcemia: serum calcium >025 mmol/L (>1 mg/dl) higher than the upper limit of normal or >275 mmol/L (>11 mg/dl)
	  ▪ Renal insufficiency: creatinine clearance <40 ml per minute or serum creatinine >177 µmol/L (>2 mg/dl)
	  ▪ Anaemia: haemoglobin value of >2 g/dl below the lower limit of normal, or a haemoglobin value <10 g/dl
	  ▪ Bone lesions: one or more osteolytic lesions on skeletal radiography, computed tomography (CT), or positron emission tomography-CT (PET-CT)
	  ○ Clonal bone marrow plasma cell percentage ≥60%
	  ○ Involved: uninvolved serum free light chain (FLC) ratio ≥100 (involved free light chain level must be ≥100 mg/L)
	  ○ >1 focal lesions on magnetic resonance imaging (MRI) studies (at least 5 mm in size

Plasma cell leukemia	Both criteria must be met: • Meets diagnostic criteria for multiple myeloma • Presence of 5% or more plasma cells in conventional peripheral blood smear white blood cell differential count

The discussed case highlights the presentation of a patient with secondary plasma cell leukemia and the challenges faced during the management. Despite using multiple lines of chemotherapy there was failure to achieve remission. It represents the refractory nature of this disease.

Silent Genes, Loud Symptoms: The Story of Hemoglobin H

Dr Muhammad Shayan Ashfaq Hematology

CASE PRESENTATION:

A five-year-old girl presented to the outpatient department with low grade fever, shortness of breath and generalized weakness. Her skin and eyes had developed a yellowish tint over the past three days. She had no history of recent travel, insect bites or medication use.

She had a history of recurrent anemia, requiring multiple red cell transfusions, the most recent one being two months earlier. She had experienced episodes of weakness and pallor throughout her life. The frequent need for transfusions had started early in childhood and she had been treated at different hospitals for this condition.

Family history revealed consanguinity between her parents and one of her brothers had transfusion-dependent thalassemia. The other siblings were reported to be healthy.

On physical examination, she appeared pale, mildly jaundiced and had difficulty breathing at rest. Her abdominal examination showed mild hepatomegaly and an enlarged spleen.

Laboratory investigations revealed a hemoglobin of 5.4 g/dL, hematocrit of 21.3 percent, and RBC count of 3.27 million/µL. Red cell indices showed microcytic hypochromic anemia with an MCV of 65.1 fL, MCH of 16.5 pg, and MCHC of 25.4 g/dL. The peripheral blood film showed microcytosis, hypochromia, anisocytosis, poikilocytosis, polychromasia, target cells, teardrop cells, basophilic stippling, fragmentation and nucleated red blood cells (Figure 1).

Hemoglobin electrophoresis by HPLC showed HbA of 99 percent while a notably decreased HbA2 of one percent (reference range: 2.4–3.2 percent). In addition, a fast-moving hemoglobin band with a retention time of less than 1 minute was noted on the chromatogram (figure 2).

As a next step, supravital staining with Brilliant Cresyl Blue was performed, which gave a characteristic "golf ball" appearance, as multiple intracellular inclusions, suggesting the presence of Hemoglobin H (Figure 3).

Based on the clinical picture, history of transfusion dependency, physical findings of hepatosplenomegaly, consanguinity in the family, a sibling with thalassemia, blood film examination, HPLC findings, and supravital staining results, the provisional diagnosis of Hemoglobin H disease was made, a form of Alpha Thalassemia.

For confirmation, molecular testing (Multiplex Ligation-dependent Probe Amplification for alpha-globin gene deletions) was advised.

