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From the Editor's Desk

Dear Readers

With the recent advancements in diagnostics, the diagnostic field is now moving from one analysis to identify one disorder to one analysis identifying multiple disorders. This has become possible with the Omics and informatics based clinical diagnostics in the area of Precision Medicine. These technologies have tremendous application in research and therapeutics as well. As these are gaining momentum, people are becoming more interested in personalized and preventive medicine. Correspondingly, along with it the use of Artificial intelligence, mobile healthy and digital pathology is revolutionizing the world of diagnostics. This Labrad is in line with this year's Pathology Research Day theme 'Omics, Artificial intelligence and Digitalization in Diagnostics'. The 3rd Pathology Research Day was conducted on October 15th 2021.

A compilation of articles covering the application of various Omics, Artificial Intelligence based technologies and digital Pathology in improving the diagnostics. We have some interesting discussions like challenges and solutions for implementing digital pathology in resource limited setting, applications of artificial intelligence in diagnostics, metabolomics and genomics in identification of different disorders. We value your opinion and feedback regarding topic selections, educational and resource materials, provide ideas on how we can better network and communicate using LABRAD.

Dr Hafsa Majid
Guest Editor LABRAD

Role of Barcoding in a Clinical Laboratory to Reduce Pre-Analytical Errors

Iffat Arman
Clinical Chemistry

The day to day progress of clinical laboratories will be dreadfully impacted by two disruptive technologies: Machine learning and artificial intelligence. Artificial intelligence and machine learning software are beginning to integrate themselves as tools for efficiency and accuracy within pathology laboratories. Artificial intelligence (AI) represents a valuable tool used to improve diagnostics through more accurate detection of pathology, better laboratory workflows, improved decision support, and reduced costs, leading to higher efficiencies.

For clinical laboratories, timely and precise specimen labelling is expected to ensure correct patient identification from collection to results reporting. Barcoding is a system of using varying width bars as a way to provide identification information. Its purpose is to ensure accurate sample identification and accession. It is an optical machine-readable representation of data, which shows certain data on certain products in the laboratory. Electronic identification such as two-dimensional barcodes can certainly include two or more person-specific identifiers to comply with this requirement.

Types of Barcoding: There are many types of barcodes, the most common ones being one dimensional and two dimensional.

Single Dimensional Barcode: In a single-dimensional barcode, the vertical lines and their spacing constitute the code, but the whole length of the vertical lines are not essential for the codes. It is said that the code is repeated in perpendicular

directions, so a symbol along with printing defects, such as spots or voids, can still be read.

Two Dimensional Barcode: Two-dimensional barcodes seek information along with the height as well as the length of the symbol. As a result of that construction, these barcodes have greater storage than the one-dimensional barcode. A two-dimensional barcode is not comprised of bars or lines but rather of black-and-white cells arranged in a matrix pattern (often laid out in a square). This square image is usually simpler to scan than linear barcodes, which fits better on curved surfaces like test tubes or patient wristbands.

Barcodes provide patient tracking and sample management. Besides laboratory samples, barcodes used on calibrators and blood products, reagents/ kits, quality controls in a clinical laboratory. Bar-coding acts as an artificial intelligence tool that helps record retrieval and is safer for patients in terms of anonymity, accuracy, and elimination of human error. Bar-coding the specimen label effectively reduces clerical errors, minimises mistakes in patient specimen handling, and increases productivity.

Congenital Dyserythropoietic Anemia: The Morphological Diagnosis

Dr Kanta Devi
Clinical Haematology

Case presentation: A 11-year-old boy presented with pallor and jaundice and had a history of regular blood transfusions for two years. Family history was unremarkable. Full blood count showed hemoglobin (Hb): 8 g/dL, white blood cells: 2.7 X 109/L, platelets 96 X 109/L and reticulocyte count of 1.5 percent. The direct antiglobulin test was negative. Liver functions tests revealed normal enzyme with high total (2 mg/ dl) and unconjugated bilirubin (1.4mg/dl). Serum ferritin was high (6561 ng/ml), while viral profile including HIV, hepatitis B and C were negative. Peripheral blood film revealed anisopoikilocytosis, elliptical cells, polychromasia, basophilic stippling, fragmented red cells, nucleated red cells, myelocytes and metamyelocytes (figure 1). Hemoglobin variant study by high performance liquid chromatography showed normal composition Hb A (96 percent) and Hb A2 (3.3 percent). Bone marrow aspiration and trephine exhibited erythroid hyperplasia with marked dysplastic changes including bi- and multinucleated erythroid precursors, nuclear to cytoplasmic asynchrony, inter-cytoplasmic and intra-nuclear bridging, and nuclear mitotic figures (figure 2). Clinical details, laboratory findings and bone marrow morphological features were indicative of congenital dyserythropoietic anemia (CDA), type II. Electron

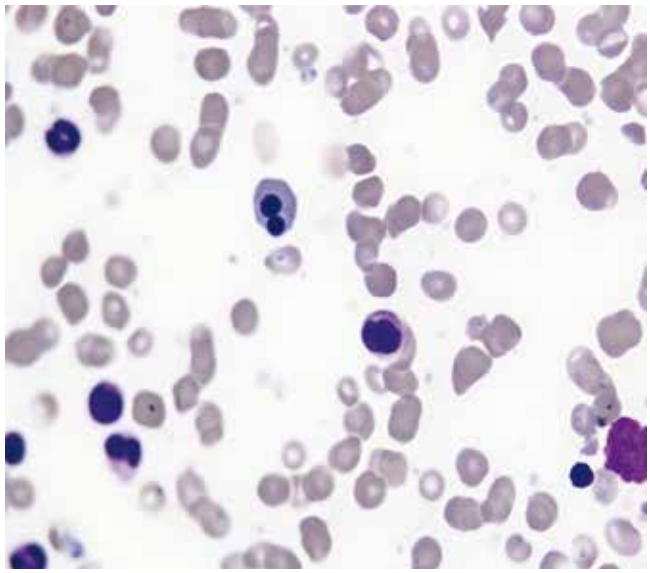


Figure1: Peripheral smear showing myelocytes and nucleated red blood cells (leucoerythroblastic blood picture)

microscopy and molecular studies were not performed due to unavailability at our facility. He was managed symptomatically with blood transfusions and iron chelation.

Discussion: CDAs are classically grouped into four sub-types based on bone marrow morphological

features. Type I has erythroblasts with internuclear bridging. Type II erythroblasts have multinuclearity of late erythroblasts while type III has gigantoblasts (erythroblasts with ≥8 nuclei), type IV causes disturbance in transcriptional activator. Inheritance is autosomal recessive and diagnosis is usually made in childhood or early adult life. The etiology is not known for all the sub-types. The diagnosis of CDA is generally considered after other chronic hemolytic anemias have been ruled out. Common clinical presentation is anemia, jaundice and splenomegaly. Although, other more common inherited hemolytic anemias have similar presentation, patients with CDA usually have high serum ferritin which causes organ damage.

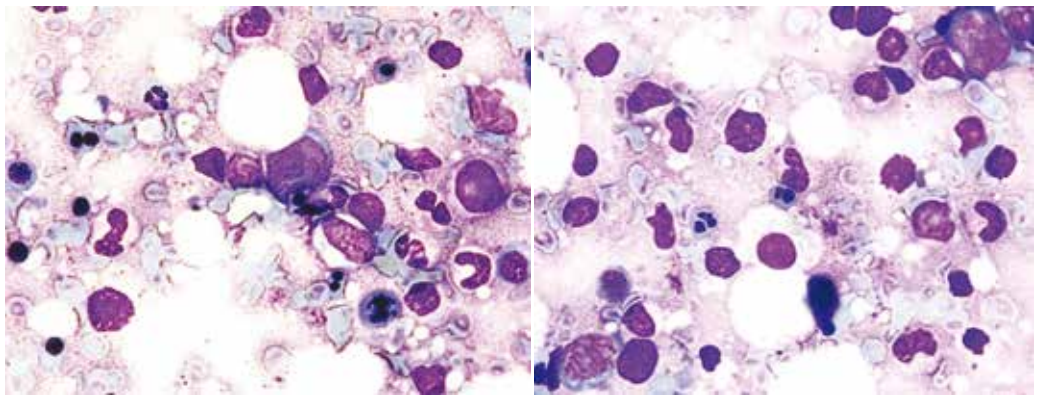


Figure 2: Bone marrow aspirate smear showing dyserythropoietic features, internuclear bridging and nuclear budding.

Bone marrow examination remains a key diagnostic tool in identification of the CDAs. CDA type II is also called hereditary erythroblastic multinuclearity with a positive acidified serum test (HEMPAS), is the most common form of CDA and is characterized by ineffective erythropoiesis, commonly involved mutation is in the SEC23B gene. Treatment is usually symptomatic and includes blood transfusions, iron chelation therapy and removal of the spleen.

How to get started? Implementation of Digital Pathology in Resource Limited setting, Need, Challenges and Solutions.

Dr. Sehar Suleman and Dr. Qurratulain Chundriger
Histopathology

A classic histopathology workflow is an amalgamation of intricate series of events, including a manual review of glass slides that ultimately results in diagnosis. An abrupt halt to this smooth running occurs when the concerned pathologist finds difficulty in judgment, they then consult with colleagues who can be in a different lab, city or country altogether. Henceforth, the sample needs to be then packaged, labeled, and mailed to the intended lab for remote consultation. This complete process can be quite cumbersome, time demanding because of days/weeks lost in transit, and expensive due to the courier costs.

The use of digital pathology in education, second-opinion consultations (real time collaboration), slide archiving/retrieval and primary diagnosis has grown drastically over the last decade. By using high-throughput, automated digital pathology scanners, it is possible to capture an entire glass slide, under bright field or fluorescent conditions, at a magnification comparable to a microscope (Whole slide imaging – WSI). Simulation of virtual microscope can be done by its dynamic mode of viewing images (horizontal and vertical movement of images, and zooming in and out), annotation and ability to view the image on a large monitor also promotes group interaction and discussion. Images can also be accessed anytime with an Internet or Intranet connected computer, or other mobile/portable devices.

On the contrary, implementing full scale routine digital pathology in low-resource settings remains a challenge today. Among the many reasons that discourage laboratories from adopting this technology include reluctance of many unenthusiastic pathologists, to embrace new modality ("Change is hard"). Absence of locally made pathology slide scanners, digital microscopes or manual whole slide image (WSI) softwares (which if available are only provided for research work), therefore, the high cost of importing equipment, both soft and hard. Limited funding/ executive sponsors, lack of high internet bandwidth, efficient IT system including internal and external network, unreliable electrical power because of frequent outages, and adverse weather events which could disrupt telecommunications.

Most of the pathologists in our setting have practiced telepathology especially during Pandemic lockdown by using microscope camera but when it comes to the diagnostic utility of these digital images, reliable consultation cannot be given due to limitations of magnifying details and quality. Since we have already gotten comfortable with virtual learning these days, it is now, best to gradually start switching gears towards digitization. For that matter, liaison with world leading biotechnology, bioinformatics organizations should be established which could be sources of provision of training; they might as well offer scanners, digital microscopes and software at a relatively negotiable and affordable price, applicable for budgets of low resource laboratories.

Following are the seven steps (Digital Pathology System Workflow Road map) that if executed can lead an organization to the forefront of Modern Pathology:

1. Getting Laboratory personnel interested: It is important to convince the pathologists and lab staff to accept the change, they can be directed towards reading up digital pathology related articles, studies, best practice recommendations, survey barriers/high level needs and understand the benefits versus risks based on evidence. At this time new visual workflow and feedback can be sought after.
2. Defining needs and goals: This requires framing a detailed new workflow and business plan, laying down how all the steps will be carried out, who will carry them out (formulation of teams, roles). A checklist can be established as an internal quality control for each stage. Concept can also be piloted.
3. Specification of infrastructure/ Laboratory information system needs: This step is crucial and determines the selection of a project technology leader, comparison of various tools, budgets (sponsors, aids, expense distribution), digital pathology system that is suitable for the concerned laboratory and can be integrated easily into the LIS. It also includes regulation of infrastructure that can accommodate the new system, provide storage and security.
4. Building a workflow: This is based on writing a statement (standard operating procedure) of workflow for each phase of digital conversion (image acquisition, preanalytics, image management and analysis).
5. Training: Offering of tutorials, training of stakeholders, hands on skill training of lab personnel is important prior to full adoption and to determine gaps and progress. Full conversion takes time. After intensive skill workshops to cover key essentials, online access to complementary materials and manuals can be provided.
6. Comparison of both systems and then rollout: Pathologists and stakeholders should review the glass slides first and then again in coming weeks WSI, concordance between the two methods should be established and turnaround time should be compared, followed by adjustment of digital workflow depending on results. After satisfactory results, fully convert to digital. Leverage benefits at full roll out volume.
7. Analysis and expansion of application: Review of speed, volume, and cost improvements, survey of customer satisfaction, exploring new insights from advanced data analytics, collaboration with providers on upgrades and refinement.

Essentials of Digital Pathology System (DPS):

Whole slide imaging systems (WSI) comprise of two major components – hardware and software. Typical hardware include microscope with lens objectives, light source (bright field or fluorescent), robotics to load and move glass slides around, digital cameras for image capturing and linked computer monitor with a software to manipulate, manage and view digital slides.

Digital image analysis software development has the potential to provide methods for quality assurance in slide review and permits quantitative interpretation of immunohistochemical staining intensity and location, and even image comparisons to archived previously diagnosed images. Under the new in vitro diagnostic medical device regulation (IVDR) of the European Parliament, manufacturers that are currently CE-IVD certified are those of Philips, Roche/Ventana, Leica/ Aperio, Hamamatsu, 3DHISTECH.

Figure 1: Whole Slide Imaging

(Reference: Aperio AT2: Leica biosystems)

Before installation of DPS in laboratory, Ergonomics, utility and convenience are vital for consideration. Recommendations laid down in literature by organisations who have completely converted to digital include testing out the interface provided by vendor prior to see if it is user friendly, easy to navigate.

