From the Editor’s Desk

What distinguishes a remarkable Pathology Department from an average one? Faculty members and technologists who are committed to provide better patient care with accurate rapid delivery of laboratory test results, faculty members and technologists who have advanced subspecialty trainings and are dedicated to teach and mentor the leaders of the future. These qualities are exactly what sets the Department of Pathology and Laboratory Medicine at Aga Khan University apart from those at other institutions.

First issue of Labrad for the year 2020 is in your hands. We always encourage residents, technologists and faculty to come together and submit articles. In this issue we have some good reads contributed by small teams. Some such examples in the current issue of Labrad are: “Role of Immunoflorescence Studies in the Diagnosis of Autoimmune Disorders” written by a team of technologists and faculty from Histopathology, similarly “Overview of Common Myeloproliferative Neoplasms” by team from Hematology, “Therapeutic Drug Monitoring of Methotrexate: factors resulting in delayed clearance” by technologist and faculty from Clinical Chemistry, “E-Cigarette, Vaping Associated Lung Injury, Histopathological Diagnosis” from team of histopathology and last and the best is the partnership of Pathology and Radiology in the article “Radiology Pathology Correlation. Bone Pathology”.

Please welcome our new associate members of Labrad who have joined our team from this year. My editorial team has several plans for improvements of Labrad during our tenure; we have already started the working. Don’t forget to take a look at two of the sections Labrad team introduced last year:
• The Best of the Past
• Polaroid

Happy reading 😊

Dr Lena Jafri
Clinical Chemistry

Role of Immunofluorescence Studies in the Diagnosis of Autoimmune Disorders

Samar Naz, Miss Iqra Saleem and Dr. Sabeeh Siddique
Histopathology

Diagnosis of connective tissue and rheumatological disorders can be challenging task for the clinicians. The term connective tissue disorder encompasses a variety of distinct diseases which have one thing in common; that is the presence of circulating autoantigens in the patients’ blood. The detection of these autoantibodies in the laboratory may help the clinicians in confirming the diagnosis. A variety of laboratory based techniques are available to detect the relevant autoantibodies in patients’ sera. However according to the American College of Rheumatology (ACR), Indirect immunofluorescence Assays (IIFA) on Hep-2 cells is the gold standard for autoimmune body screening in patients with systemic autoimmune rheumatic diseases. IIFA has a good sensitivity and it detects a wide range (approximately 100 to 150) of nuclear and cytoplasmic autoantigens. Due to its high sensitivity, it is often employed as the first line diagnostic test that detects even those autoantigens which may be missed by other commercially available multiplex ANA tests. Some of the patterns that are detected by IIFA and the associated diseases are listed in Table 1.
Traditionally Hep-2 IIFA patterns were associated with diseases. Now it has been realized that many of these associations are only valid if the antigen specificity has been confirmed by follow-up testing. Therefore The International Consensus on ANA Patterns (ICAP) advocates that disease associations should be replaced by clinical relevance. Pattern assignment in clinical laboratories has been inconsistent. That is why ICAP initiated the consensus on nomenclature & definitions of the Hep-2 patterns. ICAP consensus is based on the clinical relevance of 29 Hep-2 IIFA patterns (Figure 1). The consensus on clinical relevance is defined in the clinical context of the patient, that is, suspected disease and includes recommended follow-up testing (antigen specific immunoassay). Cytoplasmic and mitotic patterns may also have clinical relevance and therefore appropriate follow-up testing is advisable. If follow-up testing identifies the antigen, the clinical relevance can be further refined. ICAP advocates that information on Hep-2 IIFA patterns should be reported to the clinicians and should be incorporated in diagnostic and classification criteria instead of simply reporting as ‘ANA Positive/Negative’.

Recently our laboratory has acquired a complete automation equipment (HELIOS®) for processing and reporting of the ANA samples. HELIOS® is the first fully automated IIF processes and it includes an integrated optical system for automatic slide reading which not only discriminates between positive and negative samples but also detects the patterns and end point titers. The software provided with this equipment has an extensive pattern library, which the technologist or the pathologist can access for academic purposes as well as for comparing the results of the test samples. Some of these patterns are shown in Figure 2.

![Figure 1](https://www.anapatterns.org/trees-full.php)  
**Figure 1**: Taken from: ICAP International Consensus on ANA Patterns; https://www.anapatterns.org/trees-full.php

![Figure 2](https://www.anapatterns.org/trees-full.php)  
**Figure 2**: Various ANA staining patterns seen on samples performed on HELIOS®

In contrast to the manual performance, running the ANA samples on an automated machine has the following benefits:
- Provides optimum and consistent conditions for processing of samples
- Minimal human involvement minimizes chances of technical errors

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**Table 1. ANA Patterns and the Associated Diseases**

<table>
<thead>
<tr>
<th>ANA pattern</th>
<th>Associated Rheumatic Disease</th>
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<tbody>
<tr>
<td>Homogeneous</td>
<td></td>
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<tr>
<td></td>
<td>Systemic lupus erythematosus</td>
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<tr>
<td></td>
<td>Mixed connective tissue disease</td>
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<td></td>
<td>Drug induced lupus</td>
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<td>Juvenile idiopathic arthritis</td>
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<td>Speckled</td>
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<tr>
<td></td>
<td>Systemic lupus erythematosus</td>
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<tr>
<td></td>
<td>Sjogren’s syndrome</td>
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<tr>
<td></td>
<td>Polymyositis/dermatomyositis</td>
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<td></td>
<td>Systemic sclerosis/scleroderma</td>
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<tr>
<td>Nucleolar</td>
<td></td>
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<tr>
<td></td>
<td>Diffuse systemic sclerosis/scleroderma</td>
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<tr>
<td>Centromere</td>
<td></td>
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<tr>
<td></td>
<td>Limited systemic sclerosis/scleroderma</td>
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<tr>
<td>Peripheral (rim)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Systemic lupus erythematosus</td>
</tr>
<tr>
<td></td>
<td>Systemic sclerosis/scleroderma</td>
</tr>
</tbody>
</table>

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• Provides automated pattern recognition, end-point titer suggestions and reduction of interpretation bias
• Less intensive training required
• Less labour intensive, medical technologists can be spared do other chores
• Since the machine can run 106 samples in a single batch the turnaround time is significantly reduced.

As mentioned previously that IIFA performed on HEp-2 cells is a sensitive test for detecting the autoantigens in a patient, however several points are worth mentioning here:

**IFA ALGORITHM ACCORDING TO THE INTERNATIONAL CONSENSUS**

- A positive ANA result in conjunction with clinical suspicion suggests, but does not necessarily confirm the presence of an autoimmune disease
- Positive results are not uncommon in healthy individuals (particularly as they age) and those with certain infectious diseases or cancer
- In cases with strong clinical suspicion, specific antibody testing may be appropriate even if the ANA IFA result is negative
- If the ANA IFA is positive but the tested antibodies are negative, the patient may still have an autoimmune disease other than those typically associated with the antibodies tested

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**Cystathionine Beta-Synthase Deficiency**

Saba Abdul Mateen
Clinical Chemistry

Cystathionine beta Synthase (CBS) deficiency is an inherited metabolic disorder, in which the body is unable to utilize homocysteine (tHcy), leading to homocystinuria caused by a genetic mutation in the CBS gene, which results to decrease levels or absence of enzyme CBS and tHcy and methionine (Met) accumulate in the blood which leads to damage the nervous system. The pathway is shown in Figure 1.

CBS deficiency has an autosomal recessive inheritance pattern, patients have elevated levels of Plasma Met, tHcy and tHcy:Met ratio along with decreased level of cystine and cystathionine. Clinical features include ectopia lentis develop by the age of six years, osteoporosis, abnormal blood clots, generalized joint tenderness, bone X-ray abnormality, subluxation of the eyes, and osteoporosis.

developmental delay, nearsightedness, seizures, intellectual disabilities, learning disabilities and failure to thrive. CBS diagnosed by analyzing plasma amino acid and confirmed by enzyme analysis.

There are two types of homocystinuria due to CBS deficiency, one type response by Vitamin B6 and the other one does not. Treatments for CBS disorder include a protein-restricted diet, Vitamin B6, vitamin B12, folate, betaine treatment, and other supplements. With early diagnosis and treatment, individuals with CBS deficient can have normal growth and development. However, it is still possible that they could develop ectopia lentis or blood clot, osteoporosis. Sometimes the blood clots can be very serious and cause damage of organs. Regular monitoring can be done by performing 'plasma homocysteine methionine profile'.

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**Treatment of Factor VIII Inhibitor**

Dr Abdul Muqtadir
Haematology

The initial treatment of Factor VIII inhibitors consists of two steps. Firstly, control of bleeding and secondly elimination of inhibitor.

**Control of Active Bleeding**

Treatment strategies to control active bleeding include the use of desmopressin (DDAVP), factor VIII concentrates, activated prothrombin complex concentrates (aPCCs, factor eight inhibitor bypassing agent [FEIBA]), recombinant human factor VIIa and recombinant porcine sequence factor VIII concentrate.

The decision is based on the severity of bleeding and titer of the inhibitor. For non-life threatening bleeding, desmopressin has been used, however, it is rarely recommended due to overall superiority of factor bypassing agents. For life threatening bleeding, human factor VIII products (recombinant factor VIII or a factor VIII concentrate) at high doses have also been used.

For bleeding associated with a low titer inhibitor (<5 Bethesda units). The typical dose of human factor VIII is 20 IU/kg intravenously for each Bethesda unit of the inhibitor, plus an additional 40 IU/kg, with monitoring of factor VIII activity, 10 minutes following bolus injection. Intravenous bolus dosing can be repeated if the increment recovery is not adequate.

For patients with high titer factor VIII inhibitors (≥5 Bethesda units) and/or severe bleeding, the use of FEIBA or recombinant human factor VIIa is advised. Human factor VIII products generally cannot be given in high enough amounts to overcome the inhibitor.

Recommended doses for FEIBA and rFVIIa are similar to those employed in hemophilia patients with inhibitors (typical FEIBA dose 75 units/kg; rFVIIa median starting dose 90 mcg/kg, range 45 to 181 mcg/kg).

The use of FEIBA and rFVIIa should depend on local experience and their estimated cost.

