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EDITORIAL

As we unveil this year's second edition, we find ourselves at a pivotal moment in laboratory medicine. The intersection of innovation, precision, and collaboration continues to redefine what is possible in research and diagnostics.

In this issue, we feature diverse contributions, including advancements in molecular diagnostics, highlighted by an article on the *"Application of FISH Probes in Clinical Diagnostics."* Additionally, we explore the transformative role of artificial intelligence in the field of radiology.From the histopathology section, we bring you insightful reads such as *"Leadership Skills and Capacity Building in Histopathology"* and *"Diagnostics, Accuracy, and Pitfalls of Frozen Section."* The edition also covers impactful topics in diagnostics, including Anti-GBM Disease, malarial infection, and more.

This year has also been marked by incredible achievements, which we are excited to share in our Happenings section. Notably, we take immense pride in our team's success in achieving CAP reaccreditation for many of our outreach centers. This accomplishment reflects the team's unwavering commitment to excellence, adherence to the highest standards, and dedication to advancing laboratory science.
As we look ahead, we remain inspired to continue pushing boundaries and reaching new heights. Happy reading, and warmest wishes to all our readers, contributors, and colleagues for the year ahead!

Dr Sidra Arshad
Editor, LabRad

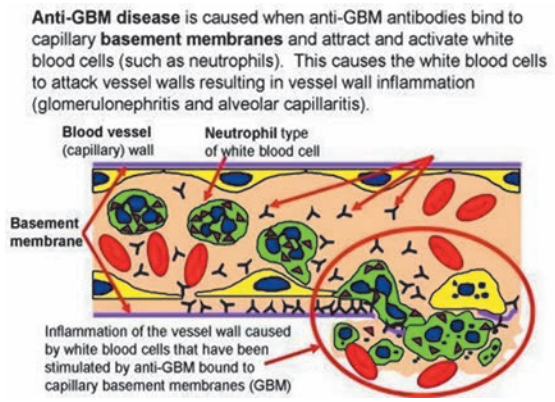
Anti-Glomerular Basement Membrane (GBM) Disease: Pathogenesis, Presentation, and Diagnostic Insights

Dr Umer Naeem Effendi, Clinical Chemistry

Anti-glomerular basement membrane (GBM) disease is a rare autoimmune disorder where circulating antibodies target an intrinsic antigen in the kidney's glomerular basement membrane, leading to acute or rapidly progressive glomerulonephritis. The primary target for these antibodies is the NC1 domain of the alpha-3 chain of type IV collagen, highly expressed in both the GBM and the alveolar basement membrane.

Anti-GBM disease is predominantly idiopathic but can follow kidney or lung injury—such as infections, hydrocarbon inhalation, or other forms of glomerulonephritis. Over 90 percent of patients present with symptoms of rapidly progressive glomerulonephritis, and between 20-60 percent experience concurrent alveolar haemorrhage. In rare cases, the disease presents solely with pulmonary symptoms. Patients may report systemic complaints like malaise, weight loss, fever, or arthralgia, typically appearing briefly in a prodromal phase.

Diagnosis of anti-GBM disease relies on detecting anti-GBM antibodies, primarily through a direct enzyme-linked immunoassay (ELISA) with purified or semi-purified antigens, or by indirect



immunofluorescence. Direct ELISA detection is the most common diagnostic method, with sensitivity varying by commercial kit from 63 percent to nearly 100 percent. False negatives can occur in the case of low antibody titers or in patients with Alport syndrome who develop post-transplant anti-GBM disease, where antibodies target the alpha-5(IV) chain. False-positive results are occasionally seen in ELISA assays that lack purified antigens. However, assays using native or recombinant human alpha-3(IV) antigen substrates offer much higher sensitivity (95-100 percent) and specificity (91-100 percent).

Advanced Total Laboratory Automation (TLA) Workflow: A Comprehensive Approach towards High-Throughput Analyzers in Clinical Chemistry

Saba Siddiqui, Clinical Chemistry

In today's clinical laboratories, TLA has become essential for optimizing workflows, particularly in clinical chemistry, where there is an increasing demand for faster, more accurate, and cost-effective diagnostic testing. Through the integration of robotics, automated analyzers, and advanced data processing systems, TLA delivers significant enhancements in efficiency, accuracy, and throughput. Our TLA system utilizes high-throughput analytical techniques specifically designed to handle a high volume of samples efficiently, establishing it as a critical asset within our chemical pathology section. Core analyzers, including automated immunoassays and advanced photometry, are precision-driven and have rapid turnaround times. TLA streamlines these processes by reducing manual intervention and standardizing workflows, thus minimizing variability and enhancing result consistency for optimal patient care.

Our TLA system features interconnected components spanning the pre-analytical, analytical, and post-analytical stages, working together to enhance the efficiency of high-throughput methods for both routine

and STAT sample processing within a specified time frame.

Advantages of TLA:
Reducing manual intervention; minimize human error; improves precision, reproducibility and reliability of results; enable processing of high sample volumes; reducing turnaround times; improved instrument flagging; minimizes manual pipetting, aliquoting, and data entry; improved lab efficiency; safer work environment; assay performance consistency; real-time monitoring; accuracy in high-throughput environments.

Precision Under Pressure: Challenges in Therapeutic Drug Monitoring for Immunosuppressants

Dr Yousra Sarfaraz, Clinical Chemistry

Therapeutic Drug Monitoring (TDM) for immunosuppressants is crucial in managing transplant patients, where precise drug levels are necessary to prevent organ rejection while minimizing toxicity. Immunosuppressants like tacrolimus and cyclosporine have narrow therapeutic indices, meaning that small variations in drug levels can lead to adverse outcomes. However, effective monitoring of these drugs is challenging due to their complex pharmacokinetics, patient-specific factors, and interactions with other medications.
At Aga Khan University Hospital (AKUH), we perform immunosuppressant assays for tacrolimus, sirolimus, cyclosporine, and methotrexate using the immunoassay technique. Dose reporting is based on both peak and trough levels; however, the laboratory encounters numerous challenges in day-to- day operations.

Narrow Therapeutic Index
One significant challenge is the narrow therapeutic index of these drugs, where sub-therapeutic dosing can lead to graft rejection, while over-dosing can result in severe toxicity, including nephrotoxicity and

hepatotoxicity.

Variable Pharmacokinetics

Drug interactions, absorption variability, and metabolic differences pose major challenges in TDM for immunosuppressants like tacrolimus and cyclosporine. These drugs often interact with other medications commonly used in transplant patients, such as antifungals, antibiotics, and proton pump inhibitors, which can inhibit or induce immunosuppressant metabolism, leading to fluctuating drug levels. Both tacrolimus and cyclosporine also have variable oral bioavailability due to differences in gastrointestinal absorption, resulting in unpredictable blood levels. Additionally, genetic variability in the cytochrome P450 enzyme system, especially CYP3A5, further complicates TDM, as variations in enzyme activity necessitate individualized dosing to maintain therapeutic levels.

Timing and Frequency of Monitoring

TDM for immunosuppressants is complex due to the need for frequent assessments and monitoring peak versus trough levels. Peak levels provide accurate drug exposure, but logistical challenges and post-transplant periods require frequent monitoring due to rapid shifts in drug levels and patient compliance.

Patient-Specific Factors Affecting TDM

Genetic variability in liver enzyme activity and organ function can affect drug clearance, necessitating individualized dosing. Frequent TDM is crucial due to changes in renal and liver function. Diet, smoking, and alcohol intake also affect drug levels.

Interpretation of TDM Results

Immunosuppressants are typically measured by immunoassays or liquid chromatography-mass spectrometry (LC-MS/MS). However, immunoassays may cross-react with drug metabolites, potentially leading to inaccurately high readings. This requires careful interpretation of results and may necessitate confirmatory testing with more specific assays. Protocols for TDM in immunosuppressants vary between institutions. This lack of standardization can lead to differences in target levels, assay methodologies, and interpretation of results, potentially impacting patient outcomes.

Strategies for Overcoming Challenges in TDM for Immunosuppressants

Advancements in laboratory techniques and standardization are crucial for improving therapeutic drug monitoring of immunosuppressants. Improved testing approaches, point-of-care monitoring, and standardized protocols can support individualized dosing based on genetic differences.

Multidisciplinary collaboration with pharmacists, clinicians, and genetic counsellors can enhance patient outcomes. A few strategies are listed below:

- Utilize personalized dosing regimens based on patient-specific factors (e.g., weight, organ function, genetic polymorphisms, co-medications, etc.)
- Standardize the time of sample collection (e.g., trough levels for drugs like tacrolimus and cyclosporine) to ensure consistency in monitoring. For drugs with long half-lives, like sirolimus, levels should be measured at appropriate intervals (e.g., weekly, bi-weekly) until steady-state concentrations are reached.
- Use validated and standardized laboratory assays for drug-level measurement (e.g., liquid chromatography-mass spectrometry (LC-MS), high-performance liquid chromatography (HPLC), or enzyme immunoassays).
- Carefully monitor potential drug-drug interactions, particularly when initiating or discontinuing drugs, or when there's a change in the patient's condition (e.g., renal or liver function).
- Genetic testing (e.g., CYP450 polymorphisms, ABCB1 gene for tacrolimus metabolism) can be used to guide dosing decisions, especially in patients who show unusual drug response patterns.
- Patient education is crucial for improving adherence to prescribed regimens. Provide clear instructions on medication timing, the importance of monitoring, and what to do if a dose is missed.

Frozen Section:
Diagnostic Accuracy, Pitfalls and Use of Digital Pathology

Dr Fatima Safdar, Dr Madiha Bilal Qureshi and Dr Nasir Ud Din, Histopathology

Frozen section analysis is a potent tool that offers a prompt diagnosis and aids in the surgical decision-making during intraoperative consultation. The need for intraoperative histologic diagnosis became significant at the turn of the 20th century as surgical methods advanced and became more intricate.

Frozen section is one of the most useful intraoperative tools for patient management, which requires collaboration between surgical and pathology teams. The process of frozen section involves several steps that start with the surgeon who excises the specimen from the patient and sends fresh tissue to histopathology laboratory, followed by intraoperative specimen processing including specimen orientation, submission, freezing, cutting, slide preparation, microscopic examination, potential specimen triage for additional workup, and diagnosis.