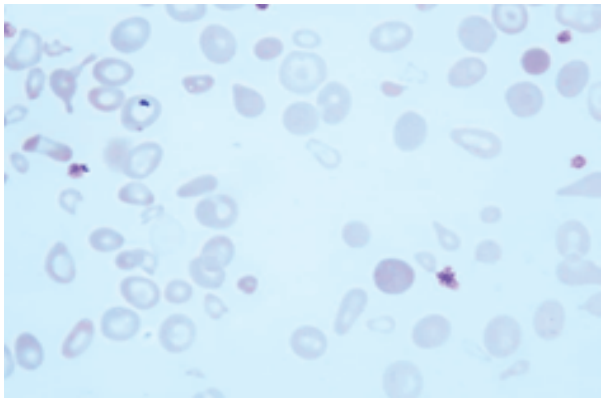


Figure 1: Peripheral blood smear of the patient with HbH disease at 100x

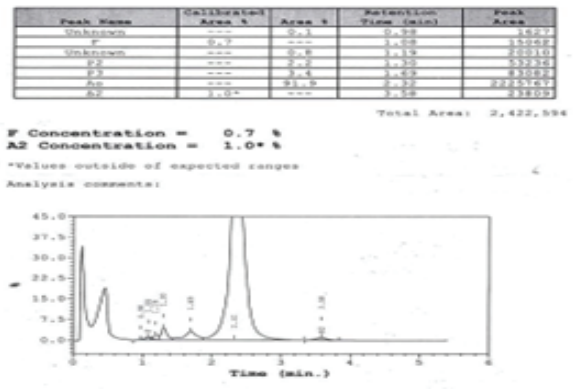


Figure 2: HPLC chromatogram showing a fast-moving hemoglobin peak before one minute, indicating HbH

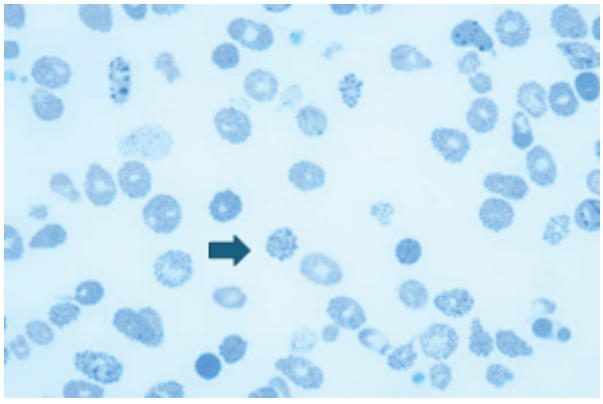


Figure 3: HbH preparation, by supravital staining, showing golf ball inclusions, at 60x

Discussion:

Hemoglobin's primary function is to transport oxygen from the lungs to tissues. Hemoglobin is a tetrameric protein composed of two α and two non-α (β, γ, or δ) globin chains, each bound to a heme group.
Alpha thalassemia results from reduced or absent synthesis of α-globin chains, which are essential components of hemoglobin (HbA = $\alpha_2\beta_2$). Humans normally have four α-globin genes (two on each chromosome 16: αα/αα). The severity of disease depends on how many α-globin genes are affected:

1. Silent Carrier (–α/αα): One gene deletion, asymptomatic.
2. Alpha Thalassemia Trait (–α/–α or —/αα): Two gene deletions, mild microcytic anemia.
3. Hemoglobin H Disease (—/–α): Three gene deletions, moderate to severe anemia with HbH formation.
4. Hemoglobin Bart's Hydrops Fetalis (—/—): Four gene deletions, lethal in utero or soon after birth

Hemoglobin H (HbH) disease occurs when three out of the four α-globin genes are deleted or dysfunctional (—/–α). The marked deficiency of α-chains results in the formation of β_4 tetramers (HbH), which have abnormally high oxygen affinity and are unstable, leading to premature red cell destruction. A common non-deletion subtype of Hemoglobin H is Hemoglobin Constant Spring, which tends to cause more severe disease due to interference with the transcription of the normal alpha gene.

Clinically, HbH disease presents with variable anemia, jaundice, pallor, and splenomegaly. The severity can fluctuate, often worsening during infections or stress. Peripheral smear findings typically include microcytosis, hypochromia, target cells, and nucleated red cells, while supravital staining with Brilliant Cresyl Blue shows the characteristic "golf ball" inclusions due to precipitated HbH.

Diagnosis is supported by HPLC or electrophoresis showing a fast-moving HbH band, and confirmed by molecular studies MLPA (Multiplex Ligation-dependent Probe Amplification) detecting three α-gene deletions.