**Eliminating the Inhibitor**

Elimination of the factor VIII inhibitor requires the use of immunosuppressive modalities. Most commonly employed initial immunosuppressive regimens are glucocorticoids alone, glucocorticoids plus cyclophosphamide and glucocorticoids plus rituximab. The highest percentage of complete remissions (undetectable inhibitor levels, factor VIII levels >70 IU/mL, and immunosuppression stopped) were noted with glucocorticoids (1mg/kg/day) plus cyclophosphamide (2mg/kg/day) regime, followed by glucocorticoids plus rituximab, respectively. The median time to complete remission is approximately five weeks for the use of glucocorticoids with or without cyclophosphamide while rituximab-based regimens
require a period approximately twice as long. Another therapy for acquired factor VIII inhibitors is intravenous immunoglobulin (IVIG). Since only few patients respond to IVIG and multiple courses are often required, it is recommended that IVIG should not be used as initial therapy.

In case of pregnancy, immunosuppression with prednisone may be used. Other agents are generally avoided unless critically needed for maternal health. Monitoring response to treatment — Bleeding should be evaluated clinically and by laboratory parameters (hemoglobin). As inhibitor titers drop very slowly following successful treatment, it is neither necessary nor advisable to check the patient’s aPTT or inhibitor titer more often than every two-four weeks once immunosuppressive therapy has been started.

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**Ergonomics In Laboratory: What You Need To Know About Repetitive Motion**

Dr Mohammad Zeeshan  
Clinical Microbiology

Carpal tunnel is a term that strikes fear into everyone that uses a computer, and we all know how much computers are used in laboratories and research in general. Carpal tunnel syndrome is a common condition that causes pain, numbness, and tingling in the hand and arm. The condition occurs when one of the major nerves to the hand — the median nerve — is squeezed or compressed as it travels through the wrist. Everything from data entry to notation and protocol documentation to grant, article, and proposal writing is digital today. But repetitive motion and associated musculoskeletal disorders (MSD) are not limited to computer users. I will discuss the technical aspects of repetition/duration and force as it applies to ergonomic risk in lab and office settings. And I will offer solutions to get the health care worker the days and weeks pain-free.

**Breaks and Rest Combat Repetitive Motion**

The definition of repetition is doing things over and over again. In repetitive work, the same motions are performed using the same parts of the body in the same way, time and time again. In activities such as typing, mousing, or entering data by source documents, the affected muscles, tendons, and joints can be used thousands of times a day, week after week, year after year. The risk of injury is even greater when repetitious jobs involve awkward posture (e.g. bent or flexed wrists) or forceful exertions such as repetitive overreaching for the mouse (which can lead to shoulder and neck pain).

Our first and foremost goal from an ergonomic standpoint is to **strive for neutral and balanced actions**. Additionally, reducing the number of repetitions experienced by each set of muscles, tendons, and joints throughout the workday and allowing time for recovery is paramount. The body has great capacity to repair itself. Problems arise, however, when the amount of damage or stress accumulated over the course of time overtakes the body’s ability to repair. This is when we experience pain. If the cumulative damage continues without allowing time to recover and heal, there is the potential for serious injury.

In order to introduce healing time, **short breaks in repetitive tasks** bring significant benefit. Break up...
data entry with variations in activity such as filing, reading, using the copier, or any other task that uses different muscles and motions than computer use. It is also good to include micro-breaks of just a minute or two every half hour or so during long data entry periods. Research has shown it is often better to take many small breaks than one long work break during the day. Try using software that tracks keystrokes and mouse movement and alerts you when breaks are appropriate.

Break Down and Analyze Tasks

It is critical to examine and analyze the work being performed. Examine the job on a task-by-task basis. In many cases we have seen unnecessary repetitive work performed due to poor process design or evolution over time. When evaluating, ask you “can parts of this process be automated? Can equipment be linked directly for data collection? Can steps be eliminated or modified to improve flow or actions?” Investigate use of barcodes and readers to reduce data entry or entry readable/scanable forms or other types of information collection. It is always worth investing time to engineer a solution that will save significant time and effort in the long run.

An Example: Mouse Use

Pain is often reported from mousing and usually attributed to over-use, and is often combined with poor mouse location. The conventional mouse requires a great amount of work to be directed through one arm, shoulder, and hand. It is a good idea to try to distribute this work and share it between both sides. One approach is the use of keyboard commands. Most operating systems contain keyboard commands or shortcuts for common tasks. Taking the time to explore and use these can greatly reduce mouse use, and once you get familiar with them will actually speed up your work. Another remedy is to try one of the many “alternative” or “ergonomic” mice now offered. Some allow one to use both hands for mousing, sharing work between hands. Software programs allow you to automate common tasks (e.g. autofill) and develop scripts called macros to perform, reduce, or eliminate many actions. Their use can significantly decrease the amount of typing you need to do.

Exertion Force

Force is the amount of muscular effort needed to perform work. Fatigue and injury track with the amount of force exerted. The more force required, the higher the risk of both. Exertion force depends on many factors, including:

- The effort used to strike an object (e.g. key depression when typing)
- The shape and dimensions of an object you are working with
- How you grip an object or tool
- The precision of motion required to perform the task
- Duration of force applied by the muscles (e.g., the amount of time spent without a muscle-relaxation break)
- Awkward postures (bending, twisting, over-reaching)

Goal number one is to always have a neutral and balanced posture. Goal two is to reduce the number of repetitions or duration of exertion experienced by each set of muscles, tendons, and joints throughout the workday.

Number three is to reduce the force applied to perform the task. OSHA provides excellent help through their eTool on ergonomics.2 Strive to recognize and reduce all the risk factors both on and off the job to effectively reduce the potential for repetitive motion pain and injury.

Figure 2: Methods to prevent Carpel tunnel syndrome due to mousing

BEFORE

WRONG!

WRONG!

AFTER

RIGHT!

RIGHT!
Science organizations, specifically in clinical laboratory context, rely on creative marketing and outreach strategies to attract top talent, showcase their products and services to potential clients, and engage the general public in science. However, many organizations overlook the importance of effective internal communications for these marketing and outreach efforts. For example, one shortcoming faced by staff is staying updated on the latest research news and developments in order to have regular content to share with the public. Thus, organizations would benefit from maintaining open lines of communication and promoting cross talk among staff to ensure information is shared throughout the organization.

Ways to Capture Content for Marketing and Outreach Efforts

One of the biggest challenges for any lab’s marketing and outreach efforts is how to capture current and relevant content to feed into external communication products (e.g., social media posts, newsletters, annual reports). In many cases, laboratories operate as individual units, which can make it difficult for the rest of the organization to know what everyone is up to. Therefore, it is paramount for lab director to make a concerted effort to keep senior managers and communications staff informed of the most recent lab developments and accomplishments. Here are some suggestions for how organizations can use internal communications to streamline this process:

- Consider setting up a project or team site that can serve as a portal for internal communication efforts and gathering content from employees. If an in-house application is not available, there are many free and low-cost options (e.g., Asana.com, bitrix24.com) that can be tailored to such needs. Be sure to include help guides and other resources to train staff on how to use the website, along with other guidelines.
- Encourage different lab’s operational units to submit periodic updates with relevant news and information. These updates can be few outlined categories (e.g., recent publications and presentations, staff awards). Introduction of new diagnostic test, procedure or change in method must be communicated with relevant one-page detail.
- Maintain a shared calendar where lab staff can add upcoming events (e.g., conferences, talks, outreach events) that they will be attending or sponsoring. This resource will allow communications staff to publicize the organization’s presence at these events. It also gives managers an opportunity to touch base with lab personnel in advance of the event to see whether they need any marketing materials or other information to effectively promote the organization.
- Ask sectional staff to regularly share already prepared materials and files that can provide ideas for promotions and/or can be repurposed for inclusion in marketing collateral. These materials may include open meeting minutes, presentation slides, and other related files (e.g., event pictures).
- Facilitate ways for staff to talk and share their science in an informal setting. Some ways to do so include adding a quick roundtable discussion at regular meetings where employees can share updates or news or scheduling coffee breaks with managers. Also consider hosting inhouse research presentations or symposia to provide a platform for employees across the organization to engage in scientific discussion about their latest work.
- From a business development standpoint, provide employees with internal communication guidelines on how to make business referrals of potential clients, collaborators, and other interested parties (e.g., event sponsors, funding agencies) to the organization. These guidelines may include holding debriefs with staff after events to collect contact information, using a shared contact management system, and making
prompt and effective follow-ups with contacts.

**Training Lab Employees to be Brand Ambassadors**

Staff members are the outward extension of any laboratory who commonly interface with target audiences, from presenting at scientific conferences to sharing updates on social media about their work. Therefore, they can be the best brand ambassadors to positively represent and promote the organization. In order to do so, it is essential to ensure lab staff are delivering consistent messages, equipped with the tools and resources to effectively communicate, and trained in science communication skills. Here are a few ideas on how to transform staff into brand ambassadors for the organization:

- Conduct in-house communications training and provide other helpful how-to guides. These topics can include everything from the art of storytelling and an intro to social media to information on how to talk to the press and on media relations.
- Leverage the networks of staff members to spread the reach of marketing efforts. For example, provide regular reminders requesting that employees share materials (e.g., forward email newsletters, share social media posts) with their contacts. Additionally, when considering what social media platforms to use, look at where employees are most active online.
- Create a downloadable communications toolkit to supply employees with the tools and resources to promote the organization's brand. Sample contents of such a toolkit may include presentation and poster templates, logos and artwork, standard text for acknowledgments, marketing materials (e.g., brochures and fact sheets), and general talking points with key messages about the organization. Also, provide a way for employees to request printed copies of any of the materials to take along to conferences and other events.

Here are some final thoughts on how managers can work with staff toward a shared goal of being effective communicators within and outside the organization. This effort ensures information is being shared with any employee who may need it to do his or her job, and it also promotes collaboration across the organization.

- Take the time to promote staff (e.g., lab member profiles, employee award announcements, best from the past etc) in the organization’s communication channels (e.g. Facebook page or twitter), and use any potential marketing content that is provided by staff whenever possible. This will help employees recognize their contributions are valued, know everyone within the organization is being promoted equally, and see how the information is being used to advertise their work in marketing efforts.
- Show the tangible results of marketing and outreach efforts to acknowledge staff efforts. These results can include summaries of relevant marketing metrics (e.g., social media statistics, number of publications downloaded) and highlights of outreach events.
- Demonstrate to employees that managers also value communications and staff time spent on these efforts by including related tasks as part of regular roles and responsibilities, allocating adequate time and resources for this work, and directly tying these activities to performance objectives.
- As communications activities take time and effort, provide staff with information on what communications resources (e.g., writing and editorial support, graphics design) are available to them, whom they can contact for help in this area, and any organizational approvals required before communicating to external audiences. In line with encouraging employees to communicate with the public, it is suggested to keep any media approvals to a minimum and instead default to training on best practices in media relations.
- Finally, seek out lab employees who may have special interest in science communications and in gaining skills in this area for career growth opportunities. These staff members can help coordinate communication efforts among laboratories, train fellow employees in science communications, prepare draft marketing materials for external use, and serve as a technical liaison to any public relations and marketing staff.
Digital Breast Tomosynthesis

Dr Kulsoom Fatima
Department of Radiology

Digital breast tomosynthesis (DBT) is a relatively new breast imaging technique where multiple low-dose mammographic images of the breast are obtained as the x-ray tube moves across an arc. These images are then reconstructed into sections or “slices” as thin as 1 mm in a plane parallel to the detector allowing three dimensional estimation of tissue distribution.