Most frozen sections are done to determine the nature and histological type of a lesion, assess the surgical margins of resection for tumor involvement/adequacy, and lymph nodes for the presence of metastatic disease. There has been a notable decline in thyroid frozen section cases in the recent years owing to the low sensitivity of frozen section for the diagnosis of thyroid follicular lesion. The cause of this is the limitation of entire capsule sampling to assess for capsular invasion and vascular invasion which are the major criteria for the diagnosis of thyroid carcinoma. The role of Fine Needle Aspiration Cytology in efficient diagnosis of thyroid swellings adds further.

Frozen sections of the parathyroid are becoming more common. The aim in most of these cases is to determine whether the tissue is actually parathyroid, thyroid or anything else. This helps the surgeon guide that he has removed the right tissue. Frozen sections for brain space occupying lesions have also increased. The diagnosis is very important because it tells the surgeon whether he is in the right area in brain and whether the tissue is representative of the tumor for a complete diagnosis or not.

Concordance between the frozen section diagnosis and paraffin sections diagnosis appears to be influenced by the surgeon's accuracy in selecting the tissue to be evaluated, the pathologist's expertise and experience, and the technical errors like freezing, crushing, or cautery artifacts in the tissue. Frozen section analysis itself also has several limitations leading to diagnostic challenges. Tissue sampling is one such restriction, which is said to be a frequent cause of inconsistencies. Viable tissue containing the lesion of interest must be sampled to make an accurate and relevant diagnosis.

Surgeons and pathologists, respectively, must exercise judgement in selecting the most representative tissue from the patient in the operating room.

Another limitation of frozen section analysis is the compromise in histologic evaluation caused by improper tissue freezing. This leads to compromised morphology of cells for making correct diagnosis. Furthermore, some tissues are challenging to cut with a microtome-like bone. Hence, there is always a risk in reporting frozen sections of tissue containing bone. Some tissues do not freeze well like fat and are very difficult to cut. This frequently results in a frozen section that is technically challenging and makes the slide unsuitable for review. All such frozen sections have a certain amount of diagnostic inaccuracy due to these factors. When a conclusive diagnosis cannot be made, the pathologist may decide to defer the frozen and postpone the diagnosis for a permanent section.

Frozen sections that have no effect on immediate patient care or operative management are deemed "inappropriate." Not only does it compromise diagnostic tissue material, but it also affects the frozen section analysis turnaround time of other cases. It compromises the diagnosis and results in needless medical expenses. Limitations encountered during a frozen section include the inability to use additional molecular/immunohistochemical studies on frozen tissue, the difficulty of cutting tissue, particularly fatty tissue, and frozen artifacts, which can be observed on both frozen and permanent histologic sections. Inaccuracies that would otherwise be prevented by standard processing may occur due to these restrictions. In addition, a frozen section needs to be done in a timely manner to be clinically useful and not significantly delay the operation. A large volume of frozen sections

received simultaneously (even 3-4 at a time) can lead to delay in diagnosis, as it takes time to process each specimen. Thus, unnecessary frozen sections from one case can impact the timely diagnosis of another case.

In conclusion, most frozen sections have an impact on operative management at our institution, and the most common frozen section specimen is for margin assessment. Subspecialty-specific factors greatly influence the rationale for sending a frozen section and how it affects operative management. Since there are many factors to consider when seeking an intraoperative consultation, there are no absolute contraindications for frozen section. To fully utilize frozen section analysis, the surgical team and pathologists must have an open and transparent channel of communication. Eliminating unnecessary frozen sections may be facilitated by interdepartmental discussions about the use of frozen sections.

The field of digital pathology has advanced significantly over the past years. As a result of this development, many pathology labs have replaced their conventional microscopes with digital microscopy and whole slide imaging (WSI). Their journey towards complete digitalization is underway. Intraoperative consultation (fast-frozen section) is one pathology area that is being digitalized. The greatest advantage is an expert opinion from a specialized pathologist in his area of specialty for frozen section analysis. For example, an expert orthopedics pathologist can report orthopedics frozen specimens from any part of the world by having the digital images at that time. This will lead to more accurate diagnostic interpretation in difficult cases.

Working Environment Perspective of a Learner

Dr Fatima Safdar and Dr Madiha Bilal Qureshi, Histopathology

Mental health is a tough and challenging aspect in a potentially high-risk profession. The reason includes workplace tensions associated with stressful and demanding lifestyle with respect to workload, multitasking, time management and frequent interaction with colleagues, patients and carers in the context of organizational environment, demand, structure and process. According to the current WHO definition, work-related or business pressure refers to: “How people ever responded to work demands and pressures that seem incompatible with their knowledge and skills and challenged their ability to adapt?”.

Work stress is one of the most important factors affecting employee performance owing to the affected mental and physical well-being. As a result, fulfilment of employee responsibilities within the organization can become more difficult, ultimately harming the productivity and causing depression in employees. There is now substantial evidence that depression can significantly affect work ability, capacity and creativity of individual and is an important cause of absenteeism.

Hence, it is essential to address these factors in a workplace. The leaders of mental health organizations should ensure that employees feel positive and motivated in their workplace maintaining good physical and psychological well-being. It is highly important to take advantage of the opportunities in workplace and beyond to promote their health and build resilience. Teaching and awareness sessions, good working environment, positive attitudes, proper supervision, timely support and treatment of depression where required is beneficial in improving performance and function.

Professionalism is a key competency in medical training and its importance is recognized by regulatory agencies at all levels. Key elements of professionalism for health care professionals in training include altruism, responsibility, excellence, honor, integrity and respect for others. While most medical institutions report integrating the teaching of professionalism into their formal curriculum, it is often taught in separate courses or rotations without supervision, continuous reinforcement or formative feedback. When professional education is not guided or provided systematically, informal or “hidden” curricula can negatively impact progress of learners. Assessing professionalism is critical to the long-term mission of medical education since errors in this regard can be harbinger of errors in future practice.

The Association of American Medical Colleges (AAMC) defines mistreatment as a deliberate or inadvertent action that degrades the dignity of people

and unjustifiably obstructs the process of learning. This kind of mistreatment frequently occurs in the medical sector, especially with trainees and minorities. A lot of research is being conducted in understanding mistreatment in residents since it is a major cause of burn out and other negative psychological effects. Peer abuse, regrettably, represents a developmental conundrum. Bynum et al. proposed that junior residents who experience mistreatment from their peers may go on to commit mistreatment themselves as a potential consequence of peer-to-peer cruelty. Residents have a big impact on clinical learning and can be both victims and perpetrators, not much is written about it in medical education.

Recent research has shown that mistreatment can be subjective and ranges from "accident-based" to "environment-based". Certain factors make a learner more likely to feel mistreated like having a bad learning experience, unjust team cohesion, being marginalized, being mistreated on the same team, seeing mistreatment, having hierarchies, being mistreated by different departments, being acclimated to uncivil behavior, and even being mistreated by the residents themselves. Different studies look at ways to fix these issues and create safer learning environments, better collaborative teams, more empathetic teachers and resilient learners.

One of the recent advances in the education system is peer learning. Simultaneously, Near-peer teaching has emerged as a beneficial way of teaching, effective for teachers, learners and residency training systems. Near-peer teachers have reported gain of knowledge, enhancement of teaching skills and development of strong relationships with learners. This describes near peers as "very relevant to their needs" and a "safe learning environment". Most residents felt more "encouraged" to ask questions and felt less "judged" than in a faculty-led education setting.

Near-peer teaching also reduces workload pressures on faculty and the initiative allows for a sustainable increase in the teaching force and fosters a culture of cooperation, teaching and learning. Near-peer programming draws on the institutional knowledge of peers who had recently been in learner's shoes. Considering the diversity of all these factors, residents should be encouraged to self- evaluate and self-reflect for future role modelling.

AI Training: A Must-Have Skill for Tomorrow's Radiologists

Dr Anam Khan, Radiology

In today's tech-driven world, AI has become an unavoidable buzzword, especially in healthcare. As Artificial Intelligence (AI) continues to reshape the landscape of medicine, radiology finds itself at the forefront of this exciting transformation. Advanced AI algorithms now have the capability to analyze medical images with impressive speed and accuracy, identifying abnormalities that might otherwise go unnoticed. However, to truly harness the power of AI, the next generation of radiologists will need more than just a surface-level understanding—they need comprehensive training. In a world where AI is integral to patient care, proficiency in using and interpreting these technologies is rapidly becoming a fundamental skill.

AI is already revolutionizing how we, as radiologists, approach our work. Machine learning (ML) has made it possible to swiftly detect critical issues like tumors and fractures, which not only streamlines our workflows but also alleviates some of the diagnostic fatigue we often face. This shift allows us to dedicate more time to complex cases, ultimately leading to improved patient outcomes.

Why AI Education Matters

1. Working With AI, Not Just Alongside It: Understanding how AI algorithms function is crucial. We need to grasp their strengths and limitations and know when to question the results. By familiarizing ourselves with the technology, we can leverage AI to enhance our expertise rather than replace it.
2. Building Trust and Transparency: A solid grounding in AI empowers us to tackle ethical issues, such as privacy and bias. By working responsibly with AI, we not only protect patient trust but also promote transparency in our clinical practices.
3. Keeping Up With Professional Standards: As AI becomes entrenched in the healthcare system, there's a growing expectation from employers and regulatory bodies for radiologists to effectively utilize these tools. Having a foundational

understanding of AI prepares us for the future demands of our profession.

Integrating AI into radiology education is not without its hurdles. We face challenges such as a lack of resources, the need for trained faculty, and the rapid pace of technological change. One practical solution could be to introduce the basics of AI early in medical education, coupled with hands-on experiences using AI tools during clinical training. Collaborating with tech experts will also help ensure our courses remain relevant and adaptable.

AI education is no longer a luxury; it's a necessity. Training the next generation of radiologists in AI is about more than keeping pace with technology; it's about empowering them to provide better patient care and lead the charge in an AI-enhanced healthcare environment. The future of radiology will undoubtedly be shaped by those who can adeptly blend their medical expertise with the transformative capabilities of AI.