Empowering Quality Through Clinical Audit: Highlights from Our Interactive Workshop

Saba Azeem Quality Assurance, Clinical Laboratory Medicine

"Improving Today's Care to Prevent Tomorrow's Harm" was the theme of our most recent Clinical and Quality Audit Workshop, which was held as a post-conference on October 16, 2025, as part of the 2nd International Allied Health Conference 2025. It was organized by the Allied Health Department of Aga Khan University and Hospital Karachi by the help of Ms Saba Sohail, Assistant Manager, Quality Assurance, Clinical lab.

Healthcare professionals from a variety of specializations came together for the four-hour interactive event to hone their abilities in conducting meaningful clinical audits, a crucial instrument for promoting patient safety and continuous improvement.

Demystifying the Audit Cycle
Guiding participants through the core of the session, Ms. Saba Sohail, (Assistant Manager, Quality Assurance Clinical Lab) presented “The Steps of the Clinical Audit Cycle.” Her session combined mini-lectures with real examples, demonstrating how each phase — from topic selection to re-audit — plays a crucial role in sustaining quality. Her engaging style kept participants attentive and inspired to apply these steps in their own departments.

Setting the Stage:

The session opened warmly with **Dr. Erum Khan** (Chairperson, Pathology and Laboratory Medicine) who broke the ice and encouraged participants to share their expectations. The room soon came alive with engaging conversation.

Building the Foundation of Clinical Audits

The first talk by **Dr. Sibtain Ahmed** (Vice Chair Pathology and Laboratory Medicine) delved into the essence of “What is Clinical/Quality Improvement Audit?” — highlighting how audits differ from research and quality improvement projects yet serve as a bridge between the two.

Fun Meets Learning

The energy didn't drop for a moment. Ms Saba Hameed led a lively Kahoot quiz, testing participants' understanding in a fun, competitive way. It was proof that learning about audits could indeed be enjoyable!

From Learning to Doing

After a short tea break, the room transitioned from listening to doing. During the interactive brainstorming activity, facilitators Ms. Mashhooda Irfan, Ms. Saba Sohail, Ms. Bushra Ahmed, and Ms. Gulnaz Wahid, guided small groups through designing their own audit proposals. Laughter, debates, and lightbulb moments filled the room as participants worked together to translate concepts into real plans and presented

The workshop concluded with Mr. Waqar Ahmed Mirza (Director, Laboratory Services) delivering the closing remarks and vote of thanks to the speakers and facilitators, a gesture of appreciation for their dedication to cultivating a culture of continuous improvement

Celebrating Teamwork and Achievement

Collective Step Forward By the end of the day, participants walked away not just with knowledge, but with a renewed sense of purpose.
Through this workshop, our department reaffirmed its mission: “Improving today’s care to prevent tomorrow’s harm.”

When an Uncommon Yeast Grows from Blood: Clinical Correlation Is Essential

Faheem Naqvi, Mohammad Zeeshan Microbiology

Background

Isolation of an unexpected yeast — such as Kodamaea ohmeri or Scheffersomyces species, Candida pelliculosa, C. rugosa, C.utilis, C.kefyr, C.guillermondii — from a blood culture presents a diagnostic puzzle. Once considered environmental organisms, these “non-classical” yeasts are increasingly recognized as emerging opportunistic pathogens specially in paediatric and immunocompromised populations. For the physician, the key challenge lies in determining whether these isolates represent true bloodstream infection. The answer requires close clinical correlation and appropriate use of laboratory tools — including β-D-glucan (BDG) testing.

Why It Matters

Kodamaea ohmeri: This environmental yeast has emerged as a cause of candidemia, especially in Asia where most reported cases have come from East and Southeast Asia. Its appearance in bloodstream infections is notable in neonates and severely ill adults with indwelling lines. Misidentification using standard systems is common and some isolates show reduced susceptibility to echinocandins, underscoring the need for accurate species identification and antifungal susceptibility testing.