Mammography is the only screening modality that has been proven to decrease breast cancer mortality. However, the major limitation of two dimensional digital mammography (DM) is the overlap of tissues which can obscure a true lesion leading to false negative examination. In addition, overlap of normal structures can create a spurious lesion resulting in a false positive result. DBT helps mitigate the masking effect of superimposed tissues allowing better visualization of true lesions and decreasing summation shadows resulting in improved cancer detection with a concomitant decrease in rate of false positive examinations. Furthermore, the increased conspicuity and better localization of lesions may obviate the need for additional mammographic views. Various studies have shown that use of DBT improves the accuracy of screening and diagnostic breast imaging. When comparing DM/DBT with DM alone, the combo imaging resulted in 29 percent increase in cancer detection rate and a 15 percent reduction in recall rate.

In a diagnostic setting, DBT may be employed to evaluate following non calcified lesions:

c) Masses: DBT can better depict the shape and margins of masses by elimination of the tissue overlap and also help in better assessment of the size. Furthermore, due to better visualization it helps in detecting multifocal or multi-centric disease which affects surgical treatment and the choice of mastectomy or breast conservation surgery.

Evaluation and characterization of calcifications may be challenging with DBT. Large calcifications often cause artifacts on multiple imaging sections while the micro-calcifications may be blurred or less conspicuous. DM spot-compression magnification views are therefore required for complete evaluation of micro-calcifications.

The average glandular radiation dose is approximately doubled when both DM and DBT are acquired although it is still below the FDA limit of 3mGy for a phantom image. Efforts are underway to address this and a software has been developed for creation of a synthesized 2D mammogram from the tomosynthesis acquisition.

Despite FDA approval in 2011, the use of DBT is inconsistent across various imaging centers in the world. As with any new technology, several issues must be considered when implementing DBT into daily practice. First there is a substantial learning curve for interpreting DBT. Secondly increased number of images increases the reading time along with increasing the storage requirements of the picture archiving and communication system (PACS). Moreover, management of suspicious findings visible at DBT alone is challenging if DBT guided biopsy technique is not available.

AKU has two state of the art mammography units, both with capability of DBT. We do not use DBT for routine screening, however it is used in selected cases mainly as a problem solving tool.

Acquiring DBT images in addition to routine DM
increases the scanning time by just few minutes.

**Example Cases**

Case number 1: A spiculated density was seen in the lower half of the left breast on digital Medio-lateral oblique (MLO) projection (Figure 1a, black circle). Corresponding DBT image (Figure 1 b), did not reveal any suspicious abnormality in that area. Normal parenchyma was seen indicating that it was not a true density but just summation of overlying tissues.

Case number 2: A 44-year old woman presented with right breast lump for one year. Digital Cranio-caudal (CC) view of the right breast showed an ill-defined increased density in the right retro-areolar region (black circle, Figure 2 a) and skin thickening overlying the right breast. Corresponding DBT image in same projection nicely depicts the spiculated high density mass in the right retro areolar region (Figure 2 b). This was proven to be invasive breast carcinoma.

Case number 3: Digital Coned MLO view of the right breast in a 38-year old woman did not reveal any focal abnormality in the lower half (Figure 3 a). Corresponding DBT image in same projection shows a well circumscribed benign appearing oval nodule with surrounding lucent halo (black circle, Figure 3 b) which was an intramammary lymph node on concomitant ultrasound. This was not evident on digital image due to overlapping of breast parenchyma.

**Therapeutic Drug Monitoring of Methotrexate: factors resulting in delayed clearance**

Ms. Humera Asif and Dr Hafsa Majid
Clinical Chemistry

Methotrexate (MTX) is an immunosuppressant drug utilized in treating post-transplant patients; cancer including breast cancer, leukemia, lung cancer, lymphoma, osteosarcoma; autoimmune diseases including psoriasis, rheumatoid arthritis, Crohn’s disease; ectopic pregnancy and for medical abortion. Methotrexate decreases the cell turnover by competitively inhibiting dihydrofolate reductase, an enzyme involved in synthesis of the nucleoside thymidine, which is in turn required for DNA synthesis.
The MTX is cleared from the body through both biliary and urinary routes, dysfunction renal (dose adjustment needed) and abnormal liver function tests are relative contraindication for MTX use. Excretion of MTX by the kidney is a result of both glomerular filtration and tubular secretion. Studies shows that the many factors which can result in delayed clearance of MTX, shown below:

- Methotrexate is water soluble drug and can accumulate in body fluids such as ascites or effusions. Methotrexate will then slowly leak out of the fluid, resulting in prolonged serum concentrations of MTX.
- Reduced renal tubular transport as a result of decreased urine pH, Folic acid, salicylates, ascorbic acid can decrease clearance of Mtx.
- Concomitant use of certain drugs also delays clearance, such as:
  - Sulfonamides, salicylates, phenytoin, tetracycline. These drugs displace Mtx from binding sites on plasma proteins
  - Aminopterin (an anti-neoplastic agent), interferes in analysis
  - NSAIDs, Penicillins, Probenecid, Gemfibrozil, Cyclosporine, Ciprofloxacin, Amiodarone, Doxycycline, Simvastatin. These drugs compete for P-glycoprotein transport, CYP450 metabolism, or renal excretion of methotrexate
  - Furosemide, when used within 48 hours of the initiation of high-dose methotrexate can delay methotrexate clearance
  - Proton pump inhibitors (Omeprazole, Esomeprazole, Pantoprazole) and Levetiractum are also reported to delay Mtx clearance.

Concentration of serum MTX is determined by Enzyme multiplied Immunoassay techniques. Results are interpreted in relation to drug dose taken and timings of last dose, so it is important to mention dose & time of MTX infusion completion and time of sample collection on every sample request forms and/or Computerized physician order entry (CPOE) while requesting MTX levels.

Overview of Common Myeloproliferative Neoplasms

Dr Hajrah Syndeed, Dr Asif Naveed, Dr Natasha Ali Haematology

Myeloproliferative neoplasms (MPNs) are a distinct group of blood disorders characterized by massive production of one or more of the blood cells. This over-production leads to various signs and symptoms and compromises the quality of life of sufferers. Moreover, the cells produced in massive numbers are dysfunctional and add to the symptomatology of the disease.

WHO Classification of MPNs: The 2016 WHO category of MPNs includes the three major subcategories of JAK2/CALR/MPL mutation-related MPNs (polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF)), as well as four other clinicopathologic entities: chronic myeloid leukemia (CML), chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia not otherwise specified (CEL-NOS) and MPN unclassifiable (MPN-U).

Another broad classification also distinguishes between these subgroups by the presence or absence of BCR-ABL1 fusion gene, a concerns that is typically helpful in clinical management and follow-up for these patients.

Incidence of MPNs: Incidence of these disorders is at a raise in our population. They usually affect individuals over 50 years of age (excluding CML). Younger individuals and even children are also affected in some cases (especially in CML). Etiological factors suggested include exposures to chemicals & radiations however majority cases remain idiopathic.
Overview, Pathogenesis, Signs and Symptoms for Common MPNs:

- **Chronic myeloid leukemia (CML)**—CML is considered to be a disease of adults with age over 50 years particularly at risk. It is also the most common pediatric MPN. People with CML initially are asymptomatic, however common symptoms are weakness, pale skin, abdominal discomfort/heaviness caused by an enlarged spleen, and unexplained weight loss. CML occurs due to abnormal chromosomes (known as Philedelphia Chromosome) which leads to a mutation BCR/ABL1. This altered gene makes an abnormally functional protein that leads to the overproduction of white blood cells.

- **Polycythemia vera (PV)** is a disease in which excessive red blood cell are produced in the bone marrow due to dysregulation of red blood cell production. Building up of RBCs in the bloodstream leads to increased viscosity of the blood causing symptoms such as headache, dizziness and visual problems. Other life threatening events such as heart attack or cerebrovascular accidents can also occur.

- **Primary myelofibrosis (PMF)** – is a disease where fibrous cells gradually replace normal bone marrow tissue. The dense fiber network impairs bone marrow function and blood cell production and can lead to production of blood cells outside the bone marrow, typically in the liver or spleen (extramedullary hematopoiesis)

- **Essential thrombocythemia (ET)**—is characterized by an increased number of megakaryocytes in the bone marrow as well as persistent increases of platelets in the blood. Excessive platelets are prone to initiate clot formation within the blood stream. Also, platelets may not function normally, leading to bleeding.

**Laboratory diagnosis for MPNs**: Diagnosis of these disorders are usually indicated by complete blood count and white blood cells differential count. Various CBC findings for MPNs are as under:

### Complete Blood Count:

<table>
<thead>
<tr>
<th>CBC parameter</th>
<th>CML</th>
<th>PV</th>
<th>ET</th>
<th>MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>Ranges from low to normal range</td>
<td>High &gt;16.5g/dL in Males &gt;16 g/dL in females</td>
<td>Normal</td>
<td>Ranges from Low to normal range; Tear drop cells are hallmark</td>
</tr>
<tr>
<td>WBC count</td>
<td>Markedly Raised in majority of cases</td>
<td>Raised</td>
<td>Normal</td>
<td>Raised</td>
</tr>
<tr>
<td>Differential Leukocyte count</td>
<td>Shows presence of immature myeloid precursors, basophils and blasts</td>
<td>Usually show few immature myeloid precursors.</td>
<td>Normal. No left shift is seen</td>
<td>Show immature myeloid precursors, varying degree of blasts</td>
</tr>
<tr>
<td>Platelet count</td>
<td>Ranges from low to normal/increased</td>
<td>Increased and shows anisocytosis.</td>
<td>Ranges from low to normal range</td>
<td></td>
</tr>
</tbody>
</table>

### Bone Marrow Biopsy

<table>
<thead>
<tr>
<th>CML</th>
<th>PV</th>
<th>ET</th>
<th>MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shows presence of hyperplastic leucosytosis, Blast count varies according to disease subtype</td>
<td>Shows Panmyelosis (marked increase in all cell lines)</td>
<td>Shows marked increase in Megakaryocytic proliferation and lakes of platelets with other cell lines within normal range</td>
<td>Shows presence of megakaryocytic atypia, variable blast percentage and other cell lines according to stage of disease</td>
</tr>
</tbody>
</table>

### Bone Core Trephine Biopsy

<table>
<thead>
<tr>
<th>CML</th>
<th>PV</th>
<th>ET</th>
<th>MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of hyperplastic leucosytosis and characteristically dwarf megakaryocytes</td>
<td>Panmyelosis (marked increase in all cell lines)</td>
<td>Marked increase in Megakaryocytic proliferation with specific clustering patterns.</td>
<td>Presence of megakaryocytic atypia and clustering.</td>
</tr>
</tbody>
</table>
Reticulin and trichrome stains further enhance the presence of fibrous/collagen bands in each of the case associated with staging and prognostic findings in each individual.