Ensuring that the digital pathology scanner fits as easily as possible into the existing laboratory infrastructure and doesnot disrupt the laboratory workflow (such as unwanted noise while operating). Ability of its integration in lab's LIS. Digital pathology images can be up to several GB in size, with higher scan magnification producing larger image files. Early communication and interchange with IT department is critical, regarding handling of image storage (on-premise or in the cloud), as well as the network bandwidth, and data security; required for such large database. It is best to research peer reviewed publications reference to seek reliable vendors with good reputation, as Digital pathology is a substantial investment.

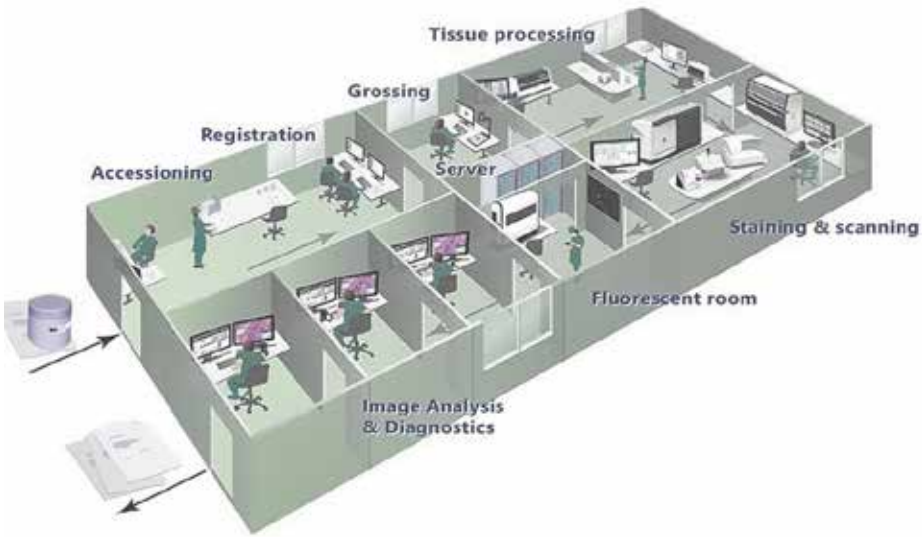


Figure 2: A Lean Digital Pathology Clinical Laboratory setting

(Reference: PANNORAMIC Pathology Management System, 3DHISTECH)

Digital Imaging in Hematology: A New Beginning

Dr Hajrah Syndeed, Dr Asif Naveed and Dr Anila Rashid
Haematology & Transfusion Medicine

Peripheral blood smear morphological analysis:

Morphological evaluation of peripheral blood smears by microscopy has a very important role for identification of benign and malignant hematological disorders. It is also important for picking up particular features in non-hematological disorders. Detailed morphological analysis includes evaluation of red blood cells, white blood cells and platelets. Any morphological abnormality is commented on complete blood count report and helps clinicians in diagnosis.

Over decades, microscopic morphological evaluation has been done by trained personnel (hematologists & lab scientists), which requires extensive training and is time consuming too. Digital morphological evaluation of peripheral blood smear is a new beginning in the modern era of diagnostics. Several analyzers are now available that provide this opportunity of evaluation through use of various software.

Principles & methods used in digital morphology:

Majority of the digital systems use innovative image analysis technology based on a prior classification of white blood cells (done on the standard counts and scatter plot). A similar characterization is present in the software for identification of red cells morphology. There are approximately 21 morphological features described for red blood cells and 17 pre-defined types for white blood cells into the system.

Benefits of digital morphological evaluation:

1. Time & labor efficient: Digital evaluation using software reduces time for evaluation of multiple blood smears. This also helps in reducing the workload on hematopathologists.
2. Inter-observer variance: Pre-defined criteria for identification of cells reduces the inter-observer difference to a large extent.

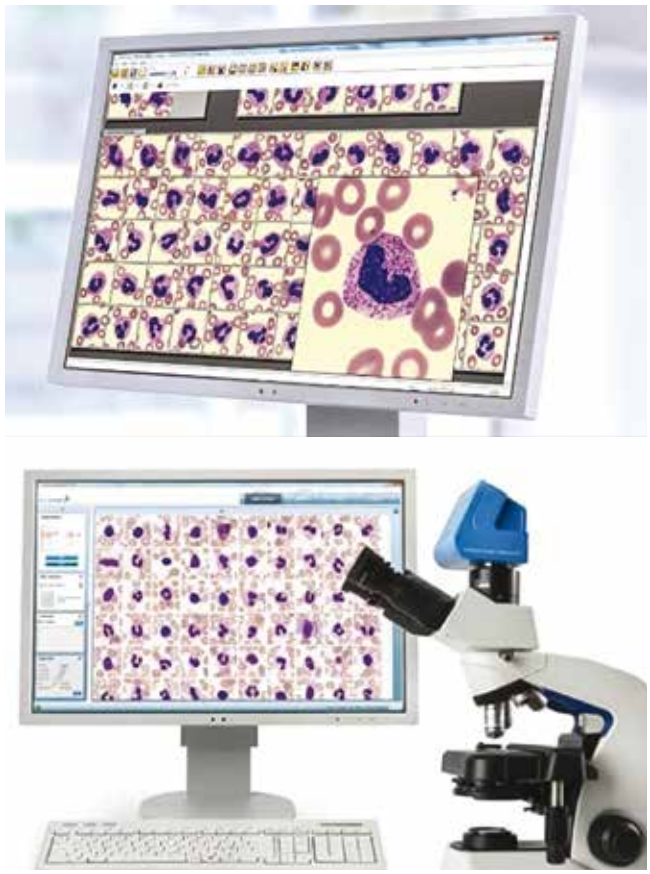


Figure 1: Digital Morphological Evaluation

(Source of Image: https://www.trademed.com/products/4289/Image-Capture-System.html)

3. Space constraints: The use of glass slides to assess morphology leads to storage and safety issues. The use of digital images instead of the slides would help in reducing these concerns and helps diagnostics to improve.
4. Remote reviews: Digital morphology/images can be remotely accessed by professionals improving academics and diagnostics.
5. Elimination of eye strain: Another benefit of using digital imaging is that, it reduces eye strain related to morphological assessment through conventional microscopy.

6. Education & Training: Digital morphological images can be saved into digital library that facilitates education on multiple academic levels.
7. Research: Ease of availability and appropriate use of images help for retrospective research for various laboratory and clinical disciplines.

Limitation of digital morphology:

1. In neonates and pediatric samples, digital imaging may not be helpful for identification of some abnormal cells. This will require a routine microscopic review (10-20 percent) of total samples.
2. Digital morphology has a limited role when it comes to identification of malignant cells, dysplastic cells, red cell fragmentation, blood born parasites and platelet clumps. This may need a review from experienced professional.
3. A limited number of standardized soft-wares are commercially available and are not very cost effective.

Although, digital imaging requires robust validation and training of personnel, its introduction has brought about a change in modern era of diagnostics allowing remote reviews, evaluation of laboratory performance through proficiency testing, education and research.

Fourier-Transform Infrared Spectroscopy; Analytical Technique to Detect Metabolites

Dr Muhammad Umer Naeem Effendi
Clinical Chemistry

Vibrational spectroscopies like Fourier transform infrared (FTIR) spectroscopy allow classification of high-throughput screening of biological specimen and equally fits the “omics philosophy” of providing unbiased, whole-system measurements. Specifically, the use of FTIR spectroscopy to monitor biochemical changes in living cells has widely gained importance in the last ten years. This technique presents the possibility to simultaneously identify various cellular biochemical targets, both in vivo and in vitro conditions, exploiting the differential infrared radiation absorption of each metabolite at a specific wave number.

Molecules and systems of biological relevance that can be detected by FTIR spectroscopy include molecules such as lipids and fatty acids, proteins, peptides, carbohydrates, nucleic acids, and biomembranes, animal tissues, microbial cells, plants, and clinical samples. However, more recently, it has been used, with the aid of sophisticated sampling techniques such as infrared imaging, to diagnose many diseases such as cervical cancer, Parkinson’s disease, Alzheimer’s disease, kidney stone and arthritis. So this technique has varied clinical application. While literature has reported that this technique can be applied for analyzing colon biopsies through FTIR spectroscopy and chemometrics in identification of colitis and colon cancer. Multiple studies have reported that infrared spectra allowed the identification of the discriminating molecules, e.g., the phosphate content and RNA/DNA ratio which can then be used in certain diseases diagnosis and monitoring. Cluster analysis of the selected spectra provided excellent classifications that correlated completely with clinical data. Although these results are preliminary, the technique appears to be promising, rapid, effective, and economic, assisting in the disease diagnosis.

At the Aga Khan clinical laboratory we have been identifying the renal and gall stone components using the FTIR analysis. FTIR spectroscopy effectively provides precise and accurate chemical variation information about the structure and the composition of the stones.

Metabolomics: Identification of Fatty Acid Oxidation (FAO) Disorders

Azeema Jamil
Clinical Chemistry

Fatty acids (FAs) are important and play a significant role in health and diseases. The detection of FA imbalances, through metabolomics can provide an overview of an individual's health status. These disorders are caused by a lack or deficiency of the enzymes needed to break down fats, resulting in delayed mental and physical development. Children with one of these disorders have a deficiency of the enzymes needed to break down fats. The lack of one of the enzyme leaves the body short of energy and accumulate products, such as Acyl-CoA.

The most common deficiency is medium-chain acyl-CoA dehydrogenase (MCAD). Other deficiencies include short-chain acyl-CoA dehydrogenase (SCAD), long-chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) deficiency, very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency, glutaric acidemia type II etc. can be identified using these metabolomics based approaches. Most of these disorders begin in infancy. FAO disorders treatment varies depending on the type of substances that accumulate in the blood.

MCAD deficiency: MCAD is one of the most common inherited metabolic disorders. Symptoms of the disease usually develop within first few months of life. Children are most likely to develop symptoms if they go without food for a while or while they have increased calories requirement, because of exercise or illness. The sugar level significantly decreases in the blood (hypoglycemia), causing confusion or coma. Children may have vomiting or seizures and become weak. Long term symptoms cause delayed mental and physical development, liver enlargement, weakness of heart muscle with an irregular heartbeat. Sudden death may occur.

LCHAD deficiency: The second most common fatty acid oxidation disorder is LCHAD. Symptoms are the same as those caused by MCAD. Affected individuals may also have progressive impairment of the structure, cardiomyopathy, nerve damage, particularly the hands and feet, and abnormal liver function. When children exert themselves, such as exercising, the muscle tissue may become destroyed. The damaged muscles may release the protein myoglobin, which turns the urine colour brown or bloody.

VLCAD deficiency: VLCAD deficiency is similar to LCHAD deficiency but presents with severe cardiomyopathy.

Glutaric acidemia type II: Glutaric Acidemia type-II is one of the condition termed as organic acidemia. Individuals with these conditions have a deficiency or absence of an enzyme that prevents the breakdown of proteins and fats in the body, resulting in several organic acids in the blood and urine. It usually appears in infancy or early childhood as a sudden episode called a metabolic crisis, in which acidosis and hypoglycemia cause weakness, change in behaviour such as poor feeding and low activities and vomiting. Common childhood illnesses or other stresses may trigger these metabolic crises (life-threatening).

Fourier Transform Infrared Spectroscopy – Attenuated Total Reflectance (FTIR–ATR) based Metabolomics for Gallstones: Development of Pakistani Gallstone Library

Drs. Muhammad Abbas Abid and Lena Jafri
Clinical Chemistry

The prevalence of gallstones is rising, possibly as a result of longer life expectancy and altered nutritional habits. Around 9-10.2 percent of Pakistani carry gall bladder stones (1). Investigation of the constituents of gallstones using spectroscopic procedures helps in understanding the pathogenesis and etiology of stone formation. In Pakistan, the diagnostic facilities for assessing gallstone composition are primitive. In the past, gallstone analysis was performed using the manual titrimetric and colorimetric technique. Not only was this method time and labor intensive, but also the results only revealed part of the actual composition. Fourier Transform Infra-Red (FTIR) Spectroscopy provides a quick and reliable method for gallstone analysis. The diamond Attenuated Total Reflectance (ATR) accessory allows for detection of dry samples without any pre-treatment. It detects light absorbance and transmittance in the wavenumber range 4,000 to 400 cm-1 (wavelengths 2.5 to 25 µm) and produces a unique absorbance pattern for each sample.

The targeted metabolomics study requires an already established reference library to match the produced absorbance pattern. Unfortunately, no commercially available neither a nationally developed library exists to match the spectra developed by analyzing gallstone samples, rendering the upgradation of gallstone analysis impossible. Identifying this resource gap, we aimed to develop a Gallstone Standard Library (GSL) and a Gallstone Real Patients' Library (GRPL) and validate them using FTIR Spectroscopy in the Section of Chemical Pathology, Department of Pathology & Laboratory Medicine, Aga Khan University. Commercially available pure standards of cholesterol, calcium carbonate, bilirubin and bile salts (figure 1) as well as gallstone specimens were analyzed using FTIR Nicolet iS-5 Spectrometer from Thermo Fisher Scientific, USA. Thermo Scientific™ QCheck™ algorithm, embedded within the OMNIC™ software, was used to identify the unique spectral fingerprint of the patient samples to match with known, standard material. Similarity of >75 percent was deemed adequate. Validation for accuracy of the library was achieved for twenty investigated gallstones at an international reference lab.

Rigorous examination was executed against the established GSL entailing 71 "pure component" spectrum separated into five subtypes to generate the library. For the GRPL, 117 patient samples from various cities of Pakistan were included. Eighty-four percent of 117 stones matched with the established GSL. Mixed stone was the most common subtype, with cholesterol being the primary component. FTIR spectrum of pure standards studied of bile salt is shown in figure 1. Outcomes of the established library were in complete agreement with the results verified from the international reference lab. The established library exhibited consistency is now effectively used for examination of gallstone configuration in our population and has replaced the labor- and time-intensive chemical method of gallstone examination.