Challenges in Blood Supply:
Importance of Voluntary Donations

Fariha Mateen, Blood Bank

Blood transfusion saves lives, but many patients face significant barriers to get safe blood at the time of need. Consequently, ensuring the availability of safe and sufficient blood must be a fundamental component of every nation's healthcare policy and infrastructure. Many low- and middle-income countries face a critical shortage of blood available for transfusion, leading to severe consequences, including significant morbidity and mortality rates. This scarcity impedes timely medical interventions and compromises patient outcomes, highlighting the urgent need for improved blood donation systems and health policies that prioritize blood safety and availability. Not only low- and middle-income countries struggle with low blood donation rates, but in January 2024, the American Red Cross also declared a national emergency due to a significant shortage of blood, reporting the lowest donation levels in two decades, with a staggering 40 percent decrease in the number of individuals contributing. Voluntary non-renumerated blood donors are a vital and preferred source for providing safe and high- quality blood for transfusions.

Here is some information to help grasp the profound impact blood donation can have on saving lives and improving health outcomes.

Every day, numerous individuals require blood transfusions, and a single blood donation has the potential to save the lives of up to three people. Your donation is crucial for transfusions and medical procedures that assist patients who have lost significant amounts of blood, and it also supports individuals whose bodies struggle to produce adequate blood cells, helping to keep them stable and healthy. Donating blood can help people with many health conditions, such as those who have internal or external bleeding due to an injury; sickle cell disease/ thalassemia or another illness that affects the blood; are undergoing cancer treatment; are undergoing surgery, such as cardiovascular or orthopaedic surgery; have an inherited blood disorder; are undergoing a transplant; need treatments involving plasma or other blood products

Benefits of Blood Donation

Donating blood not only serves as a life-saving act but also provides numerous emotional and physical health benefits for the donor. According to a report by the Mental Health Foundation, helping others can reduce stress, enhance emotional well-being, improve physical health, alleviate negative feelings, and foster a sense of belonging while reducing isolation.

Additionally, research has uncovered further evidence supporting the specific health advantages associated with blood donation.

Before you can donate blood, you must undergo a health screening conducted by a trained staff member, who will evaluate your overall health by checking your weight, pulse, blood pressure, body temperature and hemoglobin levels. Your blood is also tested for several diseases, including Hepatitis B/C, HIV, Malaria and Syphilis. Let us reaffirm our commitment to promoting voluntary blood donation and ensuring a safe, sustainable blood supply for future generations.

DONATE BLOOD SAVE LIFE

Leadership Skills and Emotional Intelligence For Professional Capacity Building in Histopathology – A Broad Perspective

Dr Madiha Bilal Qureshi, Histopathology

Professional capacity building in the field of medicine requires effort and patience. All the four components of Professional Development Plan (CART) are significant. One needs to work on improvement in clinical practice, administration work, research and teaching. Quality improvement (QI) has also become an integral part of this process.

It is highly recommended to get involve in a quality improvement project at an appropriate time in career.

CART +QI	Change required	Quality Improvement Plan
Clinical	Subspeciality expertise in 2 subspecialities Relevant Tumor Boards	Quality in subspeciality practice Tumor Board saves lives
Administration	Leadership skills in dealing with professional life	20 Leadership skills to mentor the mentors of the mentees
Research	Publishing papers in subspeciality, at least 2 per year in recognized journals Publishing books with thematic alignment	Quality research work in subspeciality
Teaching	Thoughtful lifelong learning	Education in all 4 components causes professional growth and development

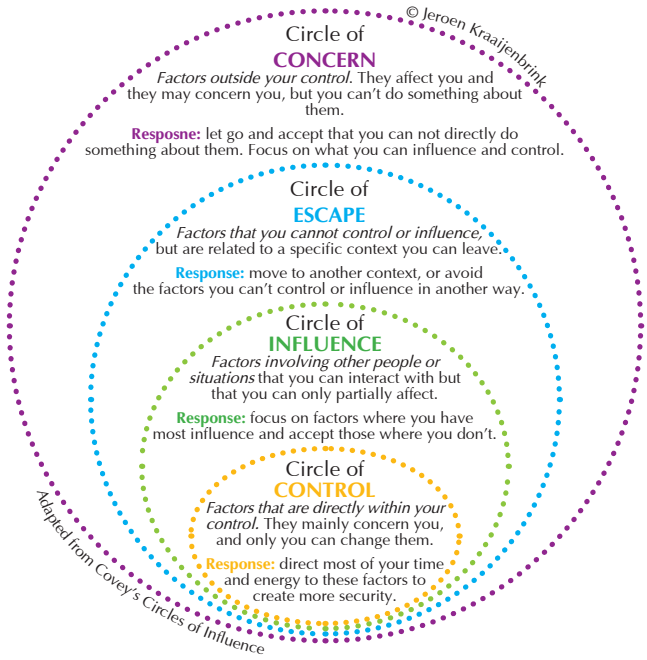
The roles of a histopathologist must be defined by him keeping in view that he stays a reflective lifelong learner, cares for the carers of the patients and mentors the mentors of the mentees. This requires emotional intelligence which describes an individual's ability to process, control, and perceive his own emotions and to recognize, understand, and influence the feelings of others. According to Daniel Goleman,

Teh Five Components of Emotional Intelligence at Work

SELF-AWARENESS	SELF-REGULATION	MOTIVATION	EMPATHY	SOCIAL SKILL
Definition: the ability to recognize and understand your moods, emotions, and drives, as well as their effect on others	the ability to control or redirect disruptive impulses and moods; the propensity to suspend judgment-to think before acting	a passion to work for reasons that go beyond money or status; a propensity to pursue goals with energy and persistence	The ability to understand the emotional makeup of other people; skill in treating people according to their emotional reactions	Proficiency in managing relationships and building networks; an ability to find common ground and build rapport
Hallmarks: • self-confidence • realistic self-assessment • self-deprecating sense of humor	• trustworthiness and integrity • comfort with ambiguity • openness to change	• strong drive to achieve • optimism, even in the face of failure • organizational commitment	• expertise in building and retaining talent • cross-cultural sensitivity • service to clients and customers	• Effectiveness in leading change • Persuasiveness • Expertise in building and leading teams

It is highly recommended to get involve in a quality improvement project at an appropriate time in career. This requires good selection of topic of interest with proper vision and ideation. The professional experience helps in selecting the right topic worthy of working on. Involvement of right team members is critical. The planning of a project requires good understanding and orientation of what does the person want to do, how can it be done, predict the likeliness of success and how would the work intervene or influence the society, what change would it bring to the patient care and working of the patient carers. Bringing change is very difficult since nobody likes to change, and acceptance of a change is challenging at all levels. Instead of expecting others to change, one should start it from his own self. Identifying the

areas to bring change for the betterment of patient care is important and the best way to bring a change is managing change. The four circles of influence (CIAA Model) are:
The change should be brought in all the three aspects: knowledge, skills and attitude. A structured strategy is needed to bring a required change. Entrepreneurship and Emotional intelligence both play a very beneficial role in this regard. Many healthcare workers do not know why they are part of the healthcare system? They are just spending minutes, hours and days in working without alignment with the ultimate vision, without realizing who they are, what is their team trying to do? Thus, presence of a specific, measurable, achievable, relevant and time-bound goal is necessary along with a strategic vision. One must be able to justify his focus and prioritization as meaningful. The enforcement of leadership skills guides at every step and these skills are mandatory to learn.



The 5Ps of entrepreneurship	
PASSION	The enthusiasm and motivation responsible for the determined work, infectious in a way that it inspires others to believe in your goals
PURPOSE	The reason of an idea like solving a problem through your service, focusing on the need of the healthcare system
PLANNING	The use of marked intelligence, research and knowledge to form plan after realization of objectives
PATIENCE	The power to control yourself and be optimistic, for change is a constant thing and requires flexibility and adaptability. Patient also when markedly enthusiastic in self-belief
PERSEVERANCE	The courage and intensity in persuasion of goal. Coming out of comfort zone and never give up, keeping in mind that one never loses, either he wins or learns

CORE SKILLS	ESSENTIAL SKILLS	IMPORTANT SKILLS	ADDITIONAL SKILLS
Change Entrepreneurship Vision	Emotional Intelligence Communication Problem Solving	Caring The Carers Mentoring The Mentors Reflective Learning Presentation Time Management	Quality Costing Succession Planning
Ideation Goal Setting Team Building	Assertiveness Negotiation Conflict Resolution		

The starting and finishing points of this journey must be aligned. The results must be revisited at intervals. Expected and actual results must be measured, and root cause analysis should be done. The factors to consider in implementation of outcome are quality, cost and succession planning which requires effective delegation thinking of long-term effect. If one is practicing the same way doing the same work as he was doing one year before, then there is no professional growth and improper delegation of work, this requires rethinking, re-evaluation and mentoring. And one should not hesitate to do so.

Ambition kills laziness, wisdom kills anger, dreams kill fear, growth kills ego, peace kills jealousy and confidence kills doubts. Teamwork has its own significance. Every team member can be effectively used if given the right task according to the capacity. A team leader should know how a particular team member can be utilized. Multidisciplinary collaboration requires effective communication skills and realization of the role of each discipline in patient care.

Social networking (National and International) is mandatory to increase the standard of care by collaboration. National level networking can bring very fruitful results since there is a lot of need to educate the healthcare professionals in multiple aspects. Even a CME done in a remote small city can bring a huge impact in patient diagnosis and management and gives a satisfaction that at least one has played his part. International networking gives recognition and more opportunities for collaboration, research and diagnostic help in challenging cases.

Books, minds and umbrellas only work when they are open. In a lifestyle where one has a lot of responsibilities on shoulder: taking care of the family, children and home, struggling with working hours, it is very hard to give equal importance to all these aspects for professional growth and improvement in patient care, but one cannot back out of the responsibilities as a health care professional. Hence making most of the time with commitment and dedication is essential.

Socrates said: “Strong minds discuss ideas, average minds discuss events, weak minds discuss people.”