Candida pelliculosa: Frequently reported in neonatal intensive-care units in Asia (e.g., Taiwan, Korea) as the cause of nosocomial fungemia clusters. The risk population is preterm or low-birth-weight infants with central lines or TPN; outbreaks reflect potential for cross-transmission, hence stringent infection control is essential.

Candida rugosa: Although rarer, C. rugosa has been documented as an emerging cause of bloodstream infections globally including Pakistan. The clinical significance lies in its unpredictable susceptibility profile e.g., high MIC of echinocandins, which makes empiric therapy more challenging.

Candida utilis: This species is an uncommon but documented cause of candidemia, including in pediatric or neonatal settings in Asia where ICU stay and central venous catheters were risk factors. Rapid identification and awareness are key; in reported cases the isolates were susceptible to common antifungals but clinical awareness remains low.

Candida kefyr : Emerging reports, including from the south Asian region, show C. kefyr causing bloodstream infections especially in immunocompromised patients (hematologic malignancy). While still rare, the trend is towards increasing recognition in tertiary centres and thus warrants inclusion in surveillance and antifungal considerations.

Candida guilliermondii: Candidemia in neonate and infants with indwelling catheters, prolonged antibiotics and parenteral nutrition has been documented in south east Asia, especially among children and the hospitalised population; and has been associated with higher MICs to azoles.

Scheffersomyces species: This rare human pathogen has been reported from blood cultures in neonatal units from Pakistan.

These organisms, though rare, can cause serious systemic infections with high morbidity if not recognized early. Yet, overtreatment with antifungals when they are merely contaminants can be equally harmful.

DISTINGUISHING CONTAMINANT FROM TRUE INFECTION

Indicator	Suggestive of True Infection
Clinical picture	Fever, hypotension, respiratory distress, or unexplained deterioration
Host factors	Neonate, low birth weight, NICU stay, malignancy, transplant, ICU stay, central venous line, immunocompromised, indwelling catheters, recent surgery, broad-spectrum antibiotic uses
Microbiology clues	Multiple positive bottles, rapid time-to-positivity, isolation from sterile sites
Species identification	Confirmed by VITEK ID CARD, MALDI-TOF or sequencing rather than conventional identification methods
Biomarker support	Positive β-D-glucan (BDG) result consistent with invasive fungal disease. In rare fungal species these might be negative

The Role of β-D-Glucan (BDG)

(1→3)-β-D-glucan is a cell wall component of most pathogenic fungi, including Candida and Aspergillus. When detected in serum, it serves as a non-culture-based marker of invasive fungal infection.

The Laboratory–Clinician Partnership

1. Immediate communication: Report unusual yeasts and discuss the patient's status and device use.
2. Repeat cultures: Confirm persistence and compare time-to-positivity from catheter vs. peripheral draws.
3. Send for accurate identification: Use VITEK ID card, MALDI-TOF or molecular sequencing if needed.
4. Request antifungal susceptibility testing: Emerging yeasts often show variable resistance patterns.
5. Correlate BDG trends: Rising BDG with symptoms → treat promptly; low BDG and stable patient → reassess and observe.

Not every yeast in the bloodstream is an infection, but every yeast deserves attention.

Incorporating β-D-glucan testing into the diagnostic pathway helps distinguish true fungemia from contamination and ensures antifungals are used judiciously and effectively. Clinical correlation, supported by laboratory expertise remains the cornerstone of safe patient care.

HPV Genotyping and its Clinical Significance with Pap Smear

Syeda Fazeelat Zehra , Dr Zahra Hasan, Ayesha Sharif, and Madiha Jamali Molecular Pathology

Introduction

Cervical cancer remains a major preventable cause of morbidity and mortality among women worldwide. In Pakistan, it is the second most common cancer amongst women, causing 3000 deaths each year. Human papillomavirus (HPV) comprises of more than 200 different types mostly associated with low-risk infections, often causing warts. There are about 14 high-risk (hr) HPV and persistent infection with these is the primary cause of cervical intraepithelial neoplasia (CIN) and invasive carcinoma. HPV is transmitted through sexual and close contact with an infected individual. HPV infections are usually clear naturally, but chronic infections can lead to neoplasia.