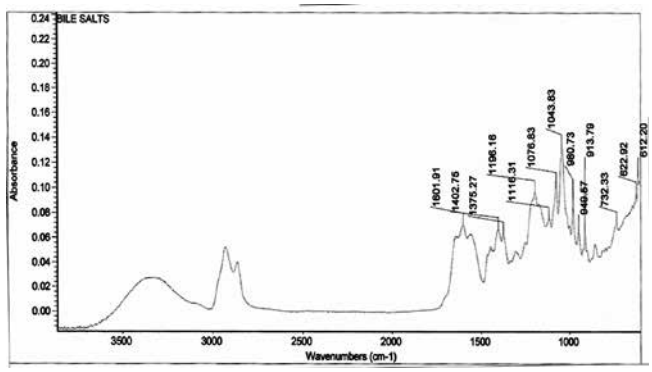


Figure 1: FTIR spectrum of pure standards studied of bile salt analyzed at Section of Chemical Pathology, AKU

Next-Generation Sequencing for HLA Genotyping

Samina Ghani
Molecular Pathology

The human leukocyte antigen (HLA) system also known as major histocompatibility complex (MHC) in humans) is an important component of the immune system. It is controlled by genes located on chromosome six. The human leukocyte antigen (HLA) genes represent the most diverse loci in the human genome with over 14,000 alleles identified as of December 2015. The high allelic diversity in the HLA genes reflects HLA protein function in binding and presenting a diverse array of peptide ligands derived from microbial pathogens. As a result of selection pressure, the HLA system now constitutes one of the major genetic differences between individuals and among different ethnic populations. Therefore, the HLA genes are clinically relevant as key determinants of compatibility in organ and bone marrow transplantation and genetic susceptibility to diseases. However, the highly polymorphic nature of HLA genes poses unique challenges for the development of molecular approaches to genotype HLA alleles. This brief review will summarize the utility of next-generation sequencing (NGS) for HLA genotyping, highlighting the advantages of this approach over other molecular methods for typing HLA alleles.

Using standard Sanger sequencing methodology, both alleles of a particular HLA locus are amplified and sequenced together resulting in multiple heterozygous positions in the electropherogram tracing. (Figure 1A).

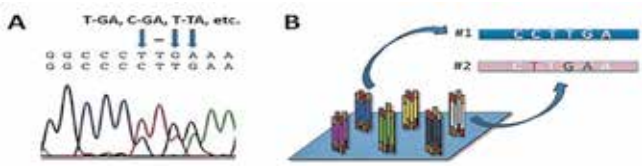


Figure 1: Sanger sequencing methodology

As the phase of the polymorphic positions cannot be visually determined, additional steps are required to assign an HLA genotype. These include the use of informatics software to query the IMGT/ HLA sequence database and assign the most likely combination of alleles, PCR amplification of only one allele, or the use of sequencing primers which anneal to only one of the two potential HLA alleles. In some instances, alternative allele pairs cannot be excluded, leading to genotype ambiguity. The additional steps required to generate HLA genotypes using Sanger sequencing are laborious and time-consuming, thus increasing the costs associated with HLA genotyping. As a consequence, only a few select exons of an entire HLA gene are routinely sequenced in clinical laboratories to determine a patient's HLA genotype and ultimately the degree of HLA match between donor and recipient.

Next Generation Sequencing: A common feature of NGS technologies is that each fragment of DNA is amplified and sequenced independently and repeatedly, thus reducing the phase ambiguities encountered using Sanger sequencing (Figure 1B). Several approaches NGS for HLA genotyping have been reported using a variety of capture strategies and sequencing platforms.

The 454 platform utilized NGS for HLA genotyping for application. The read length of this technology (~ 250-500 bp) was sufficient to cover the average size of an HLA exon Similar to Sanger approaches, the 454 HLA typing strategies utilized exon-targeted amplification (Figure 2A), which led to challenges in primer design and required numerous PCR reactions during library preparation. Although automation and microfluidic PCR technology were able to mitigate some of these issues, the amplicon-based sequencing approach was gradually replaced by a shotgun sequencing strategy (Figure 2B). In shotgun sequencing approach long-range PCR is used to amplify each HLA locus in a singlereaction. PCR amplicons are large and fragmented to produce appropriately sized sequencing templates., these templets produce the short (100-250 bp) sequencing

reads which are aligned to re-create a full-length HLA sequence. The advantage of the long-range PCR and shotgun sequencing approach is that primers can be designed to anneal in less polymorphic regions of the HLA genes (5' and 3' UTR's, for example), resulting in enhance capture and sequence of the HLA gene regions. Paired-end sequencing can also be utilized to bioinformatically phase HLA sequence data over longer genetic distances (Figure 2C), including regions between exons. Multiple commercial assays that utilize the shotgun approach for HLA NGS are now available for platforms such as Ion Torrent and Illumina.

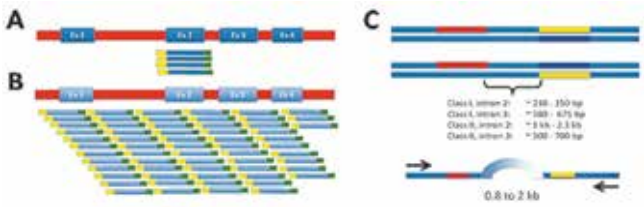


Figure 2: Exon-targeted amplification for HLA typing

Finally, bioinformatics approaches have also been developed to produce HLA genotyping information from targeted capture (exome) and non-targeted whole-genome sequence data. One of the major hurdle is the lack of sequence coverage from the HLA region obtained through exome capture techniques and pseudogene leading to misalignment of sequence reads. Despite these challenges, improved capture strategies and HLA genotyping analysis strategies offer the ability to generate accurate HLA genotyping from exome and whole-genome data. Advances in NGS methodologies and competitive bioinformatics analysis approaches for HLA genotyping, enabled us to define genes within the HLA region, identifying polymorphisms that may elucidate the evolutionary history of HLA alleles, gene expression, and disease risk.

ABL Kinase Domain Mutation Analysis by Sanger Sequencing for TKI Resistant

Kahkashan Imam, Nazneen Islam, Zeeshan Ansar

Dysregulated tyrosine kinase (TK) activity is a frequent hall- mark of multiple neoplasms. Chronic myelogenous leukemia (CML), a myeloproliferative disorder, represents a well characterized model disease for TK-dependent transformation. On the basis of the implementation of basic and clinical research into clinical practice, introduction of tyrosine kinase inhibitors (TKIs) are now the gold standard in the treatment of CML patients and a successful example of targeted therapy. The first cases of CML in leukemia patients in the late 19th century, were diagnosed through an enlarged spleen and markedly elevated leukocyte counts. It took almost one century to uncover that the Philadelphia chromosome is the typical chromosomal change in CML patients and another 13 years until Rowley and co-workers showed that this anomaly is in fact based on a translocation between chromosome 9 and 22. Another ten years later, Bartram and co-workers were able to show that the tyrosine kinase gene abl on chromosome 9 and the bcr gene on chromosome 22 are fused and generate the bcr-abl gene on the Philadelphia chromosome. The BCR-ABL oncopro-tein is characterized by constitutive and increased TK activity and leads to malignant transformation of hematopoietic stem and progenitors cells resulting in hypercellularity of the bone marrow and altered cell adhesion properties of leukemic cells both leading to typical leukocytosis and sple-nomegaly observed clinically at diagnosis. Untreated CML progresses through three distinct clinical phases. Initially, CML presents with a relatively stable chronic phase (CP) and typically develops through an accelerated phase (AP) towards myeloid or lymphoid (and rarely megakaryocytic or even monocytic) blast crisis (BC), which strongly resembles acute leukemia.

Table1: Recommended Testing Parameters for Patients on TKI Therapy per NCCN Guidelines

Test	Recommended Test Specifications	Recommended Test Frequency
Bone marrow cytogenetics	Analyze ≥ 20 metaphase cells	At diagnosis At 3 months, if RT-qPCR (IS) is not available At 12 months, if neither CCyR nor MMR is achieved At 18 months, if no MMR and no CCER at 12 months ≥1-log increase in BCR-ABL1 level without MMR
FISH	Use peripheral blood Use dual probes for BCR and ABL1 genes	At diagnosis, if collection of bone marrow is not feasible Not recommended for monitoring response to treatment
RT-qPCR	Use an RT-qPCR (IS) assay with sensitivity of ≥ 4.5 log below the standardized baseline Use peripheral blood or bone marrow	At diagnosis Every 3 months for responding patients After CCyR is achieved, every 3 months for 3 years, then every 3 to 6 months thereafter If ≥1-log increase in *BCR-ABL1* level with MMR, repeat in 1 to 3 months
BCR-ABL1 KD mutational analysis	None provided	If inadequate* response to first-line therapy or any sign of loss of response, defined as hematologic or cytogenetic relapse, or ≥1-log increase in *BCR-ABL1* level and loss of MMR If disease progression to accelerated or blast phase CML

Clinically Relevant BCR-ABL1 Threshold Levels for Predicting TKI Treatment Responses:

The clinical utility of laboratory methods for monitoring TKI therapeutic efficacy, including hematologic, cytogenetic, and PCR-based techniques, depends largely on their limits of detection. Complete hematologic response is based on normalization of peripheral blood counts [National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology (NCCN Guidelines) Chronic Myelogenous Leukemia Version 3.2013last accessed. Cytogenetic response is based on the percentage of Ph-positive (Phþ) metaphase cells observed in a bone marrow sample, typically by Giemsa staining of metaphase chromosome spreads.

Cytogenetic testing does have, however, the capability to detect other chromosomal abnormalities besides the Ph chromosome, and this may have prognostic relevance. Ancillary interphase cell fluorescence in situ hybridization (FISH), with DNA probes for BCR and ABL1, is also commonly used to monitor CML response to treatment. Because 100 to 500 interphase cells are typically examined with FISH, this approach is more sensitive (1:500 to 1:100, or 0.2 to 1%) than metaphase cytogenetics. Depending on the probes used, however, it can have a high false-positive rate. FISH lacks practical utility for MRD monitoring in the majority of TKI-treated CML patients, and therefore is not recommended by the NCCN for routine monitoring of TKI treatment response (NCCN Guidelines). Instead, guidelines from the NCCN, the European Leukemia Net (ELN), and the National Institutes of Health recommend serial BCR-ABL1 RT-qPCR assays at regular 3- to 6-month intervals for routine MRD monitoring of CML patients receiving TKI therapy. Molecular monitoring involves extraction of RNA from a bone marrow or peripheral blood specimen and subsequent RT-qPCR to measure transcript levels of BCR-ABL1 relative to those of a reference gene. Because the analyte of RT-qPCR, RNA, is labile and degradation prone, many pre-analytical variables can affect sample quality and quantitative results, including sample source (peripheral blood versus bone marrow), sample storage (temperature, type of tube), sample transport (duration of travel, temperature), and sample stabilization (lysis buffer, storage buffer). The reference gene in RT-qPCR serves as a control for overall RNA quality (with respect to degradation) and, assuming equivalent reference gene expression in all hematopoietic cells, for the number of input cells per PCR reaction. For example, in our laboratory, when the level of reference gene RNA falls two SD below the mean, RT-qPCR for the BCR-ABL1

and reference gene are repeated, and if this repeat analysis again shows low reference gene RNA levels, the sample is reported as inadequate (new sample requested). Compared with cytogenetic testing, PCR-based molecular monitoring offers exquisite analytical sensitivity, 100 to 1000 times greater than FISH or bone marrow cytogenetic analysis. It is applicable to bone marrow and peripheral blood samples, and with a short turnaround time, provides quantitative results that are associated with validated clinical response thresholds. Disadvantages of RT-qPCR include its lack of methodological and reporting standardization, its need for specialized laboratories and equipment, and the variability of analytical and reporting systems. Summarizes current NCCN testing recommendations for monitoring TKI treatment response in patients with CML.

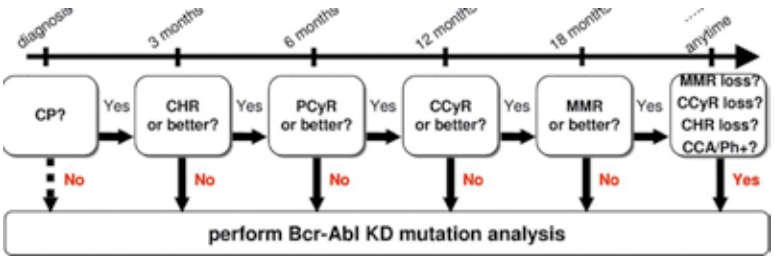


Figure 1: BCR-ABL1 KD Mutations for Therapeutic Choices

BCR-ABL1 KD Mutations for Therapeutic Choices

The emergence of BCR-ABL1 KD mutations has been shown to be associated with an increased likelihood of subsequent disease progression in patients with TKI-treated CML, Although the switch to an alternative TKI, triggered by the finding of a KD mutation, has never been shown to directly improve long-term outcomes in a prospective trial, ELN and NCCN guidelines nevertheless specifically recommend a switch to certain TKI agents when particular mutations are detected. Due to the diversity of mutations, full BCR-ABL1 KD mutation screening is done in most laboratories by a direct Sanger DNA sequencing technique that has a detection limit of approximately 20% mutant allele. A significant majority of BCR-ABL1 KD mutations cluster to one of four hot spots: the ATP-binding P-loop (amino acids 248 to 256); the imatinib-binding region (amino acids 315 to 317); the catalytic domain (amino acids 350 to 363); and the activation (A)-loop (amino acids 381 to 402).11 Differential sensitivity to imatinib, dasatinib, nilotinib, bosutinib, and ponatinib has been demonstrated by these diverse mutant BCR-ABL1 kinasesin vitro studies.49e52 Because there is often, but not always, a good correlation between mutation-specific in vitro resistance and in vivo clinical responses for some, but not all, KD mutations and TKIs, the identification of the specific mutation can help to inform the optimal management strategy. In particular, the presence of the common T315I mutation suggests that ponatinib, and no other TKI, may be effective. In addition, NCCN and ELN guidelines suggest a switch to nilotinib (not dasatinib) for patients with the V299L, T315A, or F317L/V/I/C mutations; and a switch to dasatinib (not nilotinib) for patients with the Y253H, E255K/V, or F359V/C/I mutations. Aside from point mutations, the BCR-ABL1 KD also commonly develops insertion/deletion mutations, including a 35-bp intronic insertion at the exon 8 to 9 junction, an L248V mutation with deletion of 81 bp of exon 4, an exon 7 deletion, and several others. Although the clinical and drug resistance significance of most of these insertion deletion mutations is still unclear, the very common 35-bp intronic insertion after exon 8 does not appear to mediate TKI resistance, in vitro or in vivo. The BCR-ABL1 KD also carries some common single nucleotide polymorphisms that appear to be wholly benign, including three non-synonymous (K247R, F311V, Y320C), and three synonymous (T240T, T315T, E499E) variants, each of which has no known effect on TKI binding or drug resistance.