Molecular testing: According to WHO classification of MPNs, molecular testing plays a vital role in diagnosis as well as risk stratification for the patients. The following shows details for each common MPN:

<table>
<thead>
<tr>
<th>CML</th>
<th>PV</th>
<th>ET</th>
<th>MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCR-ABL1 Positive</td>
<td>BCR-ABL1 Negative JAK2 Positive in 90% of cases</td>
<td>BCR-ABL1 Negative CAL-R MPL (with majority of cases) JAK2 (with few cases)</td>
<td>BCR-ABL1 Negative Positivity for JAK2, MPL, CAL-R may be detected as an evidence of clonality</td>
</tr>
</tbody>
</table>

WHO classification strongly emphasized for BCR-ABL1 testing to be excluded before considering any MPN other than CML, this emphasizes the importance of testing BCR-ABL testing in each suspected case of MPN.

BCR-ABL1 Testing: The testing can be performed through FISH or PCR on blood/bone marrow aspirate samples.

**Significance of BCR-ABL1 Detection:**
1. Diagnostic significance.
2. Targeted therapies are aimed at inhibiting the abnormal proteins related to genetic mutations in MPNs. For example, drugs called tyrosine kinase inhibitors (imatinib, dasatinib, nilotinib) can target the abnormal BCR-ABL protein in chronic myeloid leukemia cells.
3. Monitoring patients through BCR-ABL1 analysis responding to tyrosine kinase inhibitors helps in determination of disease status and continuation of targeted therapies.

**Continuous Monitoring and Follow-up:** are required in cases of MPNs. They have a risk of advancing to acute leukemia (especially MF, PV and accelerated phases of CML). A close clinical and laboratory correlation is thus required for a good quality of life in these patients.

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**Radiology Pathology Correlation: Bone Pathology**

Dr Nasir Ud Din and Dr Dawar Khan
Histopathology and Radiology

An 18-year-old boy was presented to an orthopedic OPD with complains of right knee pain and swelling of 3 month’s duration. An x-ray knee was done which showed a lytic lesion in the epiphysis of right distal femur with well-defined sclerotic margins and a few calcific areas in it. (Figure 1A). The patient underwent curettage and bone grafting of the lesion. Histological examination showed sheets of polyhedral cells. These cells had abundant eosinophilic cytoplasm and nuclear grooves. Scattered osteoclast type giant cells were noted (Figure 1B). Focal chicken wire calcification was seen (Figure 1C). Based on radiological and histological findings, a diagnosis of chondroblastoma was made.
Chondroblastoma is a rare, benign, locally aggressive bone tumour. The tumour typically affects the epiphyses or apophyses of long bones like proximal and distal femur, proximal tibia and proximal humerus. The tumour commonly occurs in patients between 10 and 25 years of age with a slight male predominance. Most of the patients present with history of localized mild pain of variable duration. Radiographically, chondroblastomas are seen as well defined lucent lesions, with either smooth or lobulated margins and a thin sclerotic rim. Usually arising eccentrically in the epiphysis of long tubular bones like femur, humerus, or tibia or apophyses such as the greater trochanter, greater tuberosity, calcaneus, or talus. Internal calcifications can be seen in 40-60 percent of cases. They range in size from one-ten cm.

Histologically, sheets of uniform round to polygonal cells with well-defined cell borders are seen. The cytoplasm is eosinophilic, and nuclei are round to ovoid which often show longitudinal grooves. Scattered osteoclast type multinucleated giant cells are seen. Fine pericellular, chicken-wire type calcifications are pathognomonic but not seen in all cases as decalcification dissolves these fine calcifications. Secondary aneurysmal bone cyst is seen in up to 1/3rd of cases. Tumour cells express S100 protein and SOX9. Recently, a heterozygous mutation replacing lysine 36 with methionine (K36M) in the histone H3 variant H3.3 is seen in chondroblastoma. Antibody H3K36M is a sensitive and specific marker for diagnosis of chondroblastoma. Histologically, the closest differential diagnosis of chondroblastoma is Giant cell tumour of bone (GCT). Both tumours involve epiphysis, but GCT occurs in patients after growth plate closure while Chondroblastoma mostly occurs in patients less than 20 years. Osteoclast type giant cells in GCT contain many nuclei which are fewer in chondroblastoma and these are evenly dispersed in GCT and scattered placed in chondroblastoma. Mitotic figures are frequent in GCT while few mitoses are seen in chondroblastoma. Characteristic chicken-wire calcification is not seen in GCT.

Immunohistochemically, GCT is negative for H3K36 and positive for H3.3G34W. The other differential diagnosis of chondroblastoma is chondromyxoid fibroma (CMF). CMF also occurs in the same age group but involves metaphysis of long bones. CMF has a lobular architecture and myxoid background which is not seen in chondroblastoma and CMF do not contain chicken-wire type calcification. Clear cell chondrosarcoma (CCC) occurs in older patients but it also involves epiphyses of long bones.

Histologically, cells of CCC are larger than chondroblasts of chondroblastoma and these contain abundant clear cytoplasm. Trabeculae of bone seen in CCC are not a feature of chondroblastoma.

Radiographic differential diagnoses include clear cell chondrosarcoma, osteomyelitis with abscess, e.g. Brodie abscess, intraosseous ganglion and giant cell tumor.

Chondroblastomas are successfully treated by simple curettage and bone grafting. Local recurrence rates range from 14 to 18 percent. Rarely, pulmonary metastases may develop from histologically benign chondroblastomas. These metastases are clinically non-progressive. There are no reliable histological features to predict recurrence or metastasis.
E-Cigarette, Vaping Associated Lung Injury (EVALI), Histopathological Diagnosis (literature review).

Dr Sahar Sulaiman and Dr Saira Fatima
Histopathology

E-cigarette use or “vaping” has been increasing in popularity over the past decade, and it is commonly seen as a “healthier” often marketed as a safer alternative to smoking traditional combustible tobacco cigarettes.

While they may not contain tar or some of the other carcinogens, in addition to nicotine, the vapor aerosolized by e-cigarettes is known to have many other additives and flavorings like tetrahydrocannabinol (THC- a psychoactive mind-altering compound of marijuana that produces the “high”) and other plant compounds, lipid-rich materials (essential oils, etc). In the recent past, lung disease related to vaping has risen as a public health issue in the United States, generating considerable attention in the national news media. As of January 21, 2020, a total of 2,711 hospitalized EVALI cases or deaths have been reported to CDC from 50 states, the District of Columbia, and two U.S. territories (Puerto Rico and U.S. Virgin Islands). Amongst them 66 percent were male. The patients ranged from 13–85 years, 24 being the average age.

Although several studies have described clinical features including respiratory symptoms (dyspnea, cough, and hypoxemia), constitutional symptoms (fever and gastrointestinal symptoms) along with bilateral pulmonary infiltrates on computed tomography chest imaging, literature is severely deficient in regards to the histopathologic changes observed in the lungs of these patients.

To date, histologic findings reported in presumed cases of vaping-associated acute lung injury include organizing pneumonia, lipoid pneumonia, diffuse alveolar hemorrhage, mild nonspecific inflammation, diffuse alveolar damage with foamy macrophages, interstitial and peribronchiolar granulomatous pneumonitis, and respiratory bronchiolitis.

The pathogenesis of vaping-associated acute lung injury remains poorly understood, but much attention has been given recently to the possibility that this may represent a form of lipoid pneumonia. One potential explanation is the deposition of aerosolized oils inhaled from e-cigarettes within the distal airways and alveoli of these patients, inciting a local inflammatory response impairing vital gas exchange. The presence of numerous Oil Red O–positive macrophages in bronchoalveolar lavage (BAL) fluid was the only evidence to make such a diagnosis.

(E-F) Surgical lung biopsies from a 51-year-old man vaping marijuana showed a vaguely nodular, airway-centered acute lung injury pattern with severe bronchiolitis, abundant fibrin within bronchioles and peribronchiolar airspaces, vacuolization of the bronchiolar mucosa and pneumocytes, foamy macrophage accumulation, and airway-centered organization. (Butt YM, Smith ML, Tazelaar HD, et

Finding lipid-laden macrophages on lung biopsy specimens or BAL samples may not be a completely sensitive test for vaping-associated lung injury. While foamy macrophages were nearly universally noted, Mukhopadhyay et al., did not note conspicuous foamy macrophages in every case included in their study. While, performing Oil Red O stains on BAL samples is favoured as a less invasive option that would not require tissue biopsy, it should be noted that alveolar foamy macrophages are a very nonspecific finding. Increase in number of alveolar foamy macrophages are classically associated with lung injury due to drug toxicity such as amiodarone or due to aspiration. Thus, the clinical effectiveness of Oil Red O is brought into question, at least until more robust sensitivity and specificity data are available.

EVALI with confirmation using the CDC classification and Oil Red O stain. Bronchoalveolar lavage sample shown. (A) Thin Prep specimen of BAL with large intracytoplasmic vacuoles (inset) and (B) Oil Red O staining in large and fine intracytoplasmic vacuoles. (C) Cell block sections demonstrated numerous alveolar macrophages, with many containing “soap bubble”–like vacuoles (inset). (D) Immunohistochemical staining performed for CD163 demonstrated numerous alveolar macrophages. (Anjali Saqi A, Mukhopadhyay S, Butt Y et al, E-Cigarette or Vaping Product Use–Associated Lung Injury: What is the Role of Cytologic Assessment? DOI: 10.1002/cncy.22237, wileyonlinelibrary.co)

In accordance with Anjali Saqi et al., presently knowledge of the effects of various vaping agents on the respiratory tract is in its infancy. Additional understanding of the agents implicated in EVALI as well as a correlation between BAL and lung biopsy samples is required.