Factor VIII and XI Deficiency; A Finding That Could Have Been Missed-A Case Report

Dr Wajeeha Iftikhar, Heamatology

Hemophilia A (Factor VIII deficiency) is an X-linked recessive disorder while Hemophilia C (factor XI deficiency) is an Autosomal Recessive bleeding disorder. Inheritance of these two independent abnormal genes is a co-incidental and very rare finding.

Case presentation

We received Factor XI Assay of a 34-year-old male who had history of gum bleeding and easy bruising. He suffered unprovoked intracranial hemorrhage in 2019 and hematuria in 2021 for which FFPs were given. Factor assay was sent on a post transfusion sample, and it revealed normal PT and APTT. Factor VIII was 39%, which was mildly low. Factor IX and XIII were normal. vWF antigen and Platelet aggregation studies were also normal. A diagnosis of low factor VIII levels was made at that time.

Now, he had suffered massive hemorrhage from aortic dissection and hematologist sent his factor XI assay which came to be 50% (67%-127%).

Upon inquiry, it was found that his brother was a known case of factor VIII and XI deficiency, mother and sister had complained history of easy bruising and bleeding after dental extraction. However, his father was normal.

Discussion

Factor VIII deficiency has been reported in combination with the following other inherited clotting disorders:

(1) Factor V deficiency
(2) Factor IX deficiency
(3) Thrombopathia (Disorder of Platelet signal transduction)
(4) von Willebrand’s disease and thrombocytopenia
(5) Factor XI deficiency

Some of these combined deficiencies were found to result from the coincidental inheritance of two independent clotting defects, whereas some of them, such as combined Factor V and VIII deficiency may represent distinct separate disease entity.

Symptoms of Factor VIII deficiency include prolonged bleeding, easy bruising, and joint damage. Factor XI deficiency, or hemophilia C, may cause mild to moderate bleeding tendencies, often with delayed onset. Symptoms include nosebleeds, heavy menstrual bleeding in females, and, in severe cases, joint bleeding.

Combined deficiencies of antihemophilic factor (Factor VIII) and plasma thromboplastin antecedent (Factor XI) are very rare, and this combination has been reported in the literature in only seven instances. Few examples are given below:

- Scardigli and Guidi first described the simultaneous presence of Factor VIII and Factor XI deficiency in different members of one family.

- Grasso and Quinte, Angelopoulos et al., Matsouka and Nossel subsequently reported isolated cases

of combined deficiencies of Factors VIII and XI in the same person.

Sufficiently detailed family studies were not performed in any of these reports, and the mode of inheritance is not clear.

Conclusion

If the prolongation of partial thromboplastin time (APTT) does not correlate with the degree of the clotting factor deficiency, especially after specific replacement therapy, suspicion of a second clotting factor deficiency other than an inhibitor should be raised and individual clotting factor activities should be determined.

Clinicians should request for a specific factor assay especially where there is a family history of such disorder and when symptoms do not match the already known factor deficiency to make a timely diagnosis.

Accelerated Phase of Chediak-Higashi Syndrome: Insights from a Paediatric Case

Dr Fatima Farhan, Heamtology

Case Presentation

A six-year-old male with a history of recurrent infections and multiple hospitalizations presented to emergency in June 2024 with fever, left-sided neck swelling, hepatosplenomegaly and pancytopenia for two to three months. His family history was significant for an older brother who had experienced similar recurrent infections, hospitalizations, and hepatosplenomegaly before dying in 2018 from pancytopenia and severe sepsis; the patient's parents are consanguineously married. Patient's CT scan showed extensive lymphadenopathy in the cervical, axillary, supraclavicular, mediastinal, hilar, and retroperitoneal regions. A cervical lymph node biopsy revealed necrotizing lymphadenitis, suggesting an infectious cause. The biochemical markers were derranged i.e. raised ferritin and triglycerides and low fibrinogen. Given the concern for hemophagocytic lymphohistiocytosis (HLH), a bone marrow biopsy was performed and peripheral smear showed white blood cells exhibiting large prominent cytoplasmic granules (as shown in Figure 1 & 2) and aspirate and trephine revealed hemophagocytosis, raising the possibility of Chediac Higashi Syndrome in accelerated phase. His genetic workup for LYST gene was sent and meanwhile prompting the initiation of dexamethasone and etoposide therapy.

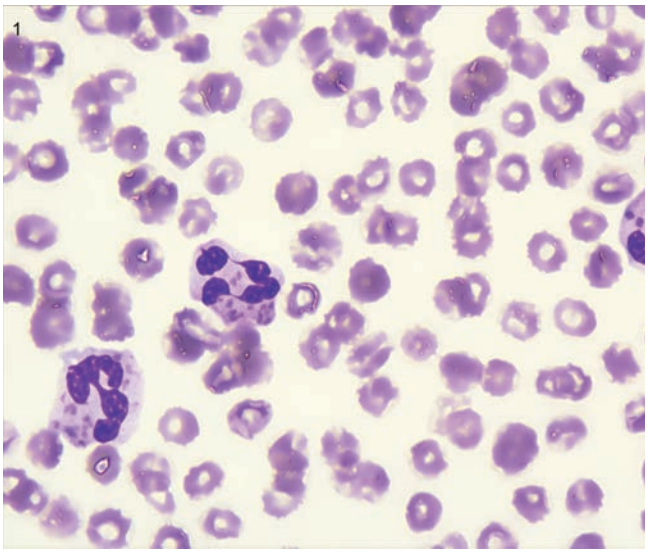
In July 2024, the patient was readmitted with recurrent fever, jaundice, pancytopenia, and gum bleeding. During the hospital stay, he developed a gluteal wound that cultured positive for pan-resistant Klebsiella pneumoniae and Pseudomonas aeruginosa, necessitating wound debridement and broad-spectrum antibiotics. Transfusions of packed red cells and platelets were given to manage anemia and thrombocytopenia. Genetic testing revealed mutation in LYST gene which confirmed the diagnosis of Chediak-Higashi Syndrome, specifically in the accelerated phase with HLH. Unfortunately, despite comprehensive treatment efforts, the patient ultimately succumbed to his condition.

Discussion

Chediak-Higashi Syndrome (CHS) is a rare, autosomal recessive disorder marked by recurrent, severe infections, oculocutaneous albinism, and immune system dysfunction. The syndrome results from mutations in the CHS1/LYST (lysosomal trafficking regulator) gene, which impairs the lysosomal trafficking regulator protein that leads to large, dysfunctional granules within cells resulting in decreased phagocytosis and predisposition to recurrent bacterial infection. Due to these cellular abnormalities, individuals with CHS face a high risk of infections and an accelerated disease phase such as hemophagocytic lymphohistiocytosis (HLH).

The discussed case highlights the severe challenges of managing Chediak-Higashi Syndrome (CHS) in its accelerated phase, complicated by recurrent, resistant infections and immune dysregulation.
Diagnosis of Chediak-Higashi Syndrome (CHS) is confirmed by examining a peripheral blood smear for characteristic large granules in white blood cells and platelets, along with genetic testing for CHS1/LYST mutations. Patients who survive initial infections often go on to develop hemophagocytic lymphohistiocytosis (HLH), marked by widespread immune cell infiltration in multiple organs.

Hematopoietic cell transplantation (HCT) is the preferred treatment. The case underscores the importance of early genetic counselling, especially in consanguineous families and tailored care for patients with CHS to improve outcomes amidst complex, life-threatening complications.



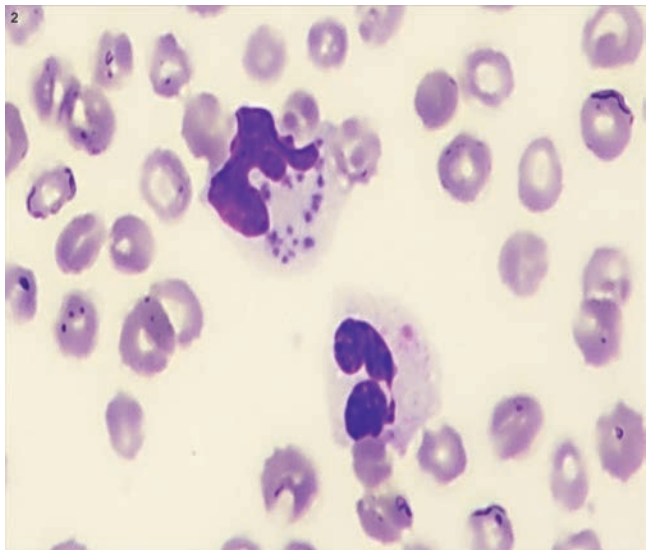


Figure 1 and 2: Neutrophils exhibiting intracytoplasmic prominent granules

Corynebacterium Diphtheriae:

From Bench Side to Bedside

Dr Ayman Shahid, Microbiology

Corynebacterium diphtheriae, historically known for causing diphtheria, has emerged as a significant pathogen leading to a rise in infections beyond its classical presentation. This article explores the evolution of C. *diphtheriae* from a well-studied bacterium to an emerging threat in contemporary clinical practice, emphasizing the factors contributing to its resurgence, the challenges it presents in diagnosis and treatment, and the implications for public health.

Corynebacterium diphtheriae is a gram-positive bacillus traditionally recognized for causing diphtheria, a serious respiratory illness characterized by a pseudomembrane formation in the throat. While vaccination efforts have dramatically reduced diphtheria incidence globally, recent observations indicate a worrying trend: C. diphtheriae is increasingly implicated in a variety of infections, including skin and soft tissue infections, respiratory infections, and even systemic diseases. This phenomenon poses significant challenges for clinicians and public health authorities.

The recent surge in cases

Several factors contribute to the resurgence of C. diphtheriae as a pathogen

1. **Vaccination Gaps:** Despite the availability of effective vaccines, there are still pockets of unvaccinated or under-vaccinated populations due to various socio-economic and cultural barriers. This has created an environment where bacterium can thrive and cause disease.
2. **Urbanization and Overcrowding:** Rapid urbanization and dense population contribute to the spread of infections. Poor sanitation and limited access to healthcare exacerbate this issue, facilitating the transmission of pathogens like C. diphtheriae.
3. **Increased Awareness and Surveillance:** Improved diagnostic capabilities and heightened clinical awareness have led to better identification of C. diphtheriae in non-diphtheritic infections. Laboratories now routinely perform advanced molecular techniques, such as PCR, enabling quicker and more accurate detection of this organism.