Standard screening for cervical cancer relies on cytology (Pap smear) testing. However, this is based on identification of an abnormal smear or ASCUS. Hence, Pap smear will identify dysplasia, neoplasia or other abnormalities at a later stage. Hence early identification of HPV, which is associated with cervical cancer, is standard of care for screening.

The Cobas HPV Test detects 14 high risk HPV types, in a single automated assay on the Cobas® 5800 system. It is an automated qualitative in vitro test for HPV that utilizes amplification of target DNA by the Polymerase Chain Reaction (PCR) and nucleic acid hybridization for the detection of 14 high-risk (HR) HPV types in a single analysis. The test specifically identifies HPV16 and HPV18 while concurrently detecting the other high-risk types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) at clinically relevant infection levels. Specimens can be collected in Roche Cell Collection Medium (Roche Molecular Systems, Inc., or Sure Path™ Preservative Fluid (BD Diagnostics-Tri Path). Hence, it is compatible with co-testing with Pap smear. In 2021, the World Health Organization recommended DNA based HPV testing as a first line test for screening of cervical cancer (7). The Centers for Disease Control (CDC), USA recommended that for women aged between 30-65 a cytology test to be conducted every three years, an HPV test alone every five years, or a cytology test plus an HPV test (co-test) every five years.

Clinical Implications of Genotyping

Combining HPV DNA testing with cytology increases sensitivity, allows longer screening intervals after negative results, and improves risk stratification when genotyping is included. HPV 16 and 18 are the most common HPV types associated with cervical cancer. Molecular assays targeting HPV DNA, particularly those with partial genotyping such as HPV16/18, are increasingly used in screening programs either as primary tests or alongside cytology.

Women positive for HPV16 or HPV18 require urgent follow up, often colposcopy. Women positive for other HPV types may undergo cytology triage, reducing unnecessary referrals. Genotyping enhances risk discrimination in co testing or primary HPV screening.

Integration with Pap Smear

Cytology remains widely used but has lower sensitivity for high grade lesions. HPV testing, either as co testing or primary screening with reflex cytology, improves detection and supports longer screening intervals. Evidence shows HPV testing identifies more advanced disease than Pap smear alone.

Advantages of HPV Genotyping

- Greater sensitivity for CIN2+ lesions.
- Strong reassurance with negative results.
- Clear risk stratification: HPV16/18 carry higher oncogenic risk.
- Enables targeted follow up and reduces system burden.

Screening Pathways

1. First Cytology, HPV genotyping is abnormal.
2. Primary HPV screening, cytology triage if positive.
3. Co testing with HPV and cytology for combined sensitivity and specificity.

Relevance in Resource Limited Settings

HPV testing can be applied in low- and middle-income countries. In Pakistan, where cytology quality varies and screening uptake is low, HPV genotyping could expand coverage, improve detection, and enhance risk stratification. This is particularly relevant as self-collection of swabs for HPV testing has been trialed in different settings and found to have equivalent efficiency for HPV testing. As access to health care providers may be difficult and there is hesitation to seek screening for female reproductive health. Recent national HPV vaccination campaigns further strengthen prevention strategies (6–8).

Limitations

False positives and negatives remain possible with HPV testing due to variability in sampling and viral load in specimens. Positive HPV without cytological abnormalities may cause anxiety or overtreatment. Effective follow-up services are essential. Cytology still detects certain lesions not identified by HPV testing. Vaccination programs may alter genotype prevalence and screening strategies.

Future Directions

- Real world studies in South Asia to assess performance and cost effectiveness
- Self-collection of swabs for HPV testing may increase the ease of testing thereby enhancing the capture of HPV positive cases in the population. Recently there is an expanded focus on HPV in Pakistan.
- A national HPV vaccination campaign was introduced in 2025 for girls aged 11 – 14 years in Pakistan in September 2025. This new initiative is with a bivalent vaccine against HPV 16 and 18 to protect against the most common type of strain.
- In the future it may be necessary to expand genotyping beyond HPV16/18 if the prevalence pattern changes.