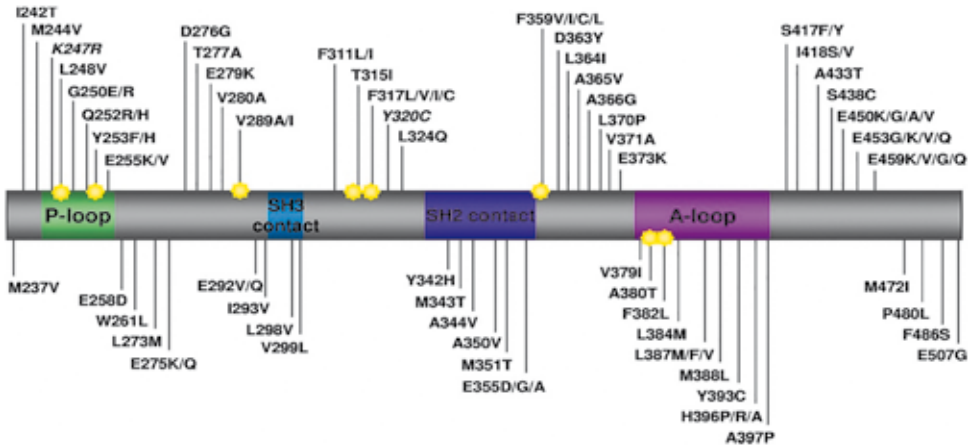


Figure 2: Map of all the amino acid substitutions in the Bcr-Abl KD identified in clinical samples from patients reported to be resistant to imatinib

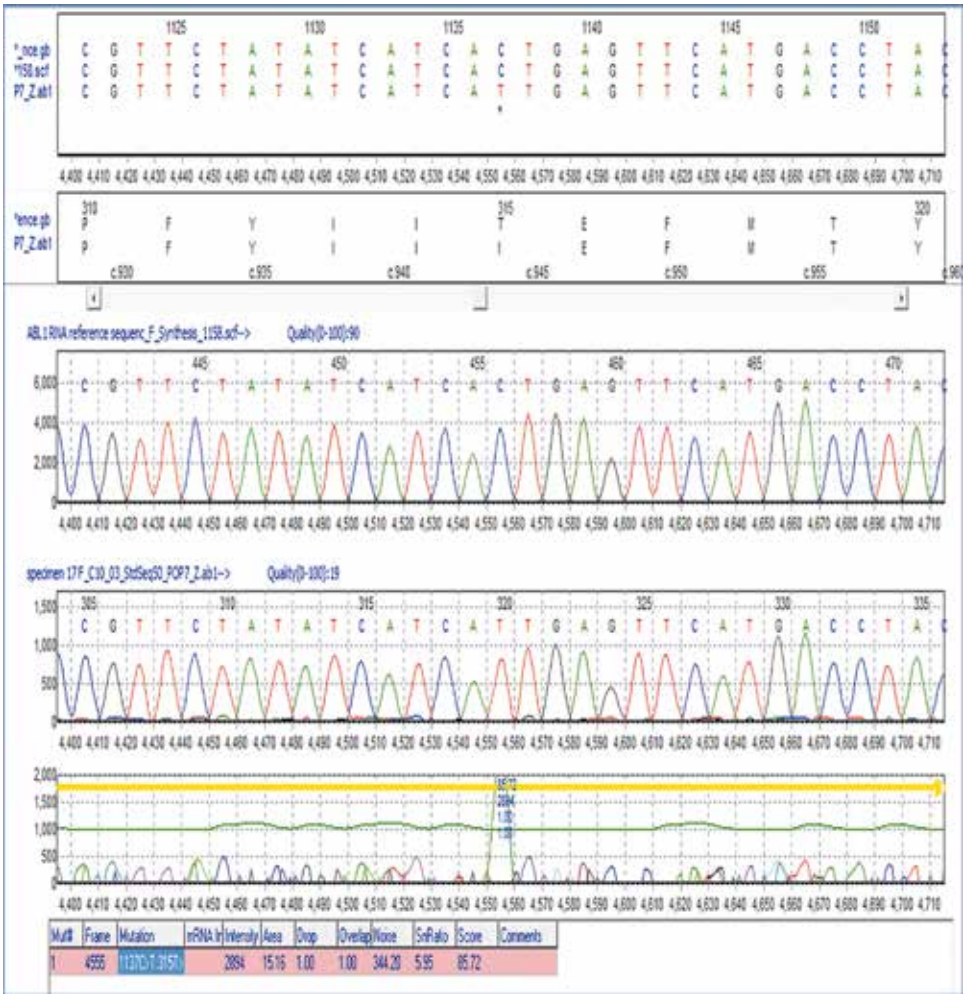


Figure 3: Interpretation of Bcr-Abl KD identified patient's samples and Reference sample

Given our evolving understanding of the molecular events mediating resistance in CML and Ph_ ALL, standards for reporting of BCR-ABL mutational studies would benefit from a greater degree of uniformity. Commercially-available reference samples and calibrators as well as a publicly available BCR-ABL mutation database are the currently needed resources to allow laboratories and clinicians to interpret the significance of BCR-ABL KD mutation studies. While these standardization efforts are proceeding, mutation studies should be based on the already developed criteria for clinical resistance (summarized above) to better ensure appropriate utilization. As shared databases become more widely available, the most appropriate statements regarding the clinical significance of specific mutations will be better defined and allow more precise guidance to be given.

Urine Metabolomics to identify Organic Academia

Saba Abdul Mateen
Clinical Chemistry

Organic acids are intermediates of amino acids, carbohydrates, lipids and biogenic amine metabolism, and any defect in a metabolic pathway may result in elevated levels of these metabolites in urine. The scientific study of chemical processes involving metabolites is known as metabolomics, so organic acid analysis in urine is known as metabolomics through organic acid.

At Aga Khan University Hospital, Karachi analysed urine organic acid samples by Gas Chromatography-Mass Spectrometry (GCMS) in Biochemical Genetic Laboratory (BGL). A spot-urine sample is required for analysis without preservative, and samples are stored at -20°C until analysis. The analysis is performed using ethyl acetate derived by bis-trimethylsilyl, using

the internal standard 3,3 dimethyl glutaric acid. The data generated from the mass detector is analyzed by Chemstation software with the help of libraries, for example, the NIST library for Organic acids. The system's precision is assessed by analyzing internal quality control samples, while for proficiency testing, ERNDRIM and CAP samples are analyzed.

One of the most common disorder in Pakistan is methylmalonic aciduria which is easily detected on GCMS. Methylmalonic acidurias are inherited metabolic disorders characterized by elevated methylmalonic acid, along with methyl citrate, 3-hydroxyisovalerate 3-hydroxypropionate, and tiglylglycine shown in figure 1. Metabolites are widely used as diagnostic and/or prognostic indicators of inherited metabolic diseases (IMDs). Urine metabolomics plays a key role in diagnosis of these disorders.

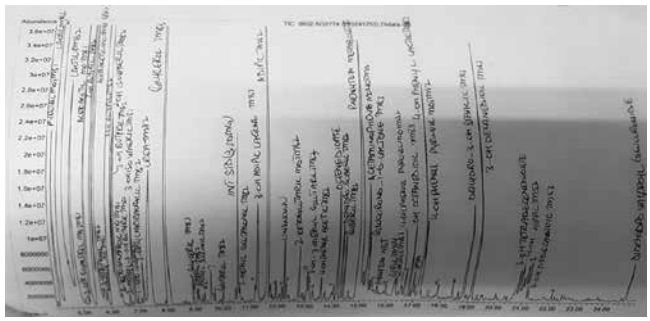


Figure 1: A Urine organic acid chromatogram for a Patient with Methylmalonic Aciduria

Next-Generation Sequencing (NGS) of Solid Tumors

Misha Ahmed, Dr Najia Ghanchi
Molecular Pathology

Next-generation sequencing (NGS) has changed the face of oncology research and diagnostics by significantly increasing the breadth, sensitivity, and specificity of information obtained within a single assay. A newer, alternative strategy called next generation sequencing (NGS) allows clinicians to simultaneous detection of several genes associated with cancer. Next generation sequencing can be performed on material from a patient's tumor that has been biopsied or surgically removed.

Next-generation sequencing (NGS) is a new technology used for DNA and RNA sequencing and variant/mutation detection. NGS can sequence hundreds and thousands of genes or whole genome in a short period of time. Deep sequencing using Next-generation sequencing (NGS) provides the highly sensitive detection of somatic variations in tumor subpopulations. Illumina sequencing by synthesis (SBS) chemistry is the most widely adopted NGS technology.

Next-generation sequencing involves four basic steps:

Library preparation is the first step of next generation sequencing. It allows DNA or RNA to adhere to the sequencing flow cell and allows the sample to be identified. Two common methods of library preparation are ligation-based library prep and tagmentation-based library preparation.(Figure 1)

Cluster Generation, the library is loaded into a flow cell where fragments are captured on a lawn of surface-bound oligo complementary to the library adapters. Each fragment is then amplified into distinct, clonal clusters through bridge amplification when cluster generation is complete, the templates are ready for sequencing.

Sequencing Illumina SBS technology uses proprietary reversible terminator–based method that detects single bases as they are incorporated into DNA template strands. As all four reversible terminator–bound dNTPs are present during each sequencing cycle, natural competition minimizes incorporation bias and greatly reduces raw error rates compared to other technologies. The result is highly accurate base-by-base sequencing that

virtually eliminates sequence context–specific errors, even within repetitive sequence regions and homo polymers.

data analysis During data analysis and alignment, the newly identified sequence reads are aligned to a reference genome. Following alignment, many variations of analysis are possible, such as single nucleotide polymorphism (SNP)or insertion-deletion (indel) identification, read counting for RNA methods, phylogenetic or metagenomic analysis, and more.

It provides the sensitivity and accuracy needed to identify low-frequency variation with confidence in samples of varying quality. High target coverage (at least 93.5 percent of bases covered at ≥ 500×) provides sensitivity and accuracy required for low-level variant calling.

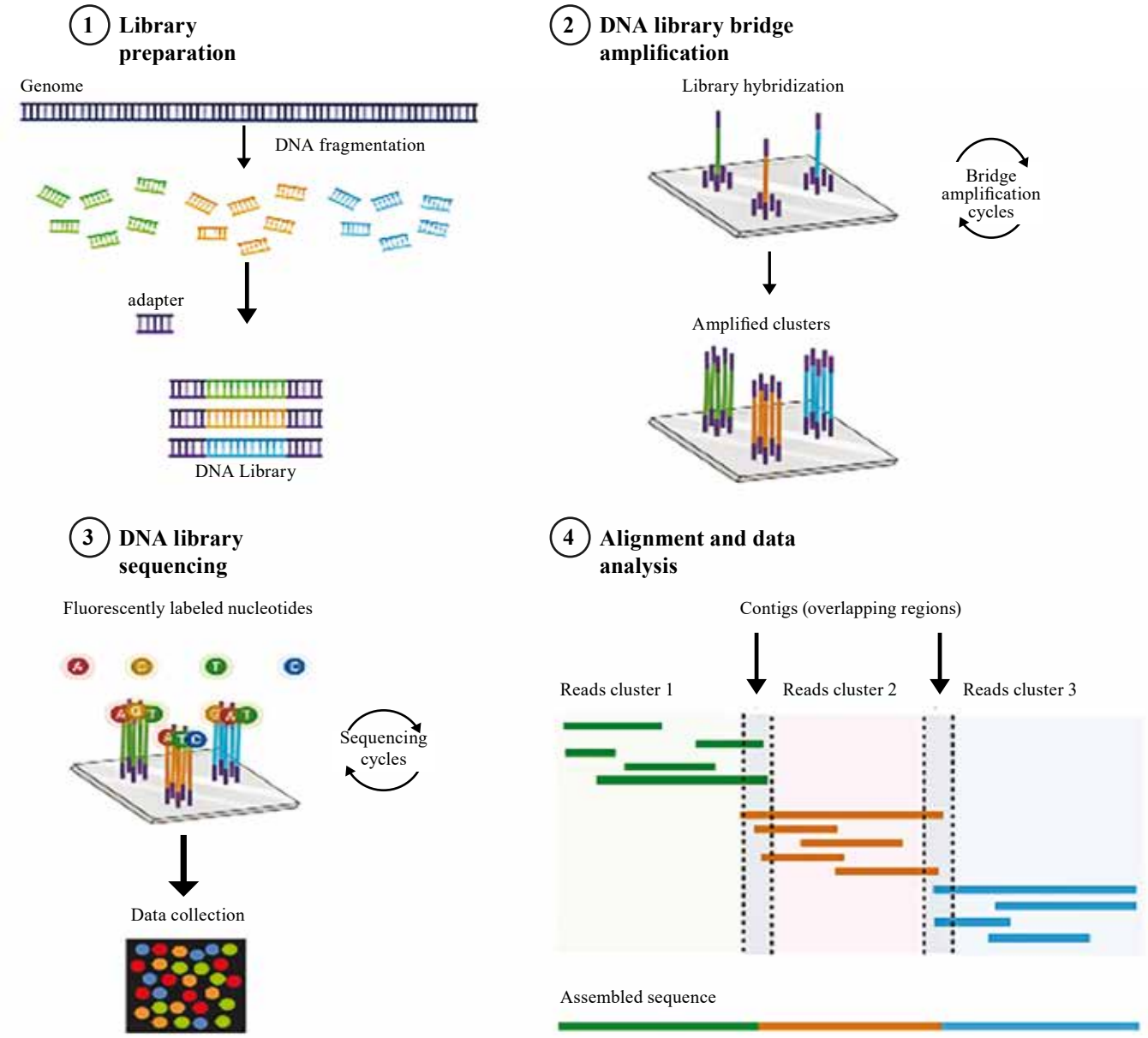


Fig 1. Next-generation sequencing involves four basic steps

Applications: In section of molecular pathology we perform 15 gene hotspot mutation panel that are commonly mutated in solid tumors or Commonly associated with prognosis of available therapies..