Clinical Manifestations

In recent years, C. diphtheriae has been associated with a spectrum of clinical manifestations beyond classic diphtheria:

- Skin Infections: Often presenting as non-healing ulcers or cellulitis, these infections are particularly common in immunocompromised individuals or those with poor hygiene practices.
- Respiratory Infections: Cases of pneumonia caused by C. diphtheriae are becoming increasingly documented, raising concerns about its potential role as an opportunistic pathogen.
- Systemic Infections: There are emerging reports of C. diphtheriae causing bacteremia and endocarditis, especially in patients with underlying health conditions.

Laboratory Identification

Here's a detailed overview of the processing, laboratory identification, and antimicrobial sensitivities associated with isolating and identifying this bacterium from a throat swab.

1. Sample Collection

- Throat Swab: Use a sterile swab to collect samples from the posterior pharynx and tonsillar areas, avoiding the tongue and saliva.
- Transport: Place the swab in a suitable transport medium (e.g., Stuart's or Amies medium) to preserve viability during transport to the lab.

2. Processing of the Sample

- Inoculation: Streak the swab onto selective media for C. diphtheriae, such as:
  - Loeffler's Medium: Promotes growth and aids in the morphology of the organism.
  - Cystine Tellurite Agar: This selective medium inhibits the growth of other bacteria and allows for the differentiation of C. diphtheriae, which produces black colonies due to tellurite reduction.

3. Incubation

- Conditions: Incubate at 35-37°C in an atmosphere of 5-10% CO2 for 24-48 hours.

4. Identification:

- Colony Morphology: On tellurite agar, colonies are black, with a dry, granular appearance.
- Gram staining of the colonies for confirmation and isolation on sheep blood agar (SBA).
- C. diphtheriae appears as gram-positive, non-spore-forming rods, often in V or L configurations (Chinese letter arrangement).
- Biochemical Tests:
  - Catalase Test: C. diphtheriae is catalase positive.
  - Urease Test: Negative.
  - Glucose Fermentation: Positive (C. diphtheriae ferments glucose).
- Setting the API coryne for species identification.

5. Toxigenicity Testing

- Elek Test: This is a crucial test to confirm the presence of diphtheria toxin. A filter paper strip impregnated with antitoxin is placed on an agar plate inoculated with the organism. A positive result shows a line of precipitation where toxin and antitoxin interact.

6. Sensitivity Testing

- Antibiotic Susceptibility: Perform susceptibility testing using:
  - Disk Diffusion Method: Apply antibiotic disks (e.g., penicillin, erythromycin, clindamycin) on Mueller-Hinton agar and observe inhibition zones.
  - E-test or Broth Microdilution: For more precise MIC (Minimum Inhibitory Concentration) values.

7. Reporting

- Report findings as C. diphtheriae confirmed on API coryne and susceptibility patterns.

Diagnostic Challenges

The diagnosis of C. diphtheriae infections can be challenging due to:

- Misidentification: Traditional laboratory techniques may fail to distinguish pathogenic strains from non-pathogenic ones.
- Low Index of Suspicion: Clinicians may not consider C. diphtheriae in differential diagnoses for various infections, leading to delays in appropriate treatment.

Treatment Options

C. diphtheriae is generally susceptible to several antibiotics, including penicillin and erythromycin. However, treatment must be guided by sensitivity patterns, which can vary. The use of antitoxin remains crucial in cases of diphtheria, highlighting the importance of prompt recognition and intervention.

Public Health Implications

The emergence of C. diphtheriae as a significant pathogen necessitates a coordinated public health response:

1. Vaccination Campaigns: Strengthening vaccination efforts to ensure high coverage rates, particularly in vulnerable populations, is vital to curbing the spread of this pathogen. According to WHO Epi schedule, 3 dose primary series of diphtheria toxoid vaccine start at the age of 6

weeks. The next two doses are given on 10 and 14 weeks.
2. Surveillance and Reporting: Enhanced surveillance systems should be implemented to monitor the incidence and spread of C. *diphtheriae* infections, allowing for timely public health interventions.
3. Education and Awareness: Healthcare providers and the public must be educated about the risks associated with C. *diphtheriae*, including the importance of hygiene practices and vaccination.

Conclusion

Corynebacterium diphtheriae represents an emerging pathogen of concern especially in Pakistan. As it causes a growing number of infections, understanding its epidemiology, clinical manifestations, and treatment options becomes critical for healthcare providers. Through collaborative efforts in vaccination, surveillance, and education, it is possible to mitigate the impact of this pathogen and protect public health in the region.

Application of Fluorescence In-Situ Hybridization Probes in Clinical Diagnostic, Advancing our Understanding and Enhancing Patient Management Strategies

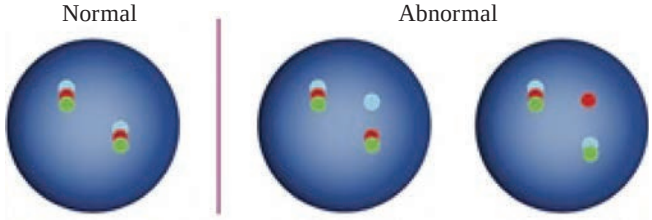
Amna Umar, Muneba Sharif, Dr. Zeeshan Ansar, Molecular Pathology

Fluorescence in situ hybridization (FISH) is a molecular cytogenetic technique that uses fluorescent probes that bind to only particular parts of a nucleic acid sequence with high sequence complementarity. It involves using fluorescent probes that bind to specific chromosome regions, allowing researchers to visualize the locations of genes or genetic abnormalities under a fluorescence microscope. FISH is often used for finding specific features in DNA for use in genetic counseling/testing, medicine, cancer research and species identification.

Types of FISH Probes

Locus-Specific Identifier (LSI) probe is designed to bind to specific gene loci on chromosomes. LSI probes target particular genes or chromosomal regions to detect abnormalities such as deletions, duplications, amplifications, and translocations. These probes are essential in identifying genetic alterations at a single-gene or locus level.

Centromere Enumeration Probes (CEP) are designed to hybridize specifically to the centromeric region of chromosomes. CEP probes target repetitive DNA sequences found in centromeres and are primarily used to enumerate chromosomes, helping detect aneuploidy in cells. These probes are labeled with fluorescent dyes, producing a distinct signal that allows easy counting of specific chromosomes.
Break-apart or Translocation Probes target two areas of a specific gene sequence. Usually, a green fluorescent label is used on one end of a gene sequence, and a red fluorescent label is used on the other. When the gene sequences are intact, the green and red signals will usually fluoresce as a yellow signal, known as a fusion signal. When a break in the gene sequence occurs, the green and red signals will not be close together anymore and thus appear as separate green and red signals. Break-apart probes are less specific than dual-fusion probes and provide less information since they do not define where the gene of interest has translocated.
Tri-Color Break-Apart Probes are used to identify complex chromosomal rearrangements, including translocations, inversions, and deletions within a specific gene or region. These probes use three fluorescent colors to target distinct, non-overlapping regions of a single chromosome or gene locus. When structural rearrangements occur, the signals separate or “break apart,” resulting in distinctive signal patterns that reveal the type and extent of the rearrangement. In a normal cell, the three colors (e.g., red, green, and blue) will appear together as closely positioned or overlapping signals. When a chromosomal rearrangement occurs, such as a



translocation or inversion, the signals will separate, indicating a "break" within the gene or locus.

Significance of FISH Technique in Clinical Oncology

The FISH (Fluorescence in Situ Hybridization) technique is a powerful diagnostic tool in cancer diagnosis to detect specific genetic abnormalities in cells. FISH enables visualization of chromosomal changes at the molecular level, allowing clinicians to identify alterations crucial for cancer classification, prognosis, and treatment decisions. Below are the main ways FISH significantly impacts cancer diagnosis and patient management:

- Detects Translocations: the BCR-ABL1 translocation in CML and PML-RARA in APL can be detected with high sensitivity.
  Targeted Treatment Selection: Identifying these fusions helps guide treatment choices, as specific therapies are developed to target these fusion proteins, such as tyrosine kinase inhibitors for BCR- ABL1 positive CML.
- Oncogene Amplification: HER2 amplification in breast cancer, for example, is detected by FISH and helps select patients for anti-HER2 targeted therapies, like trastuzumab.

Prognostic Value: The presence or absence of gene amplifications can offer prognostic information, as tumors with high levels of oncogene amplification tend to be more aggressive.

- Tumor Suppressor Gene Loss: FISH can detect deletions in genes, TP53 in many cancers, providing insight into tumor development.
- Aneuploidy Detection: By enumerating chromosomes or specific loci, FISH can detect aneuploidy, a hallmark of many cancers. Aneuploidy may be associated with tumor aggressiveness and poor patient outcomes.
- Detection of Minimal Residual Disease (MRD): FISH can be used to detect residual cancer cells with specific genetic abnormalities post-treatment, helping to monitor remission status.

Placenta Accreta Spectrum

Dr Shaista Afzal-Associate Professor, Radiology Department, AKUH
Dr Meena Hashim-Senior Medical Officer, Department of Obstetrics and Gynecology, AKUH
Dr Imrana Masroor-Professor, Radiology Department, AKUH

Introduction:

Placenta accreta spectrum (PAS) occurs when there is an abnormal invasion of chorionic villi into myometrium owing to a defect of the decidua basalis. The entity is classified as accreta, increta and percreta based on the difference in depth of the placental/ trophoblastic invasion.

The placenta can form an accreta, where it adheres superficially to the myometrium without intervening in the decidua, an "increta," where it penetrates the uterine myometrium, and a "percreta," where it penetrates the entire uterine wall and may infiltrate the surrounding pelvic organs.

A number of risk factors are associated with the development of PAS. These include previa diagnosed during the antenatal period, prior caesarean sections, other uterine surgeries, IVF pregnancies, and other medical histories. In women who have never had a caesarean section, there is also a higher risk of placenta accreta spectrum as maternal age increases.

