NEWSLETTER OF THE DEPARTMENTS OF PATHOLOGY & LABORATORY MEDICINE AND RADIOLOGY

LABRAD

DECEMBER 2023

VOL 48, ISSUE 2

آغا خان یونیورسٹی ہسپتال، کراچی

The Aga Khan University Hospital, Karachi

CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS

LABRAD

A Publication of the Departments of Pathology & Laboratory Medicine and Radiology

December 2023
Volume 48, Issue 2

Labrad Administration Office
Farhana Arshad
Noorshah Somani
Department of Pathology and
Laboratory Medicine
Aga Khan University Hospital
Stadium Road, P. O. Box 3500
Karachi 74800, Pakistan

Tel: 92 21 3486 1551
Fax: 92 21 3493 4294, 3493 2095

hospitals.aku.edu/Karachi/clinical-laboratories

EDITORIAL
Navigating Lab Excellence in LABRAD

In this edition of LABRAD, we embark on a journey through the ever-evolving landscape of laboratory sciences. This is a non-thematic issue, consisting of articles, polaroid section showcasing the pictures of different events or achievements of Departments of Pathology and Radiology. A new section 'Farewell Chronicles' is added and in 'Best of the Past' section a pediatric Radiologist has been interviewed.

In this issue we have included articles on strategies for laboratory resilience in a changing climate, emphasizing sustainability as a cornerstone for future-proof clinical laboratories. There are articles that delve into the future of autoimmune disease testing with insights from a pioneer in antinuclear antibody testing, shedding light on advancements and challenges and Journey into the world of transplant immunology. This issue covers diverse facets of diagnostics, from comprehensive insights into viral hemorrhagic fevers to the spotlight on the rare Bombay Blood Group and explore the crucial role of cytogenetics in multiple myeloma and unravel a deceivingly "blue" biopsy revealing a low-grade nasopharyngeal papillary adenocarcinoma.

Join us in bidding farewell to Dr. Anila Rashid, celebrating her remarkable contributions to the field. Take a nostalgic stroll with "THE BEST OF THE PAST" and capture the vibrant moments in POLAROID. LABRAD remains your compass in the intricate world of laboratory sciences. Happy reading!

Dr Hafsa Majid
Associate Editor, LABRAD

ARTICLES
Lab Resilience in a Changing Climate: Sustainable Strategies for Future-Proof Clinical Laboratories

Hafsa Majid
Clinical Chemistry

Climate change can have indirect effects on laboratory medicine, particularly through its impact on public health, ecosystems, and the global distribution of diseases. Some ways in which climate change may influence laboratory medicine are:

1. **Changing Disease Patterns:** Climate change can alter the distribution and prevalence of infectious diseases. Shifts in temperature, precipitation, and humidity can affect the habitat and behaviour of disease vectors (e.g., mosquitoes carrying diseases like malaria or dengue). This change in disease patterns may influence the types of diagnostic tests needed and the frequency of testing.
2. **Emerging Diseases:** As climate change contributes to environmental changes, it may facilitate the emergence of new diseases or the reemergence of existing ones. Laboratories may need to adapt by developing new diagnostic tests or modifying existing ones to detect emerging pathogens. Also changes in climate can influence the geographic distribution of disease vectors, leading to the spread of vector-borne diseases. Laboratories in new or expanded regions may need to implement testing for diseases not previously prevalent in those areas.
3. **Increased Incidence of Heat-Related Illnesses:** Rising global temperatures can lead to an

increase in heat-related illnesses. Laboratories may see an increased demand for tests related to heat stroke, dehydration, and other conditions associated with extreme heat events.

4. **Impact on Laboratory Infrastructure:** Extreme weather events, such as floods, can damage laboratory infrastructure and disrupt the supply chain for reagents and equipment. This can affect the availability of laboratory tests.