Clinical Significance of free Androgen Index (FAI)

Mr. Sherdil Aman and Dr. Siraj Muneer
Clinical Chemistry

Testosterone is the principal male sex steroid hormone also produced in smaller amounts by the ovaries in women. The majority (nearly 60 percent) of testosterone is bound to a specific high-affinity protein called sex hormone-binding globulin (SHBG), while the rest is bound to albumin (add percentage) and other proteins (percentage). A small fraction represents the physiologically active free form that mediates the biological action of the hormone at the target tissues in both sexes.
The SHBG is a plasma dimeric glycoprotein and produced by the liver. In the circulation, SHBG have higher affinity of binding to testosterone than to estrogens (affinity to testosterone is five times greater than to estrogens). An increase in SHBG concentration decreases the bioavailability of testosterone and thus decreases the free hormone levels without noticeable change in total hormone levels, and vice versa. Conditions that are associated with decreased SHBG are polycystic ovary syndrome (PCOS) in females, usually accompanied with excess testosterone action or hyperandrogenism leading to hirsutism, menstrual disturbance, or infertility. Conditions associated with increased SHBG concentration such as thyrotoxicosis, liver disease lead to decreased free testosterone status and hypogonadism.

Androgen excess is the cardinal underlying phenomenon in a variety of disorders particularly PCOS, idiopathic hirsutism, and ovarian neoplasm in females, adrenal neoplasms, congenital adrenal hyperplasia as well as insulin resistance in both the gender. Free or bioactive testosterone that is responsible for the pathogenesis of androgen excess status appears to be of more clinical relevance. However, despite all advances in technology, direct measurement of free testosterone concentration is surrounded by many technical difficulties with many assays not easily feasible. As alternatives, calculating the percentage ratio of total testosterone to SHBG concentration, called free androgen index (FAI) have increasing application as diagnostic tools for hyperandrogenism. It is measured by the formula shown below:

\[ FAI = 100 \times \left(\frac{\text{Total Testosterone}}{\text{SHBG}}\right) \]

This approach has improved the validity and efficiency of the test as a diagnostic tool. The FAI is a ratio used to determine abnormal androgen status in humans and used as a surrogate marker for free testosterone level, but it is not very accurate. Reference range of FAI is gender specific, for males 33.8-106 whereas for females levels between 0.51-6.53 are considered normal. The ratio correlates well with free testosterone level in female compared to male. It is recommended that FAI ratio should be interpreted in clinical context.

Radiology Quiz

Shayan Sirat Maheen Anwar

A 41 year old gentleman with occasional headache

- Pilocytic astrocytoma
- Hemangioblastoma
- Cystic Metastasis
- Ependymoma
Triple Negative Myeloproliferative Neoplasms

Dr Hajrah Syndeed, Dr Asif Naveed and Dr Natasha Ali Haematology

**Triple negative MPNs:** The term “Triple-Negative” is used to encompass a particular subset of Philadelphia negative MPNs [polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF)] which does not show presence of either of JAK2, CAL-R and MPL mutations, found in BCR-ABL1 negative MPNs. These cases comprise of 10-15 percent of all cases of PMF and ET especially and show a more changed course of disease.

Impact: Triple-negative status carries prognostic significance in MPNs: In PMF, triple-negative status is associated with poorer survival, while triple-negative ETs have favorable prognosis.

**Percentage of Triple Negative MPNs:**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Pathway/region</th>
<th>Type of mutation</th>
<th>Frequency of mutation (%)</th>
<th>Prognostic significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABL1</td>
<td>Tyrosine kinase</td>
<td>Missense</td>
<td>5-12% in PV &amp; ET, 11% in MF</td>
<td>Adverse in PV and MF</td>
</tr>
<tr>
<td>BRAF</td>
<td>Tyrosine kinase</td>
<td>Missense</td>
<td>6% in ET, 1-3% in MF</td>
<td>None</td>
</tr>
<tr>
<td>EZH2</td>
<td>Histone methyltransferase</td>
<td>Missense</td>
<td>3-12% in MF</td>
<td>Adverse in ET and MF</td>
</tr>
<tr>
<td>SHH</td>
<td>G protein-coupled receptor</td>
<td>Missense</td>
<td>10% in PV, 1% in ET</td>
<td>None</td>
</tr>
<tr>
<td>XIAP</td>
<td>Cullin E3 ubiquitin ligase</td>
<td>Missense</td>
<td>1-4% in MF</td>
<td>Adverse in MF</td>
</tr>
<tr>
<td>TET2</td>
<td>5-methylcytosine DNA demethylase</td>
<td>Missense or Mutation</td>
<td>0% in ET, 10-14% in MF</td>
<td>Adverse in ET and MF</td>
</tr>
<tr>
<td>SF3B1</td>
<td>Nuclear processing</td>
<td>Missense</td>
<td>2-3% in PV, 8-10% in MF</td>
<td>Adverse in ET and MF</td>
</tr>
<tr>
<td>MSH6</td>
<td>Nucleotide excision repair</td>
<td>Missense</td>
<td>0% in MF, 5% in ET</td>
<td>None</td>
</tr>
<tr>
<td>IDH1/2</td>
<td>2-oxoglutarate dioxygenase</td>
<td>Missense</td>
<td>1-2% in ET, 10-14% in MF</td>
<td>Adverse in ET and MF</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>DNA methyltransferase</td>
<td>Missense</td>
<td>2-3% in ET, 6-10% in MF</td>
<td>None</td>
</tr>
<tr>
<td>ASXL1</td>
<td>Histone deacetylase</td>
<td>Missense</td>
<td>2-3% in ET, 6-10% in MF</td>
<td>None</td>
</tr>
<tr>
<td>LNK</td>
<td>Tyr protein kinase</td>
<td>Missense</td>
<td>6% in PV, 1% in ET</td>
<td>Adverse in MF</td>
</tr>
<tr>
<td>FGFR1</td>
<td>Fibroblast growth factor receptor</td>
<td>Missense</td>
<td>4% in MF</td>
<td>None</td>
</tr>
<tr>
<td>CBL</td>
<td>CBL family member</td>
<td>Missense</td>
<td>2% in PV, 2% in ET</td>
<td>None</td>
</tr>
<tr>
<td>MPL</td>
<td>Myeloproliferative factor</td>
<td>Missense</td>
<td>2% in PV, 1% in ET</td>
<td>None</td>
</tr>
<tr>
<td>JAK2</td>
<td>Janus kinase</td>
<td>Missense</td>
<td>2% in PV, 1% in ET</td>
<td>None</td>
</tr>
<tr>
<td>MPL exon 10</td>
<td>Myeloproliferative factor</td>
<td>Missense</td>
<td>2% in PV, 1% in ET</td>
<td>None</td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Laboratory diagnosis: Laboratory diagnosis for triple negative MPNs require a similar sequence as for other MPNs. Clinical correlation is very important. Reactive causes such as: infections, immune disorders, secondary causes of polycythemia and thrombocytosis should be ruled out.

Molecular testing: As per definition, these entities does not show presence of BCR-ABL1, JAK2, MPL or CAL-R mutations. The natural query about clonal changes and pathogenesis of triple negative MPNs arises for both pathologists and clinicians. Mutations in LNK, TET2, DNMT3A, IDH1/2, CBL, and ASXL1 genes as well as atypical mutation in MPL (S204P) have been identified.

There are over 30 missense mutations that have been identified in 42 genes so far and each of them pose a separate risk/benefit for the patient.

Newer techniques: In order to determine the molecular mechanisms for this subset, whole genome sequencing and next generation sequencing are successful modalities to provide much more information about the target mutation(s) that may be driving these neoplasms.

New diagnostic challenge: Considering the prognostic role imposed by the mutations present in triple negative MPNs, incorporation of next generation sequencing, its availability and cost issues should be addressed for chalk out better diagnostic facilities as well as therapeutic targets for these patients is the need of hour.
The Best of the Past

*Radiologist #Womenimaging #Followtheirlead*

Interview recorded by
Dr Shayan Anwar

1. Considering your entire time as a women imaging radiologist, can you recall a time (any AHAA moment) when you felt most alive or most excited about your involvement in this organization?

*Prof Imrana Masroor:* I think, for me, the part that I most cherished during this time was when we first started the Women Imaging Fellowship programme and inducted our very first fellow. Since then all fellows, which we as mentors took on have been remarkable. I considered it a personal achievement when I would train the fellow and they would be able to apply their expertise elsewhere. It gave me an immense sense of pride.

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

*Prof Imrana Masroor:* For any consultant, the answer for this question will be anything but brief. My journey like everyone else’s was possible because of hard work and ambition. I graduated medical college in 1991 and completed house job in 1992. Then, like every other women in the workforce, I had to take a scheduled break to work on my family and had two children. I restarted work and soldiered through The Aga Khan University Radiology Residency Programme and graduated in October 1999. I was then taken on as an instructor in January 2000. I eventually became a consultant the following year in January 2001.

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

*Prof Imrana Masroor:* The attributes I value deeply, which in my opinion are becoming obsolete in this day and age, are punctuality; empathy towards not only my patients but my colleagues; and respect towards all medical staff from the housekeeping staff to the head of the department. I also value tolerance towards other’s opinions and perspective. What I have learned from this line of work is that even at this phase of my career, there is always something to learn. My work has instilled in me confidence and a sense of purpose that I otherwise would not have. I now have a heightened awareness of the frailty of human life through the experiences of my patients and am extremely grateful for it.

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

*Prof Imrana Masroor:* I think we have come a long way since when I first started clinical practice. Having said that, so much more needs to be done. There may be the availability of breast imaging in the form of ultrasounds in most urban hospitals and local clinics within the city. However, the problem is so much more than that, in terms of public awareness regarding breast cancer I truly believe we need a screening programme on a national level for Breast Cancer. I really hope that this gets achieved in the next 10 years, to be optimistic. Another dilemma that I’d like to point out is the fact that women tend to undermine themselves when it comes to any symptom especially related to the breast or female genital tract. I really
hope that in the next 10 years women will be aware enough to speak up about their concerns and not hide them. More so, I would like the men in our society to adopt the mindset of not undermining women’s health related problems. I really hope attitudes towards this change within the next decade.

5. Any advice for Junior Radiologists?

Prof Imrana Masroor: To all those aspiring to become radiologist, I think the best insight I can relay to you is that if you’re truly passionate about radiology, then you will get there in the end. Practice and clinical exposure will make you seasoned. However, the right sources of information will add to your accolade of knowledge, so be wary of that too. Another thing that most people will not realize is how important your role is in the care pathway of a patient, and trust me there will be instances where your work, however devoid of patient interaction, will be undermined by other specialists and doctors belonging to other fields of medicine and surgery. This is something that can happen in this country.