Conclusion

The Cobas HPV Test on the Cobas 6800 system is a reliable, high throughput assay for HPV detection and partial genotyping. Its sensitivity, specificity, and automation make it well suited for modern screening. When combined with or replacing cytology, it improves detection of precancerous lesions, enhances risk stratification, and supports efficient screening pathways. In countries like Pakistan, HPV genotyping offers a practical solution to limited cytology infrastructure, provided follow up and cost-effective implementation are ensured.

The Best of the Past

Specialist # Mammography #Radiology#Followtheirlead
Interviewee: Ms. Zareen Khan
Interview recorded by Dr. Shayan Anwar

1. Thinking back on your time as a Mammography Specialist at your organization, can you share a moment—an "aha" moment—when you felt especially excited or proud to be part of the team?

One of my proudest 'aha' moments was when I was selected from my department for an international training and attachment at Beth Israel Deaconess Medical Center in Boston, USA. This opportunity was granted through Aga Khan University Hospital with the support of hospital donors, and being chosen for this program was a great honor for me.

As a female mammography trainer, I was also given the opportunity to travel to Kabul, Afghanistan, to train radiographers at FMIC. This experience was especially meaningful because I was able to contribute to capacity building in a challenging healthcare environment.

Additionally, I completed an attachment at Shaukat Khanum Cancer Hospital, Lahore. In Pakistan, we are among the pioneers in implementing Contrast-Enhanced Mammography, a new technology that enables faster and more accurate diagnosis. These experiences made me extremely proud to be part of a progressive and impactful mammography team.

2. Could you briefly walk us through your journey with the organization, from your induction to becoming a Mammography Specialist?

I began my professional journey at Aga Khan University Hospital as a Trainee Radiographer. Over time, through continuous learning, dedication, and hands-on experience, I progressed to my current role as a Mammography Specialist. During my training, I gained exposure to multiple modalities within radiology; however, mammography quickly became my area of greatest interest. I was especially drawn to learning new technological advancements, refining imaging techniques, and developing strong hands-on clinical skills. I also developed a passion for teaching and mentoring others in mammography, which further motivated my growth in this specialty.

3. When you think about your values and the work you do, what is the one thing your profession has given you that you value the most?

The most valuable thing my profession has given me is the opportunity to work in a supportive and friendly environment with dedicated colleagues, while delivering high-quality patient care without any compromise on standards or procedures. I take great pride in being part of a team that prioritizes patient safety, accuracy, and compassion.

I also strongly believe that learning never truly stops in one's career. Every day brings new experiences, advancements, and opportunities to grow, and this continuous learning is something I deeply value in my profession.

4. As a senior radiographer in the country, how have you seen mammography practices grow among allied health professionals in Pakistan, and how do you imagine things evolving over the next 10 years?

As a senior radiographer in Pakistan, I have witnessed significant growth in mammography practices among allied health professionals. At our hospital, we adhere to international standards to ensure that patients receive high-quality and safe imaging services. This commitment has helped elevate the overall level of care and professionalism in the field.

Looking ahead over the next 10 years, I see artificial intelligence and emerging technologies playing an increasingly vital role in mammography procedures. Despite these advancements, the role of the radiographer will remain central—combining technical expertise, clinical judgment, and patient care to ensure accurate diagnoses and optimal outcomes.

5. What advice would you like to share with junior radiographers who are just starting their careers?

My advice to junior radiographers just starting their careers is to embrace the vast opportunities that radiology offers, especially if they are passionate about building a long-term career in this field. Radiology is constantly evolving, so staying curious, committed, and proactive in learning new techniques is key.

At Aga Khan University Hospital, trainees are fortunate to receive a stipend during their training, which allows them to focus fully on developing their skills. This is a unique advantage compared to many other hospitals where trainees may have to pay for their own training. I encourage juniors to make the most of such opportunities and to always value hands-on experience, mentorship, and continuous learning.