5. **Food & Water Safety and Air Quality:** Climate change can affect food and water safety. Altered precipitation patterns, temperature changes, and extreme weather events can impact the quality of water supplies and food sources. It can also contribute to shifts in air quality, including increased levels of pollutants and allergens. Laboratories may need to adapt testing protocols for food & water testing, develop tests to monitor air quality and conducting tests related to respiratory health.

While the direct impact of climate change on laboratory medicine may not be immediate, the indirect effects through changes in disease patterns, emergence of new diseases, and alterations in environmental conditions can influence the types and frequency of diagnostic tests needed. Laboratories need to adapt and innovate to address these evolving public health challenges associated with climate change. Adapting clinical laboratory practices to cope with climate change challenges involves a combination of strategic planning, infrastructure improvements, and the integration of sustainable practices.

1. **Resilience Planning:** Conduct a risk assessment to identify potential climate change-related threats to laboratory operations, such as extreme weather events, changes in disease patterns, and disruptions to the supply chain. Develop and implement a climate resilience plan covering infrastructure, personnel, and resources, that includes measures to mitigate identified risks.

2. **Infrastructure and Equipment:** Ensure that laboratory facilities are built or retrofitted to withstand extreme weather events, have backup power systems and equipment to ensure continuous operation during power outages. To avoid supply chain issues, diversify suppliers and establish contingency plans for supply chain disruptions, considering the potential impact of climate-related events on the production and transportation of laboratory reagents and equipment. Explore local sourcing options to reduce the environmental footprint associated with transportation.

3. **Remote Monitoring and Telework:** Implement remote monitoring technologies to oversee laboratory conditions and equipment status, allowing for real-time assessment and response to potential issues. Develop and implement telework policies for laboratory personnel when feasible, reducing the need for physical presence during extreme weather events.

4. **Energy Efficiency and Sustainable Practices:** Implement energy-efficient practices in laboratory operations, such as optimizing equipment usage, upgrading to energy-efficient appliances, use of renewable energy sources and adopting sustainable laboratory design principles.

5. **Disease Surveillance and Testing:** Enhance disease surveillance capabilities to monitor changes in disease patterns and emerging health threats associated with climate change. Develop flexible testing protocols that can quickly adapt to shifts in disease prevalence or the emergence of new pathogens. Educate staff on the potential health impacts of climate change and how laboratory practices contribute to broader public health resilience.

6. **Research and Innovation:** Support research initiatives focused on understanding the health impacts of climate change and developing innovative diagnostic tools and treatments to address emerging health challenges. Foster collaboration with research institutions and public health agencies to stay informed about evolving climate-related health risks.

By integrating these strategies into clinical laboratory practices, healthcare organizations can enhance their resilience to climate change challenges, ensuring the continuity of essential diagnostic services and contributing to overall public health preparedness.