Using NGS based testing method, we accurately detects variants from small amount of nucleic acid extracted from formalin-fixed, paraffin-embedded (FFPE) tumor tissue. Formalin-fixed, paraffin-

embedded (FFPE) tissue are routinely used for morphological analysis of solid tumors. Both the quality and quantity of input DNA are critical for producing high-quality data for molecular assays.

TruSight Tumor 15 assay utilize this method to detect 15 most commonly mutated genes in seven cancer types (Lung,Melanoma, Breast, Colon, Ovarian, Gastric, and Prostate).This assay can examine multiple cancer-associated genetic alterations simultaneously. Solid tumor assay targets genes and gene regions include single nucleotide variants (SNV) and insertions and deletions (indels) that have demonstrated involvement in solid tumors. The accurate somatic variant detection of five percent allele frequency can be achieved using FFPE tissue samples. Following are the list of genes detected by this method AKT1, GNA11, NRAS, EGFR, GNAQ, PDGFRA, BRAF, KIT, PIK3CA, FOXL2, KRAS, RET, ERBB2, MET, TP53. (Table 1)

Table 1. TruSight Tumor 15 (TST15) Gene Panel Content

Gene Region Content (with target) covered by TruSight Tumor 15 with potential disease state		
AKT1 Exon 3*;E17K Breast	**GNA11(guanine nucleotide-binding protein G protein)** Exon 5*;Q209L Melanoma	**NRAS(neuroblastoma RAS viral oncogene)** Exon2*,3* (partial), 4 codons12,13,59,61117,146 colon
BRAF(B-RAF protein) Exon 15*(patient);V600E/K/R/M Melanoma, colon ,lung	**GNAQ (guanine nucleotide binding protein, alpha stimulating)** Exon 5*(patient);Q209L Melanoma	**PDGFRA (Platelet Derived Growth Factor Receptor Alpha)** Exon 12,14,18 Gastric, Melanoma
EGFR (epidermal growth factor receptor) Focal Amplification, Exons12* (partial)18,19,20; G719A, G719X; Exon 21(L858R), L861Q, T790M Lung	**KIT** Exon8,9,10,11,13,14,17,18 Gastric, Melanoma	**PIK3CA (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha)** Exon 9,20 Lung, Breast, Prostate
ERBB2 (erythroblastic oncogene B) Focal amplification, p.E770_A77insAYVM Exon 14 (partial), 17,18,19,20* ,21*(partial) ,24,26 Lung ,Breast	**KRAS (Kirsten rat sarcoma viral oncogene)** Exon2*(partial),3 (partia),4 Colon, Lung , Gastric	**RET(rearranged during transfection)** Exon 16(M918T) Lung
FOXL2(forkhead transcription factor) Exon1*(partial),C134W Ovary	**MET** Focal Amplification Lung, Colon, Gastric	**TP53 (tumor protein)** Full coding sequence Lung, Melanoma, Ovary ,Colon

* = Coverage of these exons is only partial and targets specific hotspots

The concordance was found 100 percent between variants from reference material and TST15 reported variants in laboratory our studies. It shows reliable variant detection with reported clinical significance. It is important to understanding the molecular landscape of cancers and contribute to the worldwide ongoing effort to determine whether certain variants are potentially actionable variants. Next generation sequencing method provides concurrent information on multiple genes with high quality performance, appropriate turnaround times, and optimal use of FFPE tissue specimens. With the continuing discovery of new cancer biomarkers and availability of robust assay clinical services are required to implement more comprehensive molecular testing methodologies.

Importance of using Genomic Tool in Microbial Identification

Dr. Mohammad Arsalan and Dr. Sobia Khan,
Microbiology

The transition of "omics" tools utility from research to disease diagnosis have revolutionized the health care. This paradigm shift in diagnostics have opened the new avenues for therapeutic and preventive interventions. Several technologies have been employed including **transcriptomics,** which measures mRNA transcript levels; **proteomics**, which quantifies protein abundance; **metabolomics**, which signifies abundance of small cellular metabolites; **interactomics**, which unravels the whole set of molecular interactions in cells; and **fluxomics,** through which dynamic changes of molecules within a cell are established over time (figure 1). The multi-omics approach is required to unravel the difficulties of fundamental microbial biology.

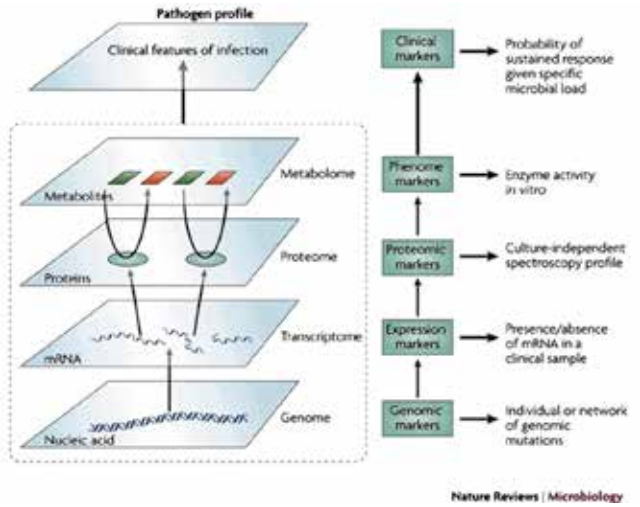


Figure 1: Multi-OMICS approach use in disease diagnosis

Medical microbiology has been transformed by the genomics in infectious disease diagnosis, treatment, and preventive approaches. Following are few exemplary situations in this regard.

1. **Infectious disease surveillance**
   In the era of emerging infectious diseases, genomic identification of causative agents aids in disease burden estimation, epidemiology, trend monitoring, and investigation of new outbreaks. This has improved public health surveillance and also filled the knowledge gaps with-in short time span. Studies done in Sub-Saharan Africa between 2012 and 2018 found that > 50 percent newly diagnosed HIV infants are resistant to mainstream non-nucleoside reverse transcriptase inhibitor (NNRTI) medications. This evidence formed the basis of developing effective surveillance guidelines and policies. World Health Organization (WHO) recently updated the recommendation and has included HIV integrase region genotype testing (along with the reverse-transcriptase and protease regions) in all specimens obtained in pretreatment or acquired HIV drug resistance.

WHO supports the use of NGS as an additional surveillance tool in below diseases. (figure 2).

	Disease control programme	Purpose	Pending concerns
HIV drug resistance	HIV	Guiding treatment policy to ensure sustained effectiveness of restricted regimens and sustained progress towards achieving epidemic control by 2030; potential for use in clinical management, especially in heavily treated patients with few therapy options	Methods depend mainly on Sanger-based sequencing; standardisation of NGS methods still ongoing, including establishment of clinically significant thresholds for minority variants and standardisation of bioinformatics pipelines and NGS-based EQA panels or programmes
Tuberculosis drug resistance	Tuberculosis	Guiding treatment policy; forecasting for second-line drugs; overall prevention efforts for minimising emergence and spread of drug resistance; individualised resistance test is essential for guiding clinical management, especially for people with multidrug-resistant or extensively drug-resistant tuberculosis	Optimisation and standardisation of laboratory methods and bioinformatics tools ongoing; need for more sensitive methods to allow for non-culture WGS-based approaches; need for optimisation of the sensitivity of resistance detection for certain drugs
Malaria drug resistance	Malaria	Ensuring sustained effectiveness of the few available regimens	Need to identify clinically significant resistant markers, and establish sampling strategies, standardisation of methods, and guidelines on use of genomic data for policy changes
Resistance to diagnostics (target sites: Pfhrp2/3 deletions)	Malaria	Guiding policy and choice of rapid diagnostic tests, ensuring their continuous effectiveness, and minimising misdiagnoses	Need to establish sampling strategies and standardisation of methods
Vector or insecticide resistance	Malaria	Guiding policy on vector control and ensuring continuous effectiveness of insecticides	Need to identify resistant markers and reference genome for the vectors; need to develop appropriate spatial sampling strategy and standardisation of methods
Transmission dynamics and elimination surveillance	Malaria	Quantification of malaria importation risk useful in elimination surveillance, determining transmission chains in elimination surveillance for targeted interventions, and characterising changing transmission intensity potentially guiding control measures	Need to standardise methods; more local, regional, and global data are needed for adequate resolution of imported vs indigenous cases and the characterisation of changes in parasite population structure
Vector dynamics	Malaria	Monitoring changes in vector species and their effect on transmission and monitoring effectiveness of mosquito gene-drive interventions	Need for a database mapping local vector species, entomology capacity building, development of appropriate spatial sampling strategy, and standardisation of methods
Antimicrobial resistance	Antimicrobial resistance	Monitoring antimicrobial resistance emergence, prevention, and transmission dynamics; identifying clusters and mechanism of resistance; reconstruction of transmission; outbreak management; vital for ensuring continuous effectiveness of antimicrobial drugs	Need to develop a more comprehensive database or set of algorithms for genotypic-to-phenotypic profiling of more organisms; need to identify resistance mechanisms for more organisms
Outbreak management and surveillance	Foodborne pathogens	Outbreak management; tracing transmission routes and networks; identifying contamination sources; surveillance and effective outbreak management ensuring food safety	Need to standardise methods; quality assurance or quality control needs to be done, including EQA
Vaccine efficacy monitoring, transmission dynamics, and elimination surveillance	Vaccine-preventable diseases (eg, polio, rubella, measles, and influenza)	Monitoring the effectiveness and impact of vaccines; outbreak management; identifying new strains, including imported cases, vaccine escape variants, and vaccine-derived variants; inform vaccine development for pathogens such as influenza	Need to standardise methods; quality assurance or quality control needs to be done, including EQA

EQA=external quality assessment. NGS=next-generation sequencing. Pfhrp2=*Plasmodium falciparum* histidine-rich protein 2. WGS=whole genome sequencing.

Table 1: WHO-recommended and other pathogen genomics use cases for disease control programmes

Figure 2: Pathogen genomics use for disease control programs

2. **Identification of therapeutic targets:**
   In order to survive, microbes regularly modify their structural and functional properties. Recognition of these characteristics at genomic level brings up opportunities for

new therapeutic options. In 1998, the genome of *Mycobacterium tuberculosis* H37Rv was sequenced, 4000 new potential therapeutic targets identified, 50 percent of which were given tentative functions.

Increasing antibiotic resistance and unavailability of a viable vaccine candidate has made *Neisseria gonorrhoeae* a "superbug". A recent study has utilized codon biasing, a tool to identify the essential genes in N. gonorrhoeae that could be utilized as novel therapeutic targets for drug or vaccine development. Through the screening of 2350 total genes, a list of 29 drug candidate genes based on codon adaptation identified. Data mining could predict the function of these 29 genes. These genes are involved in DNA replication, energy synthesis and metabolites production. A molecule/drug also identified which can be used to target essential protein DapD (succinyltransferase).

3. **Antimicrobial resistance:**
Antimicrobial resistance (AMR), which is currently the most critical public health issue, necessitates immediate global effort to counteract its negative consequences. According to a recent review published on genomics to track global AMR, around 47 freely accessible bioinformatics resources for detection of AMR determinants in DNA or amino acid sequence data have been developed.

WGS for AMR has several advantages over traditional methods, as demonstrated by a study of emerging aminoglycoside-resistant Campylobacter in the United States, where sequencing data revealed the rising trend driven by nine different resistance alleles, six of which had never been detected in Campylobacter before and would not have been easily found using PCR.

4. **Outbreak control and investigation:**
WGS also links epidemiology and pathogen biology, which is critical for determining outbreak investigation as it is crucial to determine whether an outbreak is caused by natural causes, an unintentional release of a cultured or engineered organism, or a deliberate introduction of a known pathogenic organism. WGS provides significantly more resolution than conventional methods by producing a complete database of genetic polymorphisms (particularly single-nucleotide polymorphisms, or SNPs). Methicillin Resistant *Staphylococcus aureus* outbreak in the United Kingdom, MLST (multi locus sequence typing) identified only one sequence type for a group of MRSA isolates, whereas WGS identified several distinct clusters.

Two studies of tuberculosis transmission have shown that the resolution of WGS with SNP typing is much higher than that provided by the previous gold standard typing method i.e., mycobacterial interspersed repetitive unit variable number tandem repeat (MIRU-VNTR) typing.

5. **Microbiome:**
Meta-omics-based research has revealed significant associations between the gut microbiome and human diseases such as obesity, diabetes, inflammatory bowel disease (IBD), cardiovascular disease, and various cancers in the last two decades. For instance, recent analysis of faecal metagenomic samples from patients with colorectal cancer (CRC) identified CRC-enriched bacteria, including *Bacteroides fragilis, Fusobacterium nucleatum, Porphyromonas asaccharolytica, Parvimonas micra, Prevotella intermedia, Alistipes finegoldii* and *Thermanaerovibrio acidaminovorans,* which could potentially serve as diagnostic bacterial markers across populations.

As a result, omics-based research has opened up a whole new world of diagnoses, but it will require significant expenditures in Next Generation Sequencing, bioinformatics and computer infrastructure. Monitoring, evaluation, and sustainability frameworks are critical for determining the value of NGS over other surveillance techniques, identifying gaps and areas for advancement, and ensuring that appropriate resources are available for its operations.

Diagnostic role of c-MYC, BCL2 and BCL6 rearrangement in B Cell Lymphoma

Sony Siddiqui, Muneba Sharif and Zeeshan Ansar,
Molecular Pathology

Neoplasms that were often included high-grade B-cell lymphoma in the past included blastoid or pleomorphic variants of mantle cell lymphoma, lymphoblastic lymphoma/leukaemia, Burkitt lymphoma, high-grade variants of diffuse large B-cell lymphoma (DLBCL), and neoplasms designated previously in the 2008 World Health Organization (WHO) classification as B-cell lymphoma,unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma.