It is a high-risk disease linked to serious pregnancy problems such as hemorrhage that might be fatal to the mother, the need for an extensive blood transfusion, peripartum hysterectomy, and surgical damage to nearby organs. Acute transfusion responses, acute kidney damage, renal failure, non-invasive or invasive ventilation, ARDS, and electrolyte imbalance can also result in the requirement for ICU admission.

Over the past 20 years, placenta accreta spectrum incidence has grown. Even if it's challenging to pinpoint its actual incidence. One possible treatment option for placenta accreta spectrum is a scheduled caesarean hysterectomy. In Pakistan, uterine conservation treatments are not carried out generally because of inadequate health facilities. A four-year cross-sectional study conducted at JPMC Tertiary Care in Karachi to ascertain the frequency and maternal and foetal outcomes in patients with placenta accreta spectrum revealed that the condition is linked to a high risk of maternal and perinatal morbidity and mortality. Hence, for best pregnancy outcome its recommended that such patients are managed by a multidisciplinary team in a center of excellence. The timings of delivery and surgical approach is still debated, and it's recommended to plan surgery to reduce maternal morbidity and blood loss. These risks can be reduced with accurate prenatal diagnosis and integral to this is ultrasound. The provision of sonologist who are experienced in the diagnosis of PAS is essential.

Ultrasound (US): PAS may be suspected on ultrasound

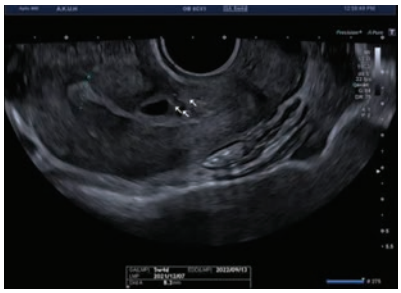


Figure 1: Gestational sac (GS) implanted at the cesarean section

as early as the first trimester and the important imaging features includes implantation of gestational sac (GS) in lower uterine segment ie the lower third of the uterus or at the cesarean section scar (figure 1) and the placental bed showing numerous lacunae / vascular spaces.

The sonographic signs in the second and third trimester includes placental lacunae- which are variably sized vascular structures in the placenta creating a “moth-eaten” or “Swiss cheese” appearance. The other sonographic markers include thinning of retroplacental myometrium (<1 mm) and loss of retroplacental clear space /hypoechoic zone (figure2). There may be uterine and bladder serosa interface abnormality. The Doppler evaluation is

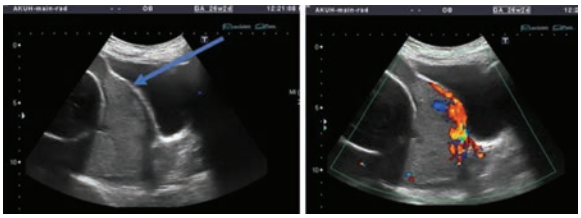


Figure 2: Placenta Accreta Thinning of retroplacental myometrium(arrow), no traversing vessels

important and shows increased placental vascularity and parallel linear vascular channels/traversing vessels extending from placental parenchyma into the myometrium which tends to exhibit turbulent flow.

MRI: Another modality that can be used for diagnosis of PAS is MRI, however the US is preferred due to its accessible and low cost. The sensitivity and specificity of MRI for the diagnosis of PAS is 80% to 85% and 65% to 100% respectively. MRI is specifically helpful when the placenta is posterior or lateral or when there

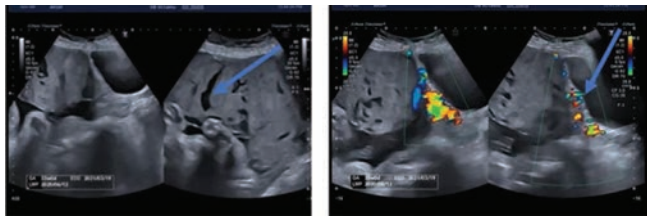


Figure 3: Placenta Percreta
3A: Placental lacunae (arrow)
3B: Increased placental vascularity and traversing vessels (arrow)

is suspicion of percreta. The imaging signs of PAS on MRI includes myometrial thinning <1mm, placental /uterine bulge, disruption of retroplacental T2 hypointense interface, (figure 4) abnormal placental vascularity, T2 dark placental bands. The placental

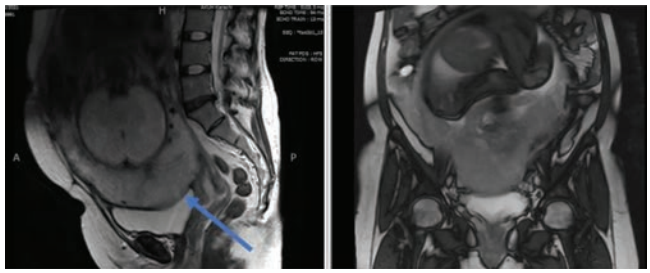


Figure 4: Placenta percreta, MRI sagittal and coronal T2WI showing placenta previa grade IV, focal areas of retroplacental myometrial thinning and disruption of retroplacental T2 hypointense interface(arrow)

location and underlying invasion should be mentioned in the report.

Conclusion:

Placenta accreta spectrum is associated with morbidity and mortality when optimal approach to management is not practiced. Increase in incidence of PAS necessitates accurate prenatal diagnosis and a multidisciplinary management approach to optimize patient outcome. Ultrasound is undoubtedly the initial preferred modality owing to its easy availability, low cost and reasonable accuracy. However, efforts are needed to standardize the sonographic markers/signs to further enhance the sensitivity and specificity.

Prader willi syndrome (PWS) and Anglman syndrome (AS) Screening and Diagnosis and Future Aspects

Syeda Mahnoor Zaidi, Dr Zeeshan Ansar, Molecular Pathology

PWS is a complex neurodevelopmental disorder caused by the lack of expression of specific paternally inherited genes on chromosome 15q11-q13. In contrast, AS results from the loss of maternally inherited gene expression in this same region, highlighting the distinct parental origin impacts on

gene expression. Several genes within the PWS region are vital to the syndrome's characteristics, including MKRN3, MAGEL2, NDN, PWRN1, NPAP1, and the small nucleolar RNA cluster (SNURF-SNRPN). The unique imprinting and methylation patterns in these genes, especially at CpG islands in their promoter regions, play an integral role in the differential expression on maternal versus paternal alleles.
PWS has an equal prevalence in males and females, with occurrence rates between 1 in 10,000 and 1 in 25,000 live births. Its hallmark features, particularly during childhood, arise primarily from hypothalamic dysfunction, manifesting in various stages of nutritional phases, as defined by Miller et al. Phase progression starts from growth restriction in utero (Phase 0), with symptoms evolving through phases marked by hypotonia, early feeding difficulties, weight gain, and ultimately, pronounced hyperphagia and insatiable appetite from childhood onward (Phase 3). Behavioral challenges, cognitive impairments, characteristic facial features, and musculoskeletal issues like spinal deformities further define the syndrome's phenotype.
Clinical and Cytogenetic Diagnosis of Prader-Willi Syndrome (PWS)

1. Clinical Diagnostic Criteria

The clinical diagnosis of PWS has evolved significantly since the establishment of consensus criteria in 1993, which were designed before the availability of genetic testing. These original clinical criteria were used to identify patients based on physical, behavioral, and developmental characteristics associated with PWS. However, with the advent of precise genetic diagnostics, the clinical criteria now primarily serve to raise suspicion of PWS, prompting further genetic testing. Recognizing this shift, Meral et al. introduced a revised clinical criterion with a lower threshold, aimed at identifying potential PWS cases. Despite these advances, genetic testing remains essential for definitive diagnosis, as clinical features alone can be insufficient due to overlapping symptoms with other genetic conditions.

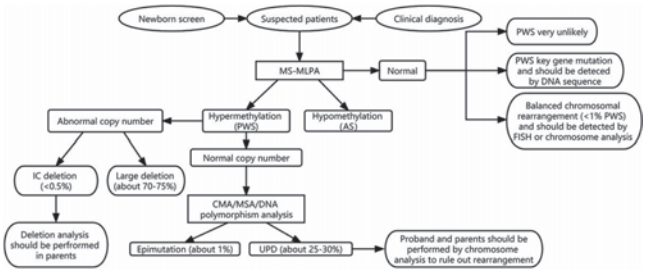


Figure 1. Genetic testing strategies for the Prader-Willi syndrome.

2. Cytogenetic Diagnostic Methods

Cytogenetic methods provide structural and functional insight into chromosomal abnormalities. Although less commonly used today due to the availability of more advanced genetic testing, cytogenetic approaches still play a role in PWS diagnostics, particularly in cases where detailed chromosomal assessment is required.

2.1 High-Resolution Cytogenetics: Before the availability of molecular diagnostics, high-resolution cytogenetics was the primary laboratory method for detecting PWS. This technique was able to detect deletions in the 15q11.2–13 region in about 60% of PWS patients, alongside other chromosomal abnormalities in around 3–5% of cases. However, this approach has limitations: about one-third of PWS patients present with normal karyotypes due to submicroscopic deletions or uniparental disomy (mUPD), which cannot be detected through high-resolution cytogenetics. Therefore, high-resolution cytogenetics has largely been replaced by more sensitive and specific molecular methods.

2.2 Fluorescence In-Situ Hybridization (FISH): Fluorescence in-situ hybridization (FISH) is a more targeted cytogenetic technique, frequently used to detect the absence of the PWS region on chromosome 15. This method relies on the hybridization of a fluorescently-labeled DNA probe specific to the PWS region to the patient's DNA. Under fluorescence microscopy, a single fluorescence signal indicates a deletion on one of the chromosome 15 alleles, whereas two signals are observed in unaffected individuals. FISH can also distinguish between Type I and Type II deletions when paired with specific probes. Despite its precision in deletion detection, FISH has limitations, including an inability to detect UPD or to differentiate between PWS and Angelman syndrome (AS) deletions. Additionally, FISH is not a high-throughput method, which limits its efficiency for large-scale screening.