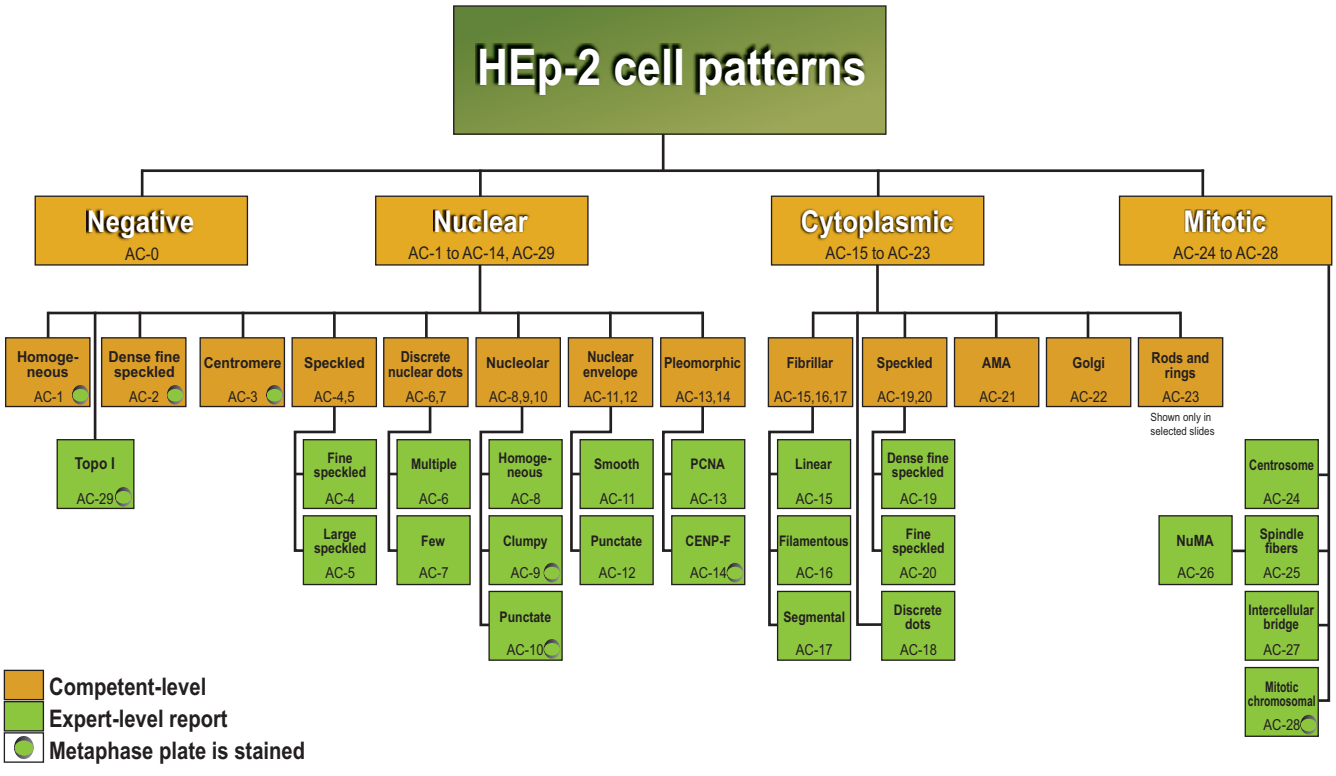
Professional Insights from a Pioneer in Autoimmune Disease Testing: The Future of Antinuclear/Anticellular Antibody Testing

Sammar Naz and Dr Qurratulain Chundriger
Histopathology

Commonly referred to as an antinuclear antibody test (ANA), Anti-Cellular Antibody test is based on the Indirect immunofluorescence Assay (IFA) which utilizes HEp-2 cells embedded on a glass slide. However, the identifiable and reported microscopy patterns are not limited to the traditional nuclear targets but rather include the numerous and clinically relevant cellular patterns including patterns observed at different stages of the cell cycle. After a patient's sample is incubated with the cells, the presence of antibodies that target cellular components are identified by automated pattern-recognition microscopy, which is verified by direct observation of the IFA images. Antibody titers are determined by a digital algorithm that has been validated in several published studies.

Anti-cellular antibodies are associated with a spectrum of autoimmune diseases including systemic lupus erythematous (SLE), mixed connective tissue disease, Sjögren's syndrome, scleroderma, inflammatory and necrotising myopathies, neurologic SLE, juvenile arthritis, autoimmune liver diseases, drug-induced autoimmunity, and other conditions.

The ANA test identifies autoantibodies that target substances contained inside cells. Although the name implies that the test detects only autoantibodies directed against components of the nucleus, the test can also be used to detect antibodies directed against cellular components that are contained within the cell cytoplasm, outside of the nucleus.



*Classification tree updated September 2021

Helios Automated IFA System (Ana Fully Autoamted Analyzer)

HELIOS is a revolutionary system that automates the complete pipetting and image capturing of IFA (immunofluorescence) tests.

It is the first and only system to automate this process completely from beginning to end - offering unparalleled process control, traceability, standardization, ease of use, and maximum efficiency by requiring technical intervention only for expert review.