Aggressive B-cell lymphomas, including diffuse large B-cell lymphoma (DLBCL), comprise the most commonly diagnosed non-Hodgkin lymphomas in the Western world and are potentially curable with standard chemoimmunotherapy treatments in up to two-thirds of patients.

In the 2016 revision of the WHO classification of lymphomas, the term high-grade B-cell lymphoma has been repurposed. Most cases in this category include so-called double and triple-hit lymphomas falling into the subgroup designated high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements. A smaller subset of cases are designated as high-grade B-cell lymphoma, not otherwise specified (NOS). This group has overlapping morphological findings with double and triple-hit lymphomas, and may carry MYC rearrangement in a subset of cases, but lacks double or triple-hit genetics as currently defined.

Diagnostic approach for high-grade B-cell lymphoma:

The diagnostic approach that can be used in practice. When we receive a high-grade B-cell lymphoma, blastoid mantle cell lymphoma is excluded with cyclin D1, SOX11 immunohistochemistry or FISH for IGH-CCND1 translocation. B-lymphoblastic leukaemia/lymphoma can be excluded with TdT and other immature markers. Of note, a subset of high-grade B-cell lymphoma can express TdT so it is very important to check other markers such as CD34, CD38, BCL6, and monotypic surface immunoglobulin. Then, we apply FISH for MYC, BCL2 and BCL6. If doublehit or triple-hit is found, the case is classified as high-grade Bcell lymphoma with MYC and BCL2 and/or BCL6 rearrangements, irrespective of morphology. If double-hit or triple-hit is not found, then the decision depends next on morphology. If lymphoma cells are large and morphology is typical for DLBCL, then it should be classified as such, irrespective of isolated MYC rearrangement. If lymphoma cells are intermediate-size and morphology is typical for BL or intermediate between BL and DLBCL, then immunophenotype such as BCL2 expression should be checked. If the immunophenotype is not typical for BL, then it can be classified as high-grade B-cell lymphoma NOS.

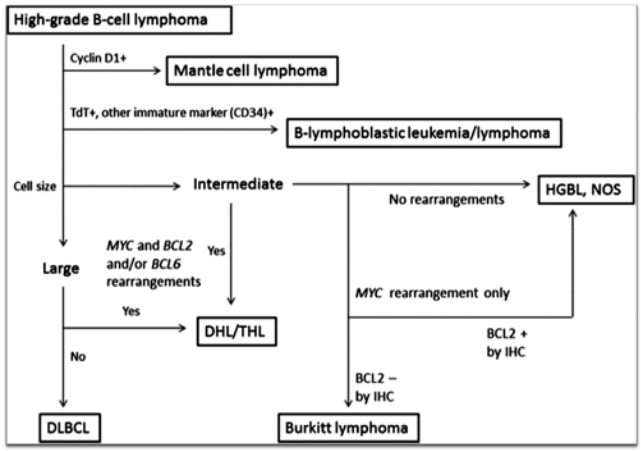


Figure 1: Algorithm for Diagnostic approach for high-grade B-cell lymphoma.

Diagnostic Importance of high-grade B-cell lymphomas harboring rearrangements of MYC and BCL2 and/or BCL6 (HGBL-DH/TH):

According to WHO 2016 classification of lymphoid neoplasms, uncommon but clinically significant subgroup of high-risk patients with a highly aggressive clinical course and dismal long-term survival. This subgroup, commonly referred to as double hit (DHL) or triple hit lymphoma (THL), has been officially classified as “high grade B-cell

lymphomas with rearrangements of *MYC* and *BCL2* and/or *BCL6*. The standard way to identify these aberrations is via fluorescence in situ hybridization (FISH) probes.

MYC expression in DLBCL drives proliferation and induces genomic instability. *MYC* is located on chromosome 8 (8q24) and is crucial for metabolism, protein synthesis, and amplification of transcription . However, *BCL2*, an oncogene located on chromosome 18 (18q21), serves to promote cellular survival by preventing apoptosis. *BCL6* normally encodes a transcriptional repressor and when overexpressed, can down-regulate several other genes, including *TP53* (tumor suppressor gene), which subsequently allows DNA-damaged cells to escape from apoptosis . A typical translocation partner for these genes is the immunoglobulin heavy chain gene (IGH) enhancer, which is located on chromosome 14 (14q32). The IGH enhancers activate efficient and accurate transcription of clonal IGH genes. Hence concurrent translocation of *MYC* and *BCL2* and/or *BCL6* molecularly generates a cellular environment of rapid growth countered by decreased apoptosis, and leads to a highly chemoresistant phenotype.

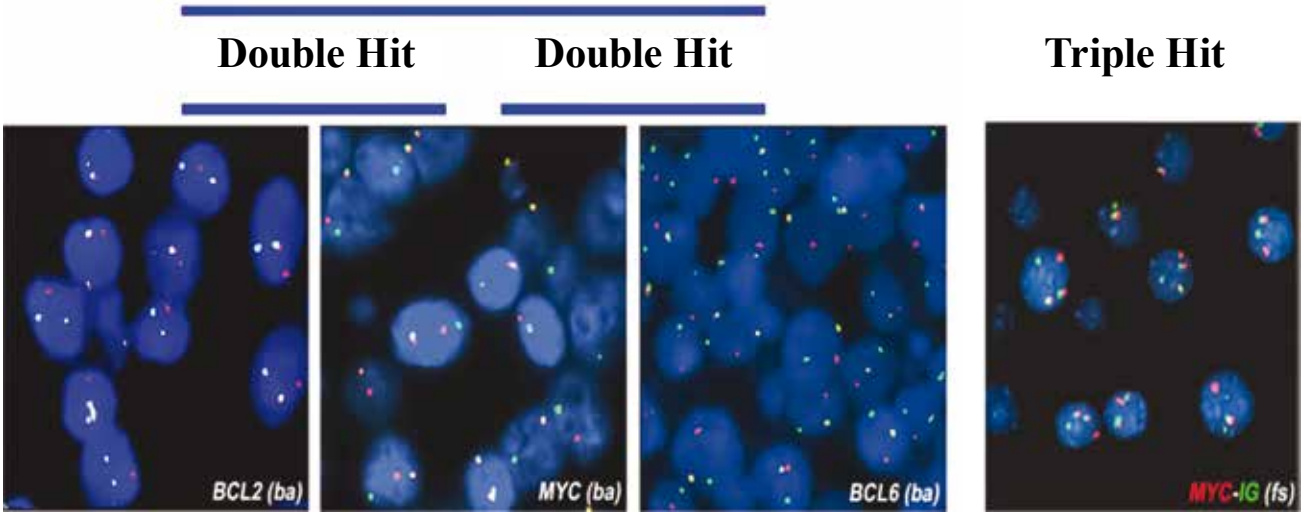


Figure 1: FISH analysis showing multiple gene rearrangements on paraffin embedded Tissue blocks. Showing (A) BCL2 gene rearrangement, (B) C-Myc gene Rearrangement, (C)BCL6 gene Rearrangement (D) MYC/IGH translocation

The poor prognosis and adverse outcomes following standard chemoimmunotherapy for patients with aggressive B-cell lymphomas harboring dual rearrangements of *MYC* and *BCL2* and/or *BCL6* is now well-established. Due to the need for more intensive induction chemotherapy than RCHOP and the potential need to implement CNS prophylaxis, it is crucial for treating physicians to know whether a patient with newly diagnosed DLBCL fits into the HGBL-DH/TH category. In a cost-conscious era, routine and widespread testing for biologic determinants of outcome may not be appropriate, and a critical appraisal of predictors is warranted. As a distinct clinical phenotype or pathologic morphology cannot be identified to accurately predict for underlying HGBL-DH/TH, the molecular features of the underlying lymphoma are a more objective means to screen for HGBL-DH/TH. Herein, we have summarized the data to support various methods of screening by molecular features including COO, protein expression, and sequential FISH testing.We recommend screening all patient samples with newly diagnosed high-grade B-cell lymphoma with a dual fusion FISH probe for MYC-IGH translocations. If the FISH study is positive, the sample can then be tested for BCL2/BCL6 translocations. Based on these data, this is both the most sensitive and cost-effective method to diagnose patients with HGBL-DH/TH and to best inform treating physicians to aid in the clinical management of these patients.

Meeting Reports: Eksath with Patients Having Rare Inherited Metabolic Disorders: An E-Portal Launching Event

Drs. Hafsa Majid
Clinical Chemistry

Launch of e-portal '**EkSath** with patients having rare inherited metabolic disorders' was conducted on March 1st, 2021 at the Aga Khan University (AKU), Karachi, Pakistan. The event was arranged by Department of Pathology and Laboratory Medicine, AKU in collaboration with Pakistan Society of Chemical Pathologists, and Pak IMD-Net.

Rare diseases affect many aspects of an individual's life including their social, educational, and employment opportunities and are an emerging global public health priority. For Pakistani families affected by IMDs there is paucity of appropriate health services and support groups and it is difficult to find and to access information. These opportunities are even more limited for families who have a child who hasn't been diagnosed yet. In addition, there is a lack of coordination and integration, and no central point of access to information on diagnostic services, treatment clinics, family support groups or up-to-date research. Bearing this in mind, an e-portal was developed by Dept. of pathology and laboratory Medicine in collaboration with Pak-IMD-Net for parents/families with children diagnosed with any IMD and physicians involved in the care of IMD patients.

This event was attended by parents/families with children diagnosed with any IMD, physicians involved in care of IMD patients, and pathologist associated with IMD diagnostic laboratories from across Pakistan.

Picture 1: Picture of the event Organizers and Guest Speakers with the Dean Medical College, Aga Khan University.

Basic Molecular techniques (Hands-on) workshop for Pathology Residents

Kiran Iqbal Masood
Molecular Pathology

As more molecular diagnostics tests are emerging with more sensitive, specific and precise diagnostic assays, these methods have assumed a niche in many clinical laboratories replacing or complementing the existing assays. Hence, it has become imperative to strengthen the foundation of molecular pathology of our residents by enabling them to be able to understand the basic, technical and clinical knowledge of the molecular based diagnostic testing. In order to achieve this, the PGME committee took an initiative by incorporating longitudinal molecular pathology course within the existing curriculum.

Post-graduate medical education (PGME) committee developed a task force whose aim was to carefully design the training program that can be used by pathology residency program as a framework that caters to enable residents to develop understanding of basic concepts of molecular biology along with its clinical applications. The committee met monthly and worked collaboratively along with representatives from different sections and from the year 2021, core curriculum for residents was revised to include the essential concepts related to molecular pathology.
As part of the curriculum, we designed a workshop on "Basic molecular techniques" for residents of the Department of Pathology and Laboratory Medicine.

The workshop comprised of hands-on training, interpretation and troubleshooting on techniques including Nucleic acid (DNA and RNA) extraction using conventional and kit based methods followed by conventional and Real-time PCR. The attendees for the workshop included in total 14 residents, mostly from year III, IV and V along with three residents from year I who were able to secure resident research grant (RRG) and were supposed to use the basic molecular techniques in their projects along with two of the residents from clinical hematology who came with special request to join the workshop.
The workshop was organized by the faculty from the section of Molecular Pathology including Drs. Kiran Iqbal and Najia Ghanchi with the help of Pathology research staff (Amna Nasir, Sarah Baber and Francis) and molecular pathology technologists (Anum Ujala and Misha Ahmed). The administrative support was provided by Ms. Shamsha Punjwani. The workshop was well-appreciated by the attendees. We are happy to share some of the snapshots and feedback received:

Very interactive and easily defined, need more hands on like these

Great session learnt basics of molecular

Hands-on experience was very helpful

Overall Great

Hands on was good experience, learnt a lot, should conduct twice a year

Activity was excellent, highly recommended for all residents

Everything was perfect at our level
Good workshop, clarified concepts
Need to organize more like these

Radiology Practice in 21st Century: Role of Artificial Intelligence

Dr Shaista Afzal, Dr Imrana Masroor,
Radiology

History of Artificial intelligence (AI)

The development of "intelligent machines" that can mimic human intelligence has always intrigued mankind. In Greek mythology, Homer wrote about mechanical servants created by Hephaestus to help masters. In the 13th century, Al-Jazri designed the first robot that worked like a human e.g., playing music and washing hands. Artificial intelligence (AI), however, is much beyond robotics and involves the processing of information and utilization of cognitive computing systems. The perception of AI describes it as an aiding tool that can improve human quality of life and support their need. [i]

In 1956 at the Dartmouth Summer Research Project, the field of AI was formally perceived. John McCarthy et al. created the term "artificial intelligence" and elaborated it as "AI is the science and engineering of making intelligent machines, especially intelligent computer programs. It is related to the similar task of using computers to understand human intelligence, but AI does not have to confine itself to biologically observable methods ".[ii] In other words, AI representing machines which were developed with a wide range of technologies, can perform tasks that are representative of human intelligence e.g., autonomous vehicle driving.

AI in Radiology

In 1980, computer-aided diagnosis (CAD) systems applied image feature-based analysis and provided the radiologist with a "second opinion" i.e., computer output to help in final decision making. The two examples of CAD utilization were for the detection of microcalcification in mammography and pulmonary nodules in digital chest radiography. However, in cases of discrepancy between the computer reader and computer aid, it is important to adopt a more acceptable approach for incorporating AI into radiological practice by leaving the final judgment to the radiologist.

In this era of AI, Diagnostic radiology applications have witnessed significant progress and growth in their workflow. AI not only involves detection of pathologies, decision making, and diagnosis but also encompasses setting of acquisition parameters, image reconstruction, pre-processing along with monitoring treatment response and hence patient management. The goal of imaging equipment manufacturers is to maximize image quality to improve diagnostic accuracy. This however comes with increased cost, time, and radiation exposure. The post-processing of acquired images using AI/machine learning software has shown promise in image quality and patient throughput.