2.3 Chromosomal Microarray Analysis (CMA): Chromosomal microarray analysis (CMA) is a highly sensitive method that identifies both microdeletions and microduplications within the genome. CMA encompasses two primary techniques:

- Array Comparative Genomic Hybridization (aCGH): This method compares the patient's DNA to a reference sample, allowing for the detection of copy number variations. In aCGH, patient and control DNA fragments are labeled with different fluorescent colors (e.g., green and red) and hybridized on an array. Fluorescence intensity is digitally measured to identify

deletions or duplications based on fluorescence ratios.

- Single Nucleotide Polymorphism (SNP) Array: SNP arrays label and hybridize the patient's sample to probes selected from known genomic locations, which allows for the identification of copy number changes and single nucleotide polymorphisms. Additionally, SNP arrays can detect long contiguous stretches of homozygosity (LCSH), making them proficient at identifying cases of uniparental disomy (UPD).

CMA offers significant advantages in detecting PWS deletion size and other chromosomal anomalies, making it a preferred method in many clinical settings. However, like FISH, CMA cannot identify balanced chromosomal rearrangements, such as balanced translocations or inversions, which may require complementary testing for complete chromosomal analysis.

Conclusion

Early diagnosis and intervention for PWS can substantially improve patient quality of life by reducing obesity, cognitive, and metabolic complications. The combination of prenatal and newborn screening is crucial in lowering the diagnostic age, which may enable timely treatment and management strategies [Citation85, Citation86]. In addition, the recent discovery of sno-lncRNAs presents a stable biomarker for PWS diagnosis, with potential applications in newborn screening due to their resilience against degradation. Future research combining sno-lncRNA biomarkers with neonatal screening technologies holds promise for advancing early and accurate PWS detection.

THE BEST OF THE PAST

#Radiologist #Diagnostic #Intervention #BodyImaging #FollowTheirLead Interviewee: Associate Prof Dawar Khan
Interview recorded by Dr. Shayan Anwar

Considering your entire time as Consultant Radiologist in Body Imaging at your organization, can you recall a time (any AHAA moment) when you felt most alive or most excited about your involvement in the organization?

On the clinical front, I was really thrilled and excited to initiate the body imaging fellowship program at Aga Khan University. Finally, the first batch was enrolled in January 2023. To the best of my knowledge, this is the first formal body imaging 2-year fellowship in a large tertiary care center in Pakistan.

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

My basic schooling was at a simple Urdu medium government school. It lacked basic facilities such as tables, chairs, or even electricity. Still, I never wavered in my pursuit to gain more knowledge. I was fortunate enough to get admitted to Sindh Medical College and after 5 years of sweat, blood and tears, I was awarded the MBBS degree.

I was trained at Karachi X-ray and Ultrasound Centre for 2 years and then at Aga Khan University Diagnostic Radiology for four years respectively to attain MCPS and FCPS degrees.

My vision has always been about equipping myself with the skills necessary to make myself the best at my craft. To this end, I decided to opt for giving FRCR in clinical radiology. I was elated to learn that I had finally passed the FRCR exam. One may ask why I went the extra mile to take this foreign exam when I could have stayed content with what I already had? The answer to this is ingrained in my perfectionistic personality. My aim is to become a respectable and professional doctor who can help any individual in need of assistance.

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

Perseverance is the key to success. My work has always taught me to never give up, no matter how difficult the odds are or however insurmountable the task at hand seems. Whatever goals you want to achieve in life, keep trying repeatedly and your efforts will eventually bear fruit.

As a senior Body Imaging Radiologist of the country, please share your experience of development of body imaging practices in Pakistan and its future in next 10 years.

One of my future career goals includes training the next generation of radiologists so that they are specialized in body imaging. This will enable them to serve patients all over Pakistan, both in urban and rural areas which traditionally have poor health care resources. I strive to continue implementing the latest evidence-based techniques in the CT section for the betterment of patients.

Any advice for Junior Radiologist?

Firstly, my advice for junior radiologists is to work hard, don't lose hope. Always prioritize your vision and goals and keep trying to the best of your ability to achieve them. Secondly, be honest with your work but most importantly, with yourself. When you are honest with yourself, you will have a sense of fulfilment that cannot be replaced and others will appreciate it, inevitably leading to your growth and those around you.

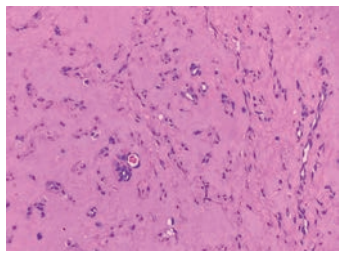
STORIES AT A GLANCE

Juvenile Hyaline Fibromatosis

Dr Alka Rani, Dr Madiha Bilal Qureshi and Dr Nasir Ud Din, Histopathology

Case Presentation

Seven year girl presented with multiple swellings on scalp, nose, gingival hypertrophy and ear swelling. Excision was done and multiple skin covered nodular tissue pieces were received ranging in size from 1.9-4.5cm. Cut surface was grey, white homogeneous. The lesion showed variable cellularity with plump to spindled uniform fibroblasts, arranged in cords imparting vessels- like appearance.
There was abundant eosinophilic non-fibrillar hyaline-like extracellular matrix. Immunostains ASMA, Desmin, CD34, S100, EMA and CD117 were negative.



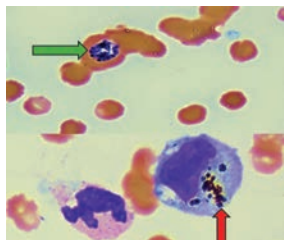
The features were diagnostic of Juvenile hyaline fibromatosis. Hyaline fibromatosis syndrome is an extremely rare autosomal recessive syndrome that typically affects infants, and causes painful, disfiguring, abnormal deposits of hyalinized matrix in the dermis, subcutaneous tissues and gingiva. Treatment is supportive with multiple excisions, recurrences are common.

Indicators of Severe Malarial Infection

Dr Komal Masroor, Hematology

The timely diagnosis of malaria is crucial for preventing disease progression and reducing its severity. The major contributors to the severity of malaria are anemia, cytopenias and leukocytosis. Presence of malarial pigment 'hemozin' (highlighted by red arrow) which is a by-product of parasite metabolism inside neutrophils and monocytes, affect host innate and inflammatory immune responses and inhibits erythropoiesis causing anemia. The presence of schizonts in a peripheral blood smear (highlighted by green arrow) is a sign of a high parasite burden increasing the likelihood of severe malarial infection.

Schizont of Plasmodium Vivax



Hemozoin pigment inside monocyte

HAPPENINGS IN PATHOLOGY

Histopathology FCPS II Mock Exam 2024

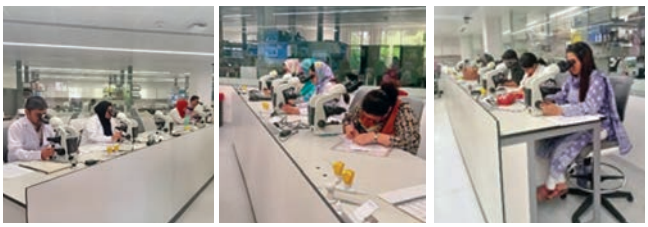
Naseema Adnan, Dr Sarosh Moeen and Dr Madiha Bilal Qureshi, Histopathology

Mock exams are essential for effective preparation of FCPS (Fellowship of the College of Physicians and Surgeons) Part II exam. They help candidates familiarize themselves with the exam format, time management and the right way to attempt questions. By simulating real exam conditions, mock exams build confidence, highlight areas of weakness and allow candidates to use their knowledge more strategically. Additionally, they reduce test anxiety and improve recall, ultimately increasing the chances of passing the FCPS exam.

In the month of September 2024, Histopathology Section organized a two-days Mock exam for FCPS II candidates. It included PGME years IV, V residents and former candidates who could not clear prior exam. The Mock Exam was held on 27th -28th September. The flyer was released a month before the exam. A total of 15 candidates participated in this event from across the city. The exam was designed to simulate the real exam experience in a format similar to that used by the College of Physicians & Surgeons of Pakistan (CPSP). The exam was conducted by professional examiners from Aga Khan University Hospital and Liaquat National Hospital. They assessed the candidates and provided valuable insights on effectively approaching the exam, highlighting techniques / strategies to maximize score.

A group photo of participants and faculty with examiners.

Day 1: The 15 candidates screened 30 Surgical, 5 IHC, and 7 Cytology cases. Five Histopathology faculty members participated as invigilators:



FCPS MOCK-EXAM

Days	Day 1 27th September 2024	Day 2 28th September 2024
Activity	Cases Screening (Surgical, IHC, Cytology)	Cytology Screening and Viva Exam (Gross & Frozen)
Organizer	Dr. Sarosh Moeen Dr. Madiha Bilal Qureshi	
Teams	1.Dr. Sarosh Moeen, Hania Naveed, Alka Rani, Anam Ghauri, Zoonish Ashfaq 2.Dr. Madiha Bilal Qureshi, Tamana Asghari, Manahil Khan	
Cases Contribution & Paper Marking	Dr. Shahid Pervez, Naila Kayani, Saira Fatima, Romana Idrees, Khurram Minhas, Aisha Memon, Zeeshan Uddin, Samia Fatima, Sidra Arshad, Madiha Bilal, Sarosh Moeen, Zoonish Ashfaq, Tamana Asghari, Hania Naveed, Anam Ghauri & Manahil Khan	
Internal and External Examiners	Dr. Naveen Faridi, Shahid Pervez, Naila Kayani, Saira Fatima, Romana Idress, Khurram Minhas, Aisha Memon, Zeeshan Uddin & Sidra Arshad	
Feedback & Results	Riaz Sirzameen, Naseema Adnan, Faisal Naeem	

Day 2: Candidates screened 5 Cytology cases, 5 Gross and 5 Frozen Cases. Examiners conducted viva for Frozen and Gross cases.

Following the exam, a case discussion session was held, where faculty members discussed the cases in detail. Candidates feedback was very positive as shown:

There was an emphasis by the candidates to conduct such exams in Karachi twice a year so that reduces their cost of travelling outside Karachi for mocks. It was an overall excellent experience with teamwork at its best.