Please do not let that get in the way of how important your contribution can be. A special mention to all those aspiring female radiologist, you will probably have to work harder than anyone else within this field, keep at it. If I made it, you will too!

Parasitic infections of the gastrointestinal (GI) tract is a significant cause of morbidity and mortality. Although they are particularly more common in underdeveloped regions with poor sanitary conditions, they are also prevalent throughout the whole world. Clinical presentation may vary depending on the parasite type and the affected parts of the GI tract. Parasites can survive in the GI tract for years without causing any symptoms. They may present important diagnostic challenges as they can mimic important GI pathologies such as eosinophilic gastroenteritis, gluten sensitive enteropathy or inflammatory bowel diseases.

The common microscopic findings include: Chronic and acute inflammation, increase of eosinophils, congestion, mucosal ulceration, necrosis, perforation, lymphoid aggregates, cryptic architectural distortion, granuloma formation, calcification, hyalinization, and presence of giant cells.

According to location the common organisms include:

**UPPER GIT:** Giardia, hook worm or fish tapeworm, strongyloides stercoralis larva, ancylostoma duodenale and diphyllobothrium latum.

**LOWER GIT:** Enterobius vermicularis, schistosomiasis, amoebiasis, ascaris lumbricoides, taenia.

**E.vermicularis (Oxyuris)**

E. vermicularis, commonly referred to as the pinworm or seatworm, is a nematode, or roundworm. Humans are the only known host, and about 209 million persons worldwide are infected. More than 30 percent of children worldwide are infected. Eggs and part of the adult worm is more likely to cause the inflammation of appendix.

**Microscopy:** Cross section has narrow lateral cuticular alae.

**Entamoeba Histolytica**

It is commonly found in the inflammatory exudate on the base of ulcers or loose on the surface of the mucosa.

**Microscopy:** Organisms are round to oval, have faintly granular cytoplasm and may be mistaken for histiocytes, but can readily be recognized by the very small pale nucleus and phagocytosed erythrocytes. They stain positive for special stains: PAS, & Immunoperoxidase stain.
**Giardia Lamblia**

Causes malabsorption with chronic diarrhea and may be associated with nodular lymphoid hyperplasia.  
**Microscopy:** Intact mucosa with blunting of villi and increased number of inflammatory infiltrate. Trophozoites have a tear drop shape with paired nuclei (‘owl-eye’ appearance) and a central longitudinal axostyle.

**Cytomegalovirus (CMV)**

It is a double stranded DNA virus belonging to the herpesvirus group. Infection is usually seen in immunocompromised and debilitated patients, usually due to reactivation of latent infection. Tissue invasive CMV results from the hematogenous spread of CMV. Gastrointestinal CMV disease is the most common manifestation of tissue invasive CMV. Most common sites of involvement are esophagus and colon. Gold standard for diagnosis is histological diagnosis with immunohistochemistry. Gastrointestinal bleeding is the most common initial presentation of CMV colitis.  
**Microscopy:** CMV infected cells are cytomegalic cells, typically two to fourfold larger than normal, containing basophilic intranuclear inclusion bodies (Cowdry bodies) surrounded by a clear halo, giving the appearance of an owl’s eye. Many of these infected cells can be seen surrounding blood vessels, as the most commonly infected cells are endothelial cells and mesenchymal cells.

**Herpes Simplex Virus (HSV)**

Patients present with painful discrete ulcers, vesicles or pustular lesions in distal rectum or perianal skin. Symptoms are similar to colitis of other causes and include watery or bloody diarrhea, fever, abdominal pain, nausea, fatigue, and weight loss.  
**Microscopy:** Multinucleated giant cells with ground glass nuclei due to intranuclear virus along with margination and moulding.

**Spirochetes**

Intestinal spirochetosis is more common in the developing world and HIV+ patients. Generally there are no or minimal endoscopic abnormalities. The infection is noninvasive, but uncommonly invasion occurs; it is also found incidentally in association with adenomatous and hyperplastic polyps, diverticular disease and inflammatory bowel disease.  
**Microscopy:** A basophilic, fringe-like, end on end attachment of filamentous densely packed spirochetes on the surface epithelium of the large intestine or appendix are seen highlighted by special stain Warthin-Starry.

**H.Pylori**

Chronic infection occurs in approximately two/three of population worldwide, prevalence is decreasing due to improved sanitation and antibiotic use. Without treatment, infection usually stays lifelong. Infection conveys 15 – 20 percent lifetime risk of peptic ulcer disease. Atrophy with concomitant intestinal metaplasia and dysplasia are seedbed for carcinoma. Presence of active inflammation after eradication therapy is sign of treatment failure.  
**Microscopy:** Bacteria is curved, spirochete-like, in superficial mucus layer and along microvilli of epithelial cells. Usually not seen in areas of intestinal metaplasia. They are highlighted by special stain Geimsa and Warthin-starry.

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![HSV](image)

![H.PYLORI (GEIMSA STAIN)](image)
Hemangioblastoma is considered to be benign neoplasm and represents 1-2% of all primary central tumors. Historically linked to von Hippel-Lindau (VHL) disease. Seventy percent do not have a family history. Peak age - between 20 and 50 years. MRI with gadolinium enhancement is the best study for screening. A cyst with a small mural nodule is the most common presentation. Cystic fluid surrounding the nodule is hypo intense on T1-weighted images and hyperintense on T2-weighted images and shows no post contrast enhancement (A). The mural nodule is isointense on T1-weighted images, demonstrates high signal on T2-weighted images (C ), shows diffusion restriction (B) and avid post contrast enhancement (A).

Retinoblastoma: Updated CAP Guidelines for Staging

Dr Qurratulain Chundriger and Dr. Shabina Rahim
Histopathology

Retinoblastoma is a malignant pediatric tumor arising from the retina of the eye. It is one of the tumors having small round blue cell morphology and is the most common intraocular cancer arising in children. The incidence globally is estimated to be roughly 3% of all cancers occurring in children less than 15 years of age with India and China having the most cases per year. It is either heritable or non-heritable; the heritable disease being more likely to be bilateral and arising in children less than one year of age; while, the non-heritable disease being more likely to be unilateral. There is increased risk of another primitive midline neuroblastic tumor (a trilateral Retinoblastoma) in patients with heritable disease, as well as risk for developing second primary tumors associated with RB1 gene mutations, i.e., Osteosarcoma and Cutaneous Melanoma.

The primary genetic abnormality in retinoblastoma is bi-allelic inactivation of the RB1 gene. This mutation can be either inherited from an affected parent or occur de novo during formation of germ cells (before conception) or during early embryogenesis. Other minor mutations include alteration in BCOR genes, amplification of MYCN or OTX2.

Multiple treatment options are available for retinoblastoma, including enucleation, local treatment
options like cryotherapy, thermotherapy, systemic, intra-arterial, intra-vitreal or sub-conjunctival chemotherapy and radiation to name a few. Cases where tumor is filling the vitreous, extending into the anterior chamber or causing neovascular glaucoma are generally treated with up front enucleation. This is also attempted when there is recurrence of progression on eye-salvage therapy. After enucleation, certain features are important for predicting disease prognosis and these need to be specifically mentioned in the surgical pathology report. The College of American Pathologists has outlined a synoptic reporting format addressing all the relevant information, based on the guidelines described in the Eighth edition of the American Joint Commission for Cancers (AJCC) staging manual.

Unique to the eighth edition TNM classification of Retinoblastoma, is the inclusion of germ-line cancer predisposition, which is denoted by an “H category (Heritable trait)” to indicate the germ-line status of the \( RB1 \) gene (\( H1 \)). The Heritable trait is reported as follows:

- **HX** Unknown or insufficient evidence of a constitutional \( RB1 \) gene mutation.
- **H0** Normal \( RB1 \) alleles in blood tested with demonstrated high-sensitivity assays.
- **H1** Bilateral retinoblastoma, any retinoblastoma with an intracranial primitive neuroectodermal tumor (i.e., trilateral retinoblastoma), patient with family history of retinoblastoma, or molecular definition of a constitutional \( RB1 \) gene mutation.

For staging after enucleation, entire eye should be submitted for examination, as mentioned in the eighth edition of the AJCC staging manual. Fresh tissue needs to be obtained for molecular and genetic studies prior to formalin fixation. The following gross and microscopic characteristics of the tumor are included in the most recent version of the CAP cancer protocols, most of which are mandatory to be included in the report (updated latest on June 2017, available at: https://documents.cap.org/protocols/cancer-retinoblastoma-17protocol-4000.pdf).

- **Procedure**
- **Specimen laterality**
- **Tumor site** – before (transillumination of the globe) and after sectioning the eye ball, including the exact quadrant of the eye, if originating in the posterior chamber
- **Tumor size**
- **Involvement of other ocular structures** – Particularly the sclera and the anterior chamber.
- **Growth pattern** – endophytic tumors grow within the vitreous cavity, exophytic tumors grow in the subretinal space towards the choroid and diffuse tumors grow laterally into the retina without significant retinal thickening.
- **Extent of optic nerve invasion** – This feature is of particular importance with regards to the status of tumor invasion in relation to the lamina cribrosa of the optic nerve. A tumor with retrolaminar invasion of the optic nerve head (beyond lamina cribrosa) will be classified as pT3b; while, if there is involvement of the cut head of the optic nerve (positive optic nerve resection margin) the tumor will qualify for a pT4 category.
- **Histologic grade** – Retinoblastoma cells may form Flexner-Wintersteiner or Homer-Wright rosettes and fleurettes or areas of neuronal differentiation, reflecting differentiation towards normal retinocytes. The extent of presence of these specialized structures defines tumor grade, whereby most poorly differentiated tumors lack these rosettes and fleurettes and grow in diffuse sheets with or without anaplasia.
- **Anaplasia grade** – Degree of anaplasia graded as mild, moderate or severe, based on the highest level of anaplasia within the tumor. This requires at least 30 percent of the tumor being able to be graded and is an optional requirement of the synoptic report.
- **Cytological features suggesting MYCN amplification** – Those being a unilateral Retinoblastoma with a loose cellular pattern, round nuclei and prominent multiple nucleoli.
- **Margins** – including the resection margin of the optic nerve and other soft tissue margins if applicable.
- **Number of lymph nodes** involved by the tumor (if present in the specimen)
- **Pathologic TNM staging** – Detailed TNM classification is available in the CAP cancer protocol document (URL provided above).

Other features of clinical and prognostic significance for survival include: invasion of sclera; invasion
of choroid (true choroidal invasion being either minimal or massive, see below); basophilic staining of tumor vessels; seeding of vitreous and involvement of anterior segment. Moreover, the number of mitotic figures in 40 fields determined by using a 40x objective with a field area of 0.152 mm² along with presence of necrosis, apoptosis, inflammation, calcifications, hemorrhage, retinal detachment and neovascularization may also be important clinically.