Several facilitators and barriers have been identified in the implementation of AI in radiology practice [iii]. The facilitating factor being "cost containment" which is expected to be achieved with the development and implementation of technological innovations to enhance efficiency and quality. The high expectations of the "potential added value" of AI are another factor. This includes improved diagnostic performance due to automation of tasks, and multiple operational benefits like reduced workload, consistency in reporting effective timesaving, and more advanced service provision. The adoption of AI applications also reflects the openness towards the adaptation of hospital-wide "innovation strategies" of the hospital management leadership. The "local champions" i.e., radiologists that possess strong knowhow of the technical aspects and interest in AI can play an important role in the initiations and implementation of AI in their department. The strategies to be adopted by the local champions to overcome the oppositions by their sceptical colleagues are sharing literature and presentations for a better understanding of AI applications and promoting experimentation by the installation of an application test version. This will not only convince and familiarize the radiologists but also the referring physicians.

One of the hindering factors identified in the implementation of AI in radiology is the inconsistent technical performance of the AI algorithm i.e., the sensitivity and specificity. [iv] This was seen with the CAD application where a large number of false positives created additional work for radiologists and the potential of overlooking a lesion. The other factors hindering the process of AI application being unstructured planning and monitoring, uncertainty about the added value in clinical radiology practice and workflow and lastly, the trust and acceptance of direct and indirect users i.e., radiologists and referring physicians.

Conclusion

AI applications are getting significant attention in radiology practice, hence insight into the facilitators and barriers in its implementation is crucial. Consequently, it is important to include all relevant stakeholders during the implementation phases of planning, execution, and monitoring.

References:

1. *El Naqa I, Haider MA, Giger ML, Ten Haken RK. Artificial Intelligence: reshaping the practice of radiological sciences in the 21st century. Br J Radiol. 2020;93(1106):20190855. doi:10.1259/bjr.20190855*
2. *McCarthy J. What is Artificial Intelligence. 2007. Available from: HTTP:// www- formal.*
3. *Stanford. edu/ jmc/ whatisai/ whatisai. HTML*
4. *Strohm L, Hehakaya C, Ranschaert ER, Boon WPC, Moors EHM. Implementation of artificial intelligence (AI) applications in radiology: hindering and facilitating factors. Eur Radiol. 2020;30(10):5525-5532. doi:10.1007/s00330-020-06946-y*

Case Quiz

Dr Sana Brohi
Haematology & Transfusion Medicine

Case presentation: A 14-month-old boy, presented in emergency department with complaints of fever and failure to thrive since birth. Physical examination revealed pallor and hepatosplenomegaly. Parents had non-consanguineous marriage and he was the first child. Family history was unremarkable. His complete blood count showed: haemoglobin: 8.9 g/dl, white blood cells: 4.72 x 10E9/L, absolute neutrophil count: 0.78 x 10E9/L and platelets: 98 x 10E9/L. Peripheral blood smear did not show any atypical cell. Bone marrow aspirate and trephine biopsy was performed

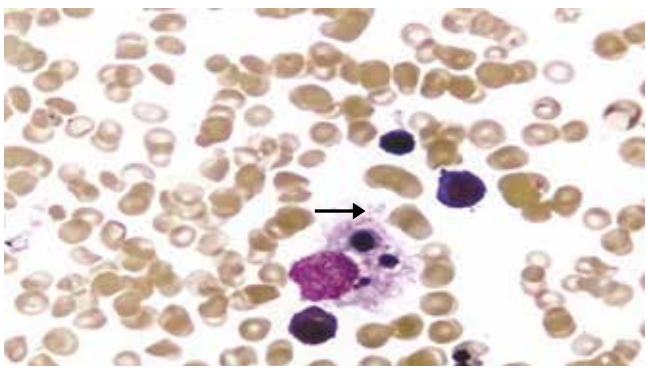


Figure 1: Bone marrow aspirate showing histiocyte engulfing erythroid and lymphoid precursor

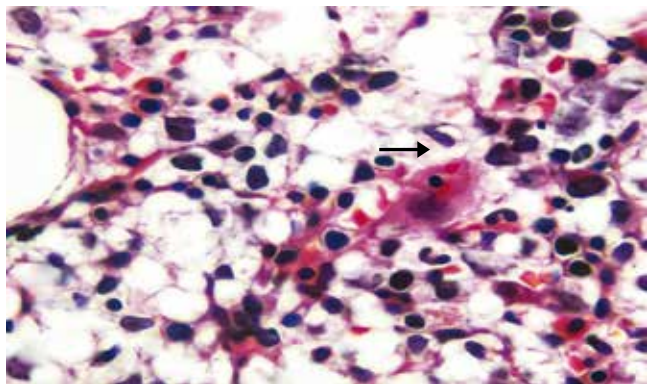


Figure 2: Bone trephine showing histiocyte engulfing erythroid, lymphoid, and myeloid precursor

for evaluation of cytopenia which showed cellular marrow with trilineage hematopoiesis and active hemophagocytosis (histiocytes and macrophages engulfing erythroid precursors) [figure 1 and 2]. Other laboratory investigations showed low fibrinogen: 93 mg/dl, high triglycerides: 431 mg/dl and high ferritin: 85797.5 ng/ml.

What is the most likely diagnosis?

Hemophagocytic Lymphohistiocytosis (HLH). It is an

aggressive syndrome of excessive immune activation. It is most common in infants and young children but can affect patients of any age. These disorders include genetic (familial and immunodeficiency-related syndromes) and acquired forms. Familial forms are autosomal recessive, without a well-defined genetic defect rarely mutations in PF1, Munc13-4 (UNC13D) and syntaxin 11 (STX11), affecting neonates and infant.

Diagnostic criteria: Five of the following nine findings:

1. Fever ≥ 38.5°C
2. Splenomegaly
3. Peripheral blood cytopenia, with at least two of the following: hemoglobin <9 g/dl (for infants <4 weeks, hemoglobin <10 g/dl); platelets <100 x 10E9/L; absolute neutrophil count <1 x 10E9/L
4. Hypertriglyceridemia (fasting triglycerides >265 mg/dL) and/or hypofibrinogenemia (fibrinogen <150 mg/dl)
5. Hemophagocytosis in bone marrow, spleen, lymph node, or liver
6. Low or absent NK cell activity
7. Ferritin >500 ng/ml (ferritin >3000 ng/mL is more indicative of HLH)
8. Elevated soluble CD25 (soluble IL-2 receptor alpha [sIL-2R]) two standard deviations above age-adjusted laboratory-specific norms
9. Elevated CXCL9

Treatment options: treatment options include Corticosteroid: dexamethasone preferred, as it crosses blood brain barrier; Immunoglobulin infusions, cyclosporine and etoposide; For neurological disease: Intrathecal methotrexate with or without corticosteroids; Definite treatment is haematopoietic stem cell transplantation.

Best of the Recent Past

#Pathologist #Followtheirlead

Interviews Recorded by Dr. Qurratulain Chundriger

The world of medicine is changing. With the advent of personalized medicine, powered and backed by evidence-based practices and more advanced diagnostic, predictive and prognostic information, the practice of medicine is in the process of a metaphorical upside-down flip. This has created challenges for pathologists, who need to conform their practice to provide more efficient, faster, and personalized test results, ultimately translating into better care for the patient.

In line with the theme for this Labrad issue, we interviewed the section heads of Department of Pathology and Laboratory Medicine, to know about their vision and foresight in adopting to the requirements of the era of modern medicine. However, the role each section of our clinical laboratory plays in clinical care are too many as well as very diverse, so we requested them to focus on two major areas only.

Interviewee:
Dr. Lena Jafri,
Associate Professor and Section Head
Chemical Pathology

1. **What Technological advancements have been brought to your section, and how have these impacted the clinical service provision?**

Advances in the techniques of analytical chemistry and metabolomics are the source of the rapid evolution of a new omics era. The introduction of metabolomics in the

Section of Chemical Pathology pertains to two important platforms: analytical techniques, (gas chromatography mass spectrometry and liquid chromatography tandem mass spectrometry) and multivariate data analysis software (Collaborative Laboratory Integrated Reports -CLIR in collaboration with Mayo Clinic, USA). With the introduction of mass spectrometers and HPLC in the section metabolic profiling of patients suspected of having inherited metabolic disorders is done. We have screened > 22000 high risk children and adults in past 8 years and have identified around 40 disorders using these cutting-edge technologies.

2. **And what is your vision for the next five years, and how will it benefit patient care?**

I propose three main objectives that will be able to configure a rendezvous point and propose future directions. These include:

- Predictive medicine: Enhanced sensitivity and improved metabolite coverage translating into faster and more accurate predictive capacity of biomarkers.
- Preventive Medicine: Structure across targeted metabolomics using mass spectrometry supplemented with bioinformatics. This can be translated into clinical care through services of genetic counselling.
- Collaborations and capacity building: In all three disciplines of service, education and research is the key to successful utilization of multiomics approach for optimal outcomes.

Interviewee: Dr Zeeshan Ansar, Assistant Professor & Section Head Section of Molecular Pathology

1. **What Technological advancements have been brought to your section, and how have these impacted the clinical service provision?**

Molecular pathology is the rapid advancement of technology has been driven by 2 primary areas: (1) automated extraction, amplification and detection platforms and (2) next-generation sequencing. As with any new advanced area, there are challenges and limitations that the laboratory medicine and public health fields must pay close attention to as these developments intersect with the care of patients and healthcare and public health policy.

2. **And what is your vision for the next five years, and how will it benefit patient care?**

I propose to work in two main areas, including: Capacity building of my faculty and technologist, train them on different genomic, transcriptomic based applications. So they are able to conduct genomic research to identify risk of diseases and translate this information, and are better able to grow the section in the times of personalized preventive medicine.

Bring in newer technologies, broader assays which can detect multiple disorders in a single run of a sample, introduce services to identify inherited genetic diseases, initiate cell free DNA analysis for detection of oncology, prenatal screening and integrate artificial intelligence into the service to improve diagnostics.

Interviewee: Dr Imran Ahmed, Assistant Professor & Section Head Section of Microbiology

1. **What Technological advancements have been brought to your section, and how have these impacted the clinical service provision?**

   FilmArray (Biofire) is a real-time multiplex PCR technology that integrates sample preparation, amplification, detection and analysis. It can simultaneously detect 14 and 22 pathogens in CSF and nasopharyngeal swab specimens respectively in a matter of hours. It reduces turn-around time, helps targeted antimicrobial therapy, antimicrobial stewardship and thus overall reduction in cost of healthcare.

2. **And what is your vision for the next five years, and how will it benefit patient care?**

My vision is to improve diagnostics by bringing in informatics and digitalization in Pathology. I plan to improve microbial identification through introduction of latest technology and bringing in laboratory automation are the next steps for microbiology.

Interviewee: Dr Arsalan Ahmed, Associate Professor & Section Head Section of Histopathology

1. **What Technological advancements have been brought to your section, and how have these impacted the clinical service provision?**

   The histopathology lab was remodelled in 2019, in accordance with the "Lean methodology" in order to maximize the productivity in response to the continually increasing demands of the workload and we have partially gone live with Novopath tracking system. The remodelled lab also houses "state of the art" grossing hall and a separate storage space for blocks and slides for at least one year. Along with it, during the past 2 years we have brought technological advancements by bringing in two 8-color flow cytometer, which has helped us to cope with increasing volumes, implemented new tests to expand our services and by reducing the turnaround time of reporting results (within 24 hours). We introduced

automation in ANA (Helios system) and Semen Analysis (Computer assisted sperm analysis). This has further improved the turnaround time, eliminated subjectivity and labour-intensive processes, increased the capacity of sample size for testing and has improved the quality of patient testing by maintaining it according to CAP standards. Liquid Based Cytology for Pap-smear was introduced using BD Sure Path to minimize the risk of missing the disease by ensuring that 100% of the cells are collected, processed leading to higher

detection rate of abnormal cells HSIL (precursor for cancer in cervix) and significant reduced cases of unsatisfactory samples. It also has the option of performing molecular testing on residual specimen.

2. **And what is your vision for the next five years, and how will it benefit patient care?**

   In future we plan to introduce Subspecialty practice in histopathology, depending on the surgical volumes. Improve our histopathology workflow processes by completely implementing the long-awaited tracking system, along with introducing voice recognition dictation and going paperless. We plan to introduce the Digital pathology and Telepathology, expand our services to other cities and countries of Middle East and Southeast Asia.