CAP Re-accreditation Inspections of Outreach Labs – An Exciting Experience

Mashhooda R. Hashmi, Quality Assurance, Clinical Lab

Laboratory accreditation is a formal process that recognizes a laboratory's competence and compliance with international standards. It is a way to ensure that a laboratory is qualified to produce valid results and maintain high quality. Laboratory Accreditation Program (LAP) of College of American Pathologist (CAP) accredits the entire spectrum of laboratory test discipline and is considered the gold seal.

Clinical laboratories of Aga Khan University Hospital initiated the process of CAP accreditation in the year 2016 when the On-campus lab gets its first accreditation. The journey won't stop here. In the year 2018, four outreach labs namely Lahore, Faisalabad, Rawalpindi and Peshawar get their first CAP accreditation followed by Multan, Sukkur, Hyderabad and Clifton Medical Services (CMS) Labs in the year 2022.

As a part of accreditation cycle, there is a requirement of re-accreditation after every two years. In this context, recently the outreach labs of Aga Khan University Hospital went through the re-accreditation cycle in two phases.
In the first phase, a team of four inspectors from Jordan visited Peshawar, Rawalpindi, Faisalabad and Lahore labs, respectively. It was an in-person inspection held from May 3 to 8, 2024. The team was very competent who did a thorough inspection using all elements of CAP checklists, but we passed with flying colors. This was the second re-accreditation cycle for the mentioned labs.

The second phase comprises of inspection of Multan, Sukkur, Hyderabad and CMS labs in the months of September and October. Two teams were assigned this time by CAP. One team from Saudi Arabia for Multan, Sukkur and Hyderabad while one team from Jordan for CMS lab. The inspection of Multan and Sukkur was held virtually based on the security and accommodation challenges of the region. However, the inspection of Hyderabad lab was decided to be conducted in-person.

The outcome of inspections of Multan and Sukkur labs were also favorable. Unfortunately, the inspection of Hyderabad lab was postponed and is now scheduled in November, after the inspection of main lab.
The accreditation cycles provide us the opportunity to build liaison with the regional team. In addition,

they gain the confidence that they are working at internationally acceptable standard. A team from Karachi comprises of Chair, Vice-Chair, QA Manager, Chief Safety Officer and relevant QA Assistant Managers accompany the inspection team and were active part of the inspection cycle. All in all, it was a mutually beneficial and learning experience. Some glimpses of inspections are shared below:


Peshawar Outreach Lab


Rawalpindi Outreach Lab


Faisalabad Outreach Lab


Lahore Outreach Lab


Sukkur Outreach Lab


CMS Lab


Multan Outreach Lab

5th Annual Research Day of Pathology and Laboratory Medicine 2024: The Future of Pathology: Adapting to a Changing Landscape

Dr Nayab Afzal, Ms. Kanwal Amin, Department of Pathology and Laboratory Medicine

The 5th Annual Research Day of Pathology and Laboratory Medicine was organized on October 12th, 2024, at the Movenpick Hotel in Karachi, Pakistan, with the theme "The Future of Pathology – Adapting to a Changing Landscape,". The event showcased cutting-edge research and advancements in the field of laboratory medicine, and it was an astounding success.

This interdepartmental and interdisciplinary initiative brought together distinguished national and international experts in the various fields of pathology and research. The speakers shared insightful findings of their recent research on emerging infections in changing climate, diagnostic and research applications of artificial intelligence and multi-omics.

The day began with a formal welcome address by Dr. Hafsa Majid, Chair of the Organizing Committee. She emphasized the importance of multidisciplinary collaborations in the field of research medicine. Dr. Najia Ghanchi, Vice-Chair for Research at the Department of Pathology and Laboratory Medicine, then provided a detailed overview of the research projects and milestones achieved by the department. The Guest of Honor, Prof. Syed Asad Ali, Associate Dean of Research and Chair of the Department of Community Health Sciences, delivered an address highlighting the role of cutting-edge pathology research in molding healthcare practices for the future. This was followed by another encouraging talk by Dr. Asim Belgaumi, Chief Medical Officer at Aga Khan University Hospital, who spoke on the importance of integration of modern research methodologies with clinical applications in order to improve overall patient outcomes.

The first scientific session of the day focused on global health, specifically the interaction of climate change and emerging infections. Dr. Wes Van Voorhis, Director of the Center for Emerging and Re- emerging Infectious Diseases at the University of Washington, delivered a compelling keynote address on global trends in emerging infections and the critical role of unprecedented climate change in rapid spread of vector-borne diseases in Pakistan. Next, Dr. Kauser Jabeen, presented a comprehensive situation analysis of emerging infections in Pakistan and its impact on global health, with special focus on emergence of resistant fungal strains in Pakistani population.

After a tea break and a vibrant poster presentation session, the conference resumed with a session on the applications of machine learning in clinical care. Dr. Julianne Meisner from the University of Washington presented an engaging talk on the applications of big data and statistical modelling in clinical research, highlighting how these new methodologies can help in improving patient outcomes. This session was followed by Dr. Muhammad Abbas Abid and Ms. Rabiya Owais, who shared insights into big data, data analytics and its role in clinical research at the university's Advanced Translational Research Center (ATRC). Their). Their talk highlighted the ATRC unit is advancing data driven research in Pathology and lab Medicine.

The next session delved into the world of multi-omics, with Dr. Fyezah Jehan, discussed how genomics and proteomics are revolutionizing diagnostics and shared her experience of a multi-Omics based research project. This comprehensive talk was followed by a presentation by Professor Dr. Aysha Habib Khan who elaborated on the vast potential of metabolomics in better understanding of disease mechanisms and showcased the metabolomics-based research work done at the Dept. of Pathology. Dr Erum Khan, Chair Dept. of Pathology and Laboratory Medicine, shared details about partnership with the D43 Fogarty International Centre, high lighting the capacity building trainings conducted for vector borne diseases, sero surveillance and vector surveillance in Pakistan.

After a networking lunch, the focus shifted to digital innovations in pathology. Dr. Anil Parwani, Donald A. Senhauser Chair, Department of Pathology at The Ohio State University, joined via Zoom. He spoke on integration of artificial intelligence (AI) and latest digital innovations in enhancing the diagnostic accuracy of histopathology reports. He also emphasized how AI algorithms can precisely analyze complex pathological images to improve current diagnostic practices. Then, Dr. Zeeshan Uddin, shared valuable insights on epidemiologic of surveillance using postmortem minimally invasive tissue sampling (MITS) in low resource settings like Pakistan. His talk highlighted how MITS can prove to be an invaluable tool for determining accurate cause-of-death in infants.

The session on precision medicine featured a compelling presentation by Dr. Salman Kirmani, on how precision medicine is leading the way to more personalized treatment for genetic diseases. He also emphasized the impact of genetic screening and timely medical interventions. This session was followed by an elaborate talk by Dr. Natasha Ali, AI diagnostic capabilities in hematology. Her talk explored how the traditional labor-intensive task of identifying abnormal cell morphology can be replaced by more accurate AI algorithm and other applications of AI in hematological diagnosis.

Oral paper presentation session included talks on azole resistance in Aspergillus fumigatus isolates, role of AntiB2GPI in antiphospholipid syndrome, histology slide digitization, and the phylogenetics of Crimean-Congo Hemorrhagic Fever virus strains in Pakistan.

Picture 1: A group Picture of speakers and participants of the Research Day

Picture 2: Plenary Speakers and Presenters of the free Paper Abstracts Research Day.

The conference concluded with the distribution of awards to the winners of both oral and poster presentations, ending on a high note with a final vote of thanks by Professor Erum Khan, Chair Dept. of Pathology and Laboratory Medicine. This enthusiastic local and international participation shows substantial interest in changing the landscape of clinical research. Discussions held during the day underscored the importance of adapting to the rapidly changing landscape of healthcare. This successful event set a high standard for future research initiatives.

Polaroid

Radiology

21st Health Asia Conference and 8th Annual Seminar on Radiology – Faculty and Technologist Session, Held on October 23, 2024, at Expo Centre Karachi.

"Mastering Mammography" Workshop Organized by the Department of Radiology on October 6, 2024, in the Radiology Seminar Room, as part of Breast Cancer Awareness Month.

Hematology

Team Coagulation analysing the final CAP Inspection Checklist 2024

Team Hematology reviewing the final CAP Inspection Checklist 2024

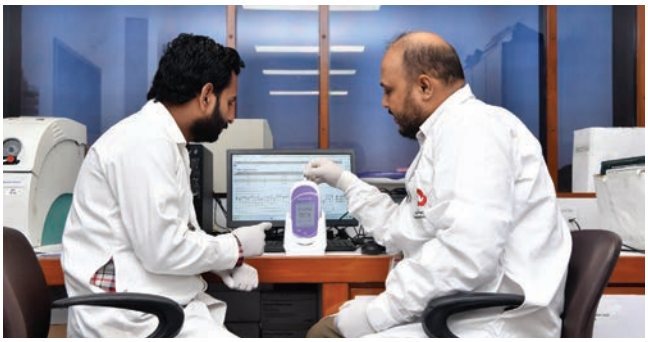
Histopathology

Section of Histopathology has recently acquired the Dako Omnis machine that is a fully automated state-of- the-art instrument .It can perform immunohistochemistry (IHC), double staining, immunofluorescence (IF), and in situ hybridization (ISH) simultaneously .

Molecular Pathology

Prenatal Diagnostic workshop at Sir Gangaram Hospital Lahore

Chemistry



The Point of care testing (POCT) coordinator is providing training to the POCT user on how to operate the Accu-Chek Inform II glucometer. POCT is clinical testing performed near the patient, offering rapid results that enable timely treatment, potentially improving clinical and economic outcomes compared to traditional laboratory testing.

Maximizing Precision and Speed: Technologists Utilizing Total Laboratory Automation (TLA) for Routine Chemistry Testing. TLA plays a crucial role in improving workforce efficiency, reducing operational costs, and optimizing the management of routine testing processes.

hospitals.aku.edu/Karachi/clinical-laboratories