True choroidal invasion is defined as one or more solid nests of tumor cells that fills or replaces the choroid and has pushing borders. Invasion of the sub-retinal pigment epithelium (RPE) space, where tumor cells are present under the RPE (but not beyond Bruch’s membrane into the choroid) is not choroidal invasion.

Minimal/Focal choroidal invasion is defined as a solid nest of tumor that measures less than 3 mm in maximum diameter (width or thickness).

Massive choroidal invasion is defined as a solid tumor nest 3 mm or more in maximum diameter (width or thickness) in contact with the underlying sclera.

Residual Tumor (R) Tumor remaining in a patient after therapy with curative intent (e.g., surgical resection for cure) is categorized by a system known as R classification as described below:

RX Presence of residual tumor cannot be assessed.
R0 No residual tumor
R1 Microscopic residual tumor
R2 Macroscopic residual tumor

The R classification may be a useful indicator of the completeness of a surgical excision. Tumor involving the resection margin on pathologic examination may be assumed to correspond to residual tumor in the patient and may be classified as macroscopic or microscopic according to the findings at the specimen margin(s).

All of these features serve as prognostic and clinical indicators of overall survival of Retinoblastoma patients and also serve to guide treatment. With newer treatment options becoming available and the option to be enrolled in ongoing clinical trials, there may be more scientific data available in the future which may alter the classification and staging systems. Remaining updated with the newest is the key to ensure the best practices for patients’ benefit.

Figure 1. Gross appearance of Retinoblastoma (Picture taken from Ocular Pathology 6th Edition – M. Yanoff et al.)

Figure 2. H&E 4x. Retrolaminar invasion of optic nerve by tumor.
Section Of Histopathology: Newer Horizons

Dr. Qurratulain Chundriger and Dr. Arsalan Ahmed
Histopathology

The Histopathology section in the clinical laboratories of the Aga Khan University Hospital (AKUH) is one of the largest histopathology centers in Pakistan and the only lab in the country accredited by the College of American Pathologists (CAP). Currently, we are reporting close to 90,000 histopathology specimens, 20,000 cytology and over 1500 flow cytometry specimens per year – along with ANA testing and semen analysis. We also get a large number of cases including blocks and slides for second opinion from labs all across Pakistan and also from Afghanistan. Unlike most centers, immunohistochemical studies at AKUH are not charged separately from the patient. With approximately 25 faculty members, 15 residents, five medical officers and more than 150 technical staff, we are the largest section of the department of Pathology and Laboratory Medicine.

The histopathology lab has been recently remodeled, under our section head Dr. Arsalan Ahmed and inaugurated by the President Mr. Feroz Rasul. It comprises of a dedicated area for receiving samples (Figure 1), areas for grossing specimens (Figure 2), processing, cutting, staining and distribution benches (Figure 3). Previously we used to have three separate gross rooms, each having a grossing station along with cupboards and cabinets for storing specimens and bench supplies. This caused congestion and a claustrophobic environment for anyone working inside and therefore resulted in observation by CAP during the initial inspection. Therefore, the idea of constructing a spacious grossing hall along with remodeling of the rest of the histopathology lab occurred to fulfill CAP’s phase II requirement, cope with an increasing volume and implementation of a “Lean method” in our section. As a result, these rooms have now been combined and turned into a large grossing hall, which has a capacity to accommodate up to six grossing stations. Looking at the statistical increase in the number of specimen reported annually from 2000 to 2019, this was the need of the hour (Figure 5). Being the largest histopathology laboratory in the country, it should be able to handle up to 200,000 specimens per year.

This grossing hall has also merged with the cytology screening and flowcytometry areas previously located immediately behind the grossing rooms. This hall is a negative pressure area, maintained by exhausts connected with the grossing stations. The cryostats and staining of frozen section can now also be done within the grossing hall, which was previously done in two steps at two different benches. This has significantly helped to decrease the turnaround time for reporting frozen sections. The predictive “mammoth” increase in volumes may also lay a foundation for a physician assistant “PA” program, which will be the first ever in Pakistan.

The highlight of this hall is the automatic sliding door system. This was so important because there is a constant traffic of people in and out of the hall; technologists bringing the specimen inside for grossing, going back and forth with baskets of specimen and blocks for processing, faculty and residents coming and going. Now the residents or technologists don’t have to take off their gloves every time they need to go out or come in. Believe it or not, this used to feel like “climbing the Mount Everest” when one had to rush out to respond to a beeping pager.

The cytology screening area has now shifted to a separate room, in accordance to the requirements of CAP. Flowcytometry reporting bench has also shifted to one of the larger areas of the lab. Since cytogenetics was shifted to Supariwala building in the molecular section, the dark room has now been converted in to semen DR reporting bench with immunofluorescence and our new gadget – the “Helios HELMED integrated optical system” which is an automated immunofluorescence machine – in close proximity (Figure 4). Liquid based cytology is another highlight and our Surepath equipment being under validation to be soon fully operational. Another newer member of the team is the bone processing equipment, including the bone cutter and Decal mate (automated bone decalcification) which is going to dramatically reduce the reporting time of bone specimen from
The histopathology section is the powerhouse of clinical and academic activities. Our team of faculty is in the process of expanding, which is essential to cope with increasing workload. With proper logistics and the largest faculty in the country under one roof, we will be a regional power. Our ambition and goal is to explore newer frontiers like the Gulf countries.

Figure 1: The receiving bench

Figure 2: The new grossing hall

Figure 3: Embedding, cutting and distribution areas

Figure 4: Helios HELMED integrated optical system

Figure 5. Line graph indicating the increase in the number of specimen received from the year 2000 to the year 2019.

Our goal was to increase the functionality and maximize workflow efficiency within the same available space. The section is thankful to the entire team behind this process, especially Dr. Arsalan Ahmed in successfully achieving this goal in record time. We aim to continue providing the best of diagnostic and predictive services which are the backbone of any patient care system.
Aspects of Quality Assurance in Laboratory Medicine: A Road to Quality?

Sujjawal Ahmad
Molecular Pathology

The top objective of any clinical laboratory is to give way to correct diagnosis and effective treatment of the patients. It is therefore imperative that diagnostic results produced are reliable. Quality laboratory services serve as bedrock for the decision-making capacity of the clinicians, healthcare workers and public health authorities to take appropriate curative as well as preventive measures for control of the disease. Principles of Quality management, assurance, and control have become the foundation by which clinical laboratories are managed and operated giving reasonable assurance that quality laboratory services are being provided to the patients. This implies to a wide-ranging concept of proper management skill and good laboratory practice that covers all activities both inside and outside laboratory.

The total testing chain in clinical laboratory involves several professionals and organizations in healthcare from the clinical decision to order a test through the pre-analytical, analytical and post-analytical phases to the value of the test result in the on-going clinical decisions and healthcare process.

Defining the quality objectives is first step in designing and implementing a quality plan. WHO summarizes quality requirements as:
• right result, at the
• right time, on the
• right specimen, from the
• right patient, with result/interpretation based on correct reference date, and at the
• right place. (1)

Error at any of the stage - be it pre-analytical, analytical or post analytical - invalidates the quality of analysis causing laboratory to fall short of its quality goals. Procedural errors are related to unclear and ineffective management and planning, inadequately trained staff, lack of adequate Quality assurance (QA) and other problems that pertain to how the work gets done. Systematic or technical errors, on the other hand, are directly related to the methods and technologies employed in a testing procedure. Avoiding or minimizing procedural errors will reduce likelihood of technical errors.

Quality assurance is the practice that encompasses all activities, procedures and endeavors that a laboratory takes to ensure that desired quality goals are being achieved and maintained. ISO, therefore, defines it as a part of quality management, providing confidence that quality requirements will be fulfilled. ISO 9000:2005, Clause 3.2.11. The primary objectives of QA, thus, include identification and as well as qualification of errors which may cause improper patient results compromising quality objectives. These may be random, systematic or procedural errors, occurring in pre-analytical, analytical or post analytical phases of diagnostic procedures employed for a specific test. QA is aimed at ensuring quality test results.

Quality control (QC) on the other hand covers the part of quality assurance which primarily concerns
the control of errors in the performance of tests and verification of test results, ensuring detection, reduction and correction of deficiencies in producing patient results. Quality control must be practical, achievable and affordable. Quality control can be implemented in two ways: **Internal QC**-performed by individual labs at their own levels. It forms the day to day basis working quality assurance, involving daily QC chart preparation. **External or inter laboratory QC**-performed by many labs at the same time, monitored by one. Bias (or Analytical error) is estimated by participation in proficiency testing schemes, using certified reference materials of surveys provided by various accrediting international organizations, including CAP, RIQAS, EQAS and many others. Comparisons are commonly made on the basis of Standard Deviation Index (SDI) or Z-score in some cases.

The laboratory must establish and follow written quality control procedures for monitoring and evaluating the quality of the analytical (examination) testing process of each method to assure accuracy and reliability of patient test results and reports.” It is therefore imperative that written procedures describing the validation process for new instruments and methods, as well as the data collected should be documented before a method is implemented for patient testing.

**A Webinar Series ‘Rare Links’**

Dr. Hafsa Majid  
Clinical Chemistry

A Webinar based educational series ‘Rare Links’ was initiated by Section of Chemical Pathology, from the platform of Pakistan Inherited Metabolic Disease Network (Pak-IMD-Net), a working group of Pakistan society of Chemical Pathology. Aim of Rare links webinar series is to provide a platform where national and international experts can discuss the inherited metabolic disease (IMD) in focus and share their experience of managing its patients. First Webinar of this series was conducted via Zoom video conferencing on ‘Diagnosing Urea Cycle Disorders (UCD)’ on 10th December 2019.

The focus of workshop was on understanding Hyperammonemia, natural history, pathophysiology and management of UCD. Webinar started with introduction of all participants followed by a welcome address by chair Pak-IMD-Net, Dr Lena Jafri. She briefed the participants about the aims and objectives of this working group. This was followed by introduction to “rare links’ webinar series by Dr. Hafsa Majid. She highlighted the importance of knowledge sharing in the area of IMDs and how this webinar series can help bringing together health care persons working at different centers and are involved in managing patients with rare diseases.