Polaroid

The 2nd International Allied Health Conference, held on 14th October, was a wonderful success! The event saw enthusiastic participation from allied health staff, trainees, students, and faculty members across various disciplines, making it a truly enjoyable and memorable day. Chair, Conference: Dr. Ayesha Habib Khan Chair, Scientific Committee: Dr. Shayan Anwar

46th International Conference of Pakistan Association of Pathologists 2025 - 11th Joint Conference of Societies of Pathology | 10-12 October 2025 | PC Hotel Lahore. The theme for this year's conference was "Pathology Meets AI: Innovation, Challenges & Future Directions." There was significant participation from AKUH Pathology and Laboratory Medicine teams. The event provided an excellent platform for learning and collaboration, offering valuable insights into the latest advancements in research and pathology. It greatly enhanced our understanding of emerging trends and the integration of artificial intelligence in the field.

Professors Shahid Pervez, Naila Kayani, and Afia Zafar being honoured with the Lifetime Achievement Award at the PAP Conference 2025.

Department of Pathology and Laboratory Medicine faculty and staff celebrating 40 years of excellence at Aga Khan University

Hematology

The Section of Hematology celebrated World Thrombosis Day with great enthusiasm, organizing a CME seminar to promote awareness about thrombosis and its prevention.

Dr. Rimsha Imran, Resident II, Hematology, presented her poster titled “Defining Maximal Aggregation Responses in Patients with Low Platelet Counts: A Study on Light Transmission Aggregometry” at the European Congress on Thrombosis and Hemostasis (ECTH 2025), held in Prague, Czech Republic, from October 22–24, 2025.

Molecular Pathology

A dissemination seminar for the project "SARS-CoV-2 Genetic Variation and its Impact on COVID-19 Immunity" was held at AKU as a hybrid event, supported by the Grand Challenges Fund, HEC Pakistan. Over the past 3 years, our national and international collaborators have uncovered critical insights into the COVID-19 pandemic—through pathogen genomics, host transcriptomes, and vaccine studies.

Chemical Pathology

"Co-Creating Preventive Education Strategies to Combat Drug Abuse in Schools". World Café session as part of the AKU Scholarship of Teaching and Learning (SoTL) Grant Workshop, led by Dr Sibtain Ahmed, alongside Dr. Tasneem Anwar (AKU IED), Dr. Lena Jafri, Dr. Yumna, and Dr. Yousra. This interactive session brought together a dedicated group of schoolteachers for a deep, collaborative dialogue on developing preventive education lesson plans aimed at addressing drug abuse in school settings.

آغا خان یونیورسٹی ہسپتال
The Aga Khan University Hospital

Myeloid Neoplasms by Next Generation Sequencing

Department of Pathology and Laboratory Medicine

UPDATE NO:10,VOL NO:XXXI,2025

November 2025

The information contained in this flyer is intended for healthcare professionals.

INTENT OF USE:

Myeloid NGS panel is intended for the molecular evaluation of myeloid neoplasms, including myeloproliferative neoplasms (MPN) myelodysplastic syndromes (MDS), myelodysplastic/myeloproliferative neoplasms (MDS/MPN), acute myeloid leukemias (AML), systemic mastocytosis, and myeloid neoplasms associated with eosinophilia and gene rearrangements. This assay, that interrogates 58 genes of interest, is most relevant to myeloid cancers.

INTRODUCTION:

Next-generation sequencing (NGS) is a comprehensive molecular diagnostic approach that allows simultaneous analysis of multiple genomic regions in tumor DNA within a single assay. While many hematologic neoplasms share morphologic or phenotypic features, they may harbor distinct somatic mutations that enable more precise classification. Moreover, several myeloid neoplasms present with a normal karyotype at diagnosis but can still be identified, confirmed, and classified based on their mutational profiles. Patients with unexplained cytopenia may exhibit acquired genetic alterations in hematopoietic cells—referred to as clonal cytopenia of uncertain significance (CCUS)—which carry a risk of progression to overt myeloid malignancies. The detection and interpretation of gene mutations in suspected or confirmed myeloid neoplasms provide essential diagnostic, prognostic, and therapeutic insights that guide clinical management.