Interviewee: Dr Natasha Ali, Associate Professor & Section Head Section of Haematology and Transfusion Medicine

1. **What Technological advancements have been brought to your section, and how have these impacted the clinical service provision**

   Introduction of automation in the routine test performed in hematology has considerably improved the accuracy of results and also the efficiency of the laboratory. The automation platform performs numerous tests from a single EDTA tube. Process improvements such as delta checks, pre- and post-analytical sample sorting/ archiving etc. have resulted in smart deliverables with much decreased turnaround time leading to better patient satisfaction

2. **And what is your vision for the next five years, and how will it benefit patient care?**

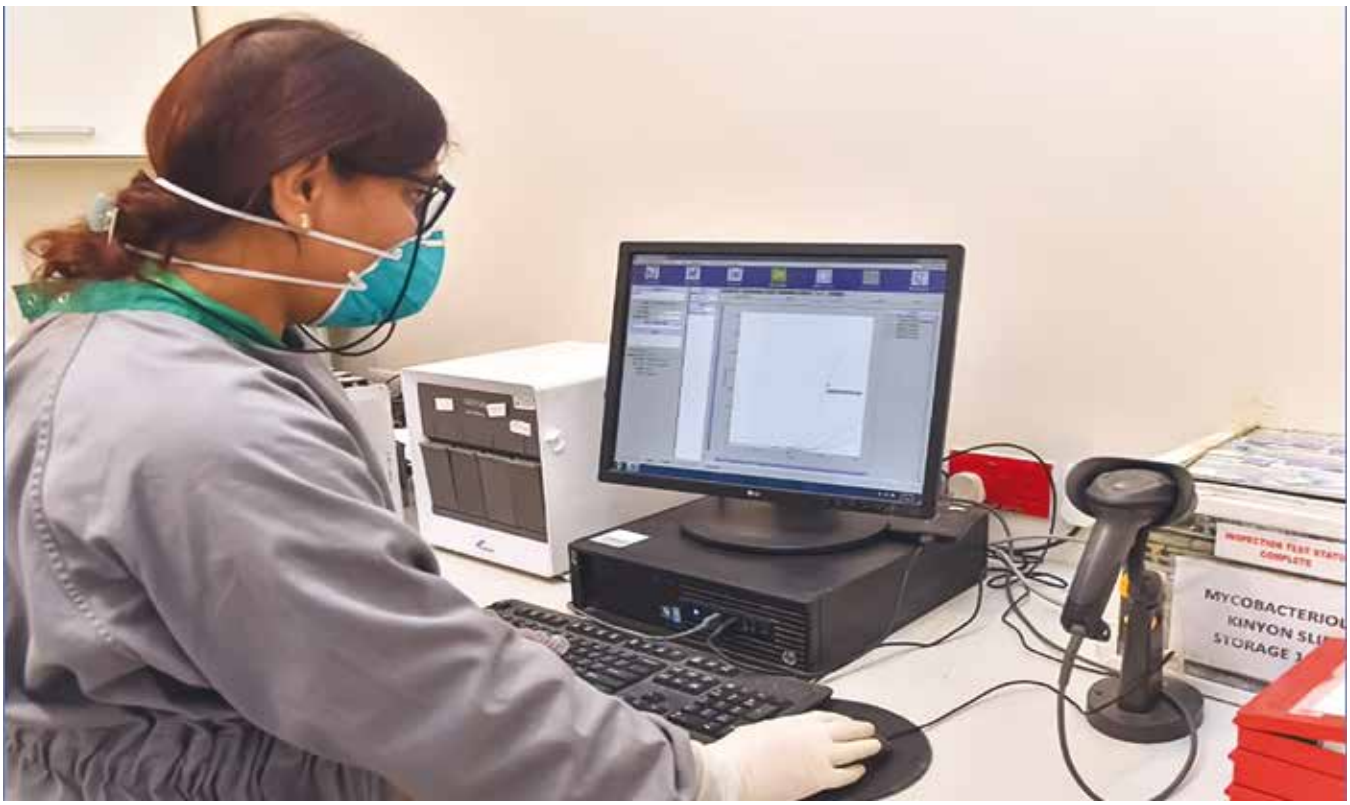
   Innovation in the area of clinical information will save laboratory time and labor, while enhancing patient care. With improvements in optics, electronics, computing algorithms and reagent systems – the insights into cellular health and physiology also grow. The power of digital morphology cannot be underestimated. Digital morphologic analysis of haematology specimens requires images scanned at high magnification under oil immersion. Compared to glass slides, digital images are easy to store, retrieve, replicate, annotate or distribute and do not deteriorate with time.

This technique combined with artificial intelligence where a software pre-classifies and pre-characterizes cells by morphological abnormalities using image analysis algorithms, opens a new door to the way we interpret and diagnose hematological disorders. The future for hematology diagnostics is exciting indeed!

Polaroid

(Picture is Worth a Thousand Words)

Picture 1: Integration of urine chemical and microscopic analysis. System perform picture analysis by neural network-based algorithm



Picture 2: Technical staff evaluating the graph of Xpert MTB/RIF which detects DNA sequences specific for Mycobacterium tuberculosis and Rifampicin resistance by polymerase chain reaction

Polaroid

Picture 3: Haematology resident analyzing hemoglobin variant chromatograph generated by high performance liquid

Picture 4: Haematology residents reviewing bone marrow aspirate smear under microscope.

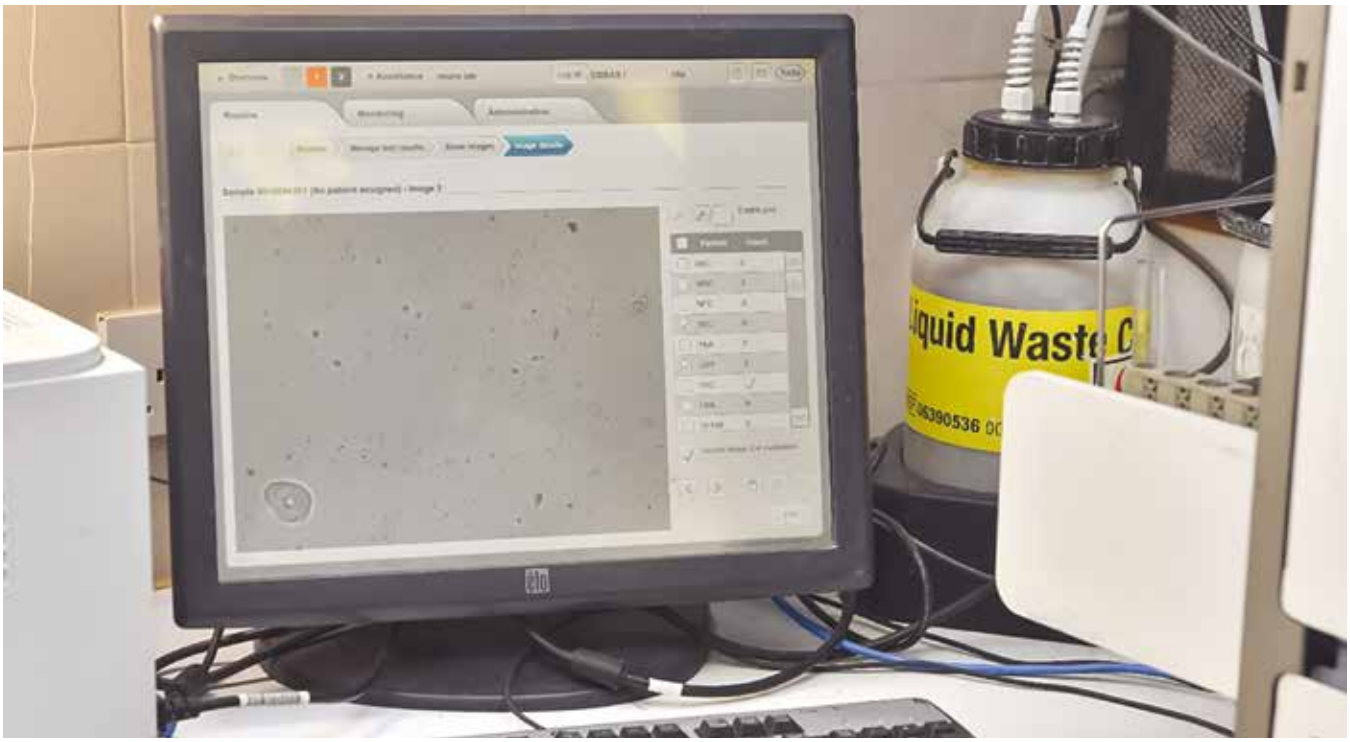
Polaroid

Picture 5: Chemical Pathologist interpreting lead spectra analyzed by Atomic absorption mass spectrometry

Picture 6: Chemical Pathologist reviewing the results of plasma Acylcarnitine analyzed by Liquid Chromatography-Mass Spectrometry

Polaroid

Picture 7: Technologist analyzing dried blood spot specimen for Acylcarnitine on Liquid Chromatography-Mass Spectrometry



Picture 8: Sequencing cartridge is ready for processing and analysis in Miseq.

Polaroid

Picture 9: Molecular pathology technologist setting up Next generation sequencing assay for solid tumor testing.

Picture 10: The Helios HelMed is an automated platform for indirect immunofluorescence studies, which provides AI assisted reporting using software and a library of images for structured reporting. In addition, it also suggests the next step in testing/diagnosis by recognition of the pattern of positivity.

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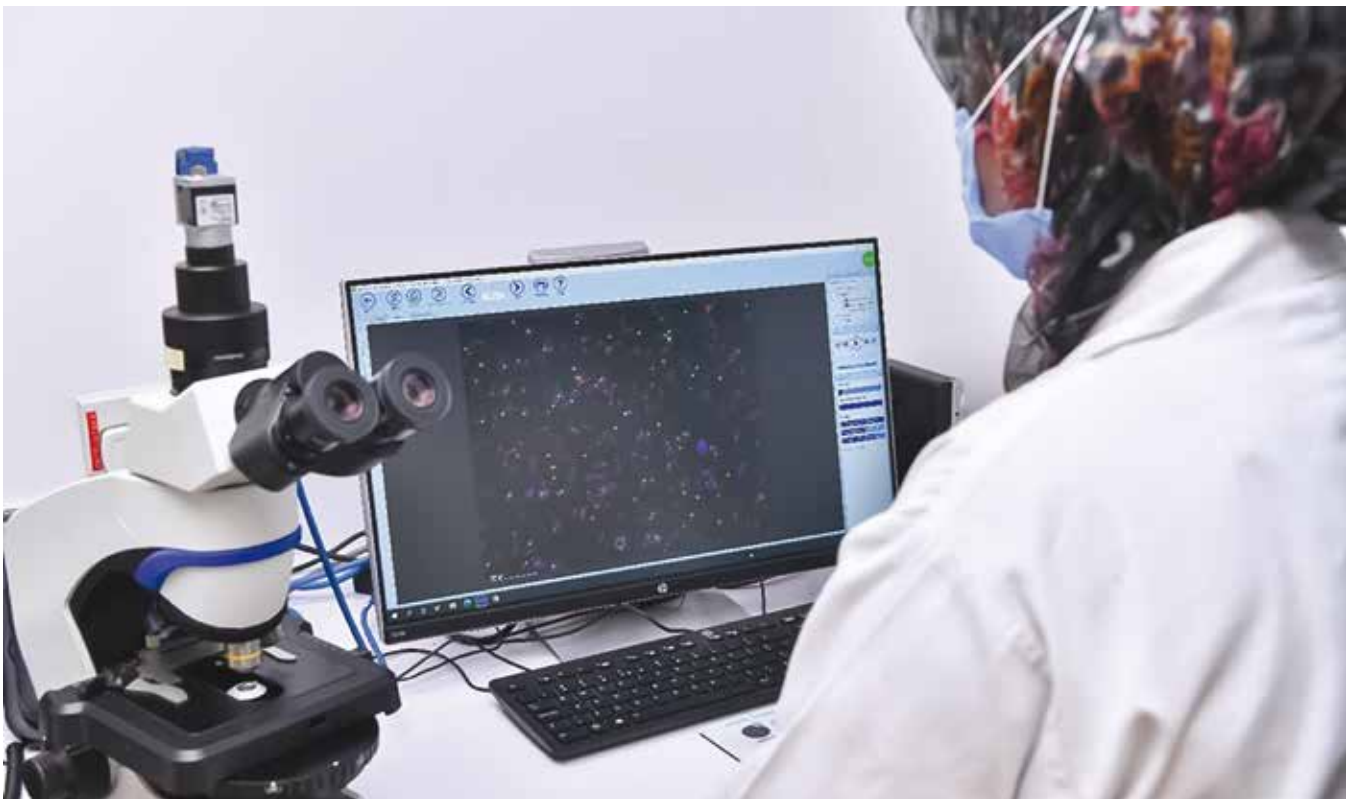
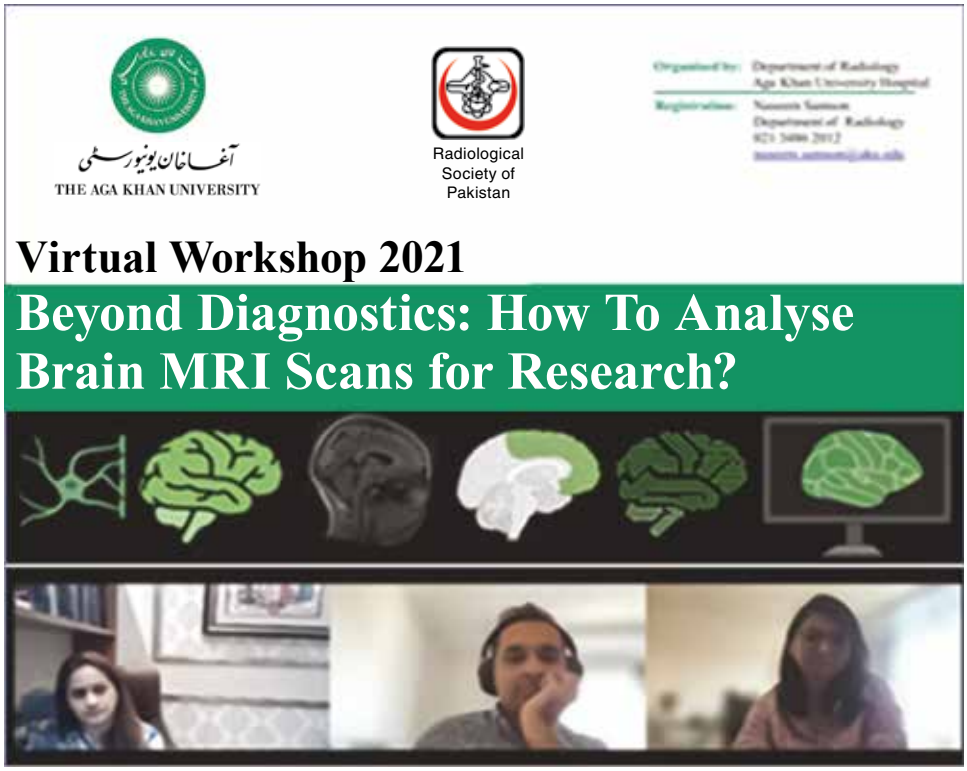


Picture 11: AI assisted reporting of Semen DR specimen is crucial for reporting not only the viability and mobility index of the spermatozoa but also their speed and structural abnormalities.

Picture 12: The 8 color BD flowcytometer is the most advanced technology for assessment of antibody panels in leukemia/lymphoma diagnosis and calculating minimal residual disease burden to name a few examples.

Polaroid

Picture 13: Grossing stations fitted with DRAGON speech recognition software, result in quicker turnaround times, minimizing the chances of human error at the same time.



Picture 14: A virtual workshop conducted by the Radiology team in September 2021.

Polaroid

Pictures 15-17: A few glimpses of the Research Day of Pathology and Laboratory Medicine.

hospitals.aku.edu/Karachi/clinical-laboratories