This was followed by lecture about approach to the diagnosis and management of hyperammonemia by Dr Aamir Ijaz, co-chair, Pak-IMD-Net. Next was a talk given by Dr Lena Jafri to further our understanding of the natural history, pathophysiology and management of UCD. Drs Sibtain Ahmed and Hafsa Majid presented case studies of UCD. An International expert on IMDs Dr Samir Munir, Metabolic Physician, working at Great Ormond Street Hospital for Children, London, United Kingdom gave a talk about management of patients with UCDs. In the end Dr Aysha Habib presented the data of IMDs patients presenting at a clinical laboratory, Aga Khan University.

It was a very successful event, with more than 80
participants from 14 different institutes and 7 cities of Pakistan. This was first webinar of this series, we plan to conduct more such activities under the banner of ‘Rare Links’

Figure: Participants from London, United Kingdom and different Cities of Pakistan attended the Rare Links Webinar via Zoom Video Conferencing

A Look into the Eye can Unleash Vital Diagnostic Pearls for Rare Disorders

Dr Sibtain Ahmed
Clinical Chemistry

Ophthalmologic manifestations are common in various inherited metabolic disorders (IMDs). Literature review revealed that the eye is the fourth most common organ system affected by IMDs. The age of onset of ocular abnormalities is however variable, ranging from childhood to infancy or early juvenile years. The following table enlists the common ocular manifestations that can serve as important diagnostic clues for certain IMDs. The following table enlists various IMDs and the associated ophthalmologic findings.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Ophthalmologic manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabry’s disease</td>
<td>opacities in the corneal epithelium, engorged conjunctival vessels and congested retinal vessels</td>
</tr>
<tr>
<td>Cystinosis</td>
<td>opacities in the stroma, deposition of distinctive iridescent crystals in the cornea</td>
</tr>
<tr>
<td>Wilson’s disease</td>
<td>opacities in the descemet’s membrane, sunflower cataract and pigmented corneal rings called as Kayser-Fleischer (K-F) rings</td>
</tr>
<tr>
<td>Mucopolysacharidosis</td>
<td>corneal clouding, thickening of the sclera at the lamina cribrosa, optic nerve head deformation and impingement of the nerve, and often optic nerve atrophy</td>
</tr>
<tr>
<td>Partial Lecithin carnitine acyl transferase (LCAT) deficiency</td>
<td>Fish-eye disease, impaired vision due to corneal opacification</td>
</tr>
<tr>
<td>Galactosaemia</td>
<td>Galactitol leads to toxic opacification and lens swelling; shift of water into the lens and disruption of lens structure</td>
</tr>
<tr>
<td>Cerebrotendinous xanthomatosis</td>
<td>bilateral cataracts, optic disc pallor, proptosis and fleck lenticular deposits</td>
</tr>
<tr>
<td>Zellweger syndrome</td>
<td>corneal opacification, cataract, glaucoma, pigmented retinopathy and optic atrophy</td>
</tr>
<tr>
<td>Marfan’s syndrome</td>
<td>ectopia lentis, iridodonesis, retinal detachment and glaucoma</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>ectopia lentis</td>
</tr>
<tr>
<td>Sulphite oxidase deficiency and molybdenum cofactor deficiency</td>
<td>opacities of the crystalline lens and ectopia lentis</td>
</tr>
<tr>
<td>Ehler-Danlos syndrome</td>
<td>keratoconus, fragility of the eye, dry eyes and tunnel vision</td>
</tr>
<tr>
<td>Retinitis pigmentosa</td>
<td>pigment deposits in the fundus and progressive death of photoreceptors, alteration of retinal pigment epithelium and retinal degeneration</td>
</tr>
<tr>
<td>Ornithine aminotransferase deficiency</td>
<td>gyrate atrophy of the choroid and retina, myopia which progresses to night blindness</td>
</tr>
<tr>
<td>Batten’s disease (Juvenile Neuronal Cereoid Lipofuscinosi)</td>
<td>hallmarks of the disease include rapid vision loss due to retinal degeneration</td>
</tr>
</tbody>
</table>
**Lab & Rad Mesh up for the Early Recognition of Rare Disorders**

Dr Sibtain Ahmed  
Clinical Chemistry

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Radiological Findings</th>
<th>Biochemical Findings</th>
</tr>
</thead>
</table>
| Urea cycle disorders           | • Conventional Magnetic Resonance Imaging (MRI): diffuse generalized cytotoxic edema, abnormal signal of Basal Ganglia (T1-/T2-hyper of the globi pallidi, T2-hyper of putamina and caudate nuclei)  
• Magnetic resonance spectroscopy: prominent glutamine/ glutamate peaks  
• Diffusion Weighted Imaging- MRI: scalloped ribbon of restriction at the depth of sulci | ↑ ammonia, glutamine and citrulline levels on plasma amino acid analysis  
↑ Orotic acid on urine organic acid analysis in few of the UCDs  
↑ Plasma Ammonia  
Often Respiratory alkalosis |
| Maple syrup urine disease      | • Conventional MRI: increased signal of myelinated structures  
(cerebellar and perirolandic, dorsal brainstem, cerebral peduncles, Posterior limb of internal capsule, thalami and globi pallidi)  
• Magnetic resonance spectroscopy: branched-chain amino and ketoacids (0.9 ppm)  
• Diffusion Weighted Imaging- MRI: Diffusion restriction of myelinated structures (cerebellar and perirolandic white matter, dorsal brainstem, cerebral peduncles, Posterior limb of internal capsule, thalami and globi pallidi), superimposed vasogenic edema of unmyelinated White Matter | ↑ Branched chain aminoacids in plasma  
↑ Alpha ketoacids in urine  
↑ Alloisoleucine in plasma is diagnostic  
↑ Ketones in urine  
Metabolic Acidosis |
| Glutaric aciduria type 1       | • Conventional MRI: enlarged fronto-temporal CSF spaces, wide Sylvian fissures          | ↑ Glutaric acid, 3-OH guiaric acid and glutaconic acid in urine  
↓ glutaryl-CoA dehydrogenase enzyme activity in cultured fibroblasts or leukocytes |
| Nonketotic hyperglycinemia     | • Conventional MRI: T2-hyper lesions of the myelinated white matter tracts, Corpus callousm agenesis, vermian hypoplasia  
• Magnetic resonance spectroscopy: glycine peak | ↑ glycine in plasma and CSF |
| Methylmalonic Acidemia         | • Conventional MRI: Diffuse swelling, volume loss, delay in myelin maturation, calcification of the basal ganglia, and focal necrosis of the globi pallidi | ↑ ammonia  
↑ Glycine on plasma Amino acid  
↑ Ethylmalonic acid, methylcitrate, propionic acid, 3-hydroxy isovaleric acid, propionylglycine, tiglylglycine and 3-hydroxy propionate on urine organic acid |
| L-2-Hydroxyglutaric (L-2-HG) aciduria | • Conventional MRI: nonspecific subcortical white matter loss, cerebellar atrophy, changes in dentate nuclei and putamen | ↑ L-2-HG in body fluids |
| Hyperphenylalaninemia          | • MRI T2/FLAIR: high signal in affected areas occurring firstly in the periventricular/parieto-occipital white matter before eventually affecting the subcortical white matter, atrophy and compensatory ventricular dilatation may occur eventually  
• Diffusion Weighted Imaging- MRI: reduced diffusion in acutely affected areas  
• Magnetic resonance spectroscopy: phenylalanine peak | ↑ Phenylalanine levels on plasma amino acid analysis  
↑ Phenylalanine to Tyrosine ratio in Plasma |
| X-linked leuokdystrophy        | • Conventional MRI: Symmetric peritrigonal abnormality with involvement of the splenium of the corpus callousm, abnormal peripheral enhancement | ↑ very long chain fatty acids by gas chromatography/ mass-spectrometry |
News from Chemical Pathology: Section of Chemical Pathology conducted a three, half days course on ‘Fundamentals of Quality Control to improve patient safety’ on 4-6th December 2019. Shown above is group picture of participants, facilitators and organizers of the course.

Dr. Natasha Ali presented poster on “Outcome of aplastic anemia using GCSF primed blood and bone marrow stem cells” at Transplantation & Cellular Therapy meeting held in Florida (February 2020)
Polaroid

Dr Saira Fatima and Muhammad Usman Tariq from section of Histopathology presented their posters at “The 59th IAP Thailand Annual Meeting”

Group of doctors and technical staff from Middle East region has been trained to perform Line probe assay (LiPA) for the diagnosis of tuberculosis by microbiology section
Inauguration of the remodeled lab (histopathology section) by the President Mr. Feroz Rasul

Haematology Faculty of AKU attended 22nd Annual meeting of Pakistan Society of Haematology, held in Lahore (February 2020)
Learning through Assessment: 1st FCPS part II Histopathology Mock Examination, Conducted on November 6-7, 2019.
Dr Sheema receiving token of appreciation by the Dean, Adil Haider, Interim CEO Shagufta Hassan and Departmental Chair Dr Afia Zafar

Party in the honor of Professor Sheema Hasan - retired on 21st of November 2019
Detection of Novel Coronavirus SARS-CoV for COVID 19 - by Real Time PCR

Introduction

Coronaviruses, named for the crown-like spikes on their surface. The SARS-CoV-2 has now been identified as the causative agent of COVID 19 disease. Around the world, most common mild to moderate respiratory coronavirus infections in humans are by 229E, NL63, OC43, and HKU1. Two zoonotic coronaviruses, SARS-CoV (2002-2003) and MERS-CoV (since 2015), are known to cause severe illness in humans. A recent example of such emergence from Wuhan, China is the novel coronavirus, SARS-CoV-2. Person-to-person spread of SARS-CoV-2 is via respiratory droplets produced when an infected person coughs or sneezes. The incubation period is 2-14 days. Symptoms include fever, cough and shortness of breath; suspicion increases when patient has history of travel to affected areas (especially China, Iran, Iraq and other countries which have reported COVID 19) in the last 2 weeks. All such patients who need hospitalization for management, should be tested at the earliest and contact isolation precautions instituted till SARS-CoV-2 infection has been ruled out.

Reliable diagnosis of virus can only be done using a targeted PCR based test. Serology or blood based antibody testing is an indirect measure of infection/exposure and cannot be used for confirmatory diagnosis of SARS-CoV-2.

Principle of the Assay:

Real-time (RT-PCR) assays for the in vitro qualitative detection of Novel Coronavirus-2019 (nCoV-2019) is used. Results interpretation is based on detection of fluorescent signals.

Specimen Collection:

- Upper respiratory specimens including nasopharyngeal or oropharyngeal swabs in a special tube containing Universal Transport Medium (available from the clinical laboratory)
- Lower respiratory specimens including Broncho-alveolar Lavage (BAL), tracheal aspirates and sputum in sterile container.

Reporting Schedule:

SARS-CoV-2 virus RT-PCR is performed and reported daily if specimen is received before cut off 11:00 am