IMPORTANT NOTE:

This test is for the evaluation of known or suspected hematologic neoplasms of myeloid origin (e.g., AML, MDS, MPN, MDS/MPN, and unexplained cytopenia) at the time of initial diagnosis or at disease relapse.
Its clinical utility includes:

- Assisting in accurate diagnostic classification
- Providing prognostic and therapeutic information to guide patient management
- Detecting newly acquired, clinically significant gene mutations at relapse

PRINCIPLE:

Next Generation Sequencing (NGS)

SPECIMEN TYPE:

Whole Blood in EDTA tubes

CHARGES:

PKR 150,000/ *Revisions may apply

SCHEDULE:

Test is performed 1st Monday of the month. Report will be issued after 20 days.

ADDITIONAL INFORMATION :

Myeloid Panel (58 genes)

ABL1	BRAF	CEBPA	ETV6	HRAS	KDM6A	NPM1	PTEN	SMC1A	TP53
ANKRD26	CALR	CSF3R	EZH2	IDH1	KIT	NRAS	PTPN11	SMC3	U2AF1
ASXL1	CBL	CUX1	FLT3	IDH2	KMT2A	PDGFRA	RAD21	SRSF2	WT1
ATRX	CBLB	DDX41	GATA1	IKZF1	KRAS	PHF6	RUNX1	STAG1	ZRSR2
BCOR	CBLC	DNMT3A	GATA2	JAK2	MPL	PIGA	SETBP1	STAG2	
BCORL1	CDKN2A	ETNK1	GNAS	JAK3	NF1	PPM1D	SF3B1	TET2	

Gene marked in green fully covers the coding sequence

For more information please call: 021 3486 1620
or Email: laboratory@aku.edu

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CAP ACCREDITED COLLEGE of AMERICAN PATHOLOGISTS

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The Aga Khan University Hospital

ROS1 gene rearrangement by FISH in formalin-fixed paraffin embedded (FFPE)tissue

Department of Pathology and Laboratory Medicine

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The information contained in this flyer is intended for healthcare professionals.

INTENT OF USE:

This test is based on the **Fluorescent In-situ Hybridization** technique using a specialized **ROS1(6q22) Gene Fusion Probe Kit** to detect ROS1 gene rearrangement and is useful for identifying tumors that may be sensitive to targeted therapy.

INTRODUCTION:

ROS1 rearrangements occur in ~1–2% of Non-Small Cell Lung carcinoma (NSCLC), typically in younger, non-smoking patients with adenocarcinoma. A positive result identifies patients eligible for ROS1-targeted TKIs (e.g., crizotinib, entrectinib, lorlatinib treatment), which yield high response rates. The ROS1 gene, located on chromosome 6q22, encodes a receptor tyrosine kinase. Chromosomal rearrangements involving the fusion of the 3′ region of ROS1 with the 5′ region of diverse partner genes were initially described in non-small cell lung carcinoma (NSCLC). Since then, ROS1 fusions have been reported in a spectrum of neoplasms, including inflammatory myofibroblastic tumors and cutaneous melanocytic tumors, among others.

From a clinical perspective, evidence indicates that tumors harboring ROS1 fusions are potentially sensitive to targeted therapy, underscoring their relevance as a predictive biomarker.

IMPORTANT NOTE:

In non-small cell lung carcinoma (NSCLC), ROS1 rearrangements are most frequently seen in adenocarcinomas, often with solid, acinar, or papillary growth patterns and signet-ring cell features.
The identification of a ROS1 fusion has important predictive and therapeutic implications, as such tumors may respond to ROS1-targeted tyrosine kinase.

ADDITIONAL INFORMATION :

ROS1 fusions are enriched in younger patients, never-smokers or light smokers, and those with advanced-stage lung adenocarcinoma.
Positive ROS1 results provide a predictive biomarker for eligibility for TKI therapy, which can lead to significant and durable clinical responses.
Negative results do not exclude other actionable fusions (e.g., ALK, RET, NTRK, MET), for which comprehensive molecular profiling may be considered.

PRINCIPLE:

Fluorescent in situ hybridization (FISH)

SPECIMEN TYPE:

Formalin-fixed paraffin-embedded (FFPE) NSCLC specimen.

CHARGES:

PKR 30,000/ *Revisions may apply

SCHEDULE:

Test is performed every Thursday; report will be issued after 7 days .

For more information please call: 021 3486 1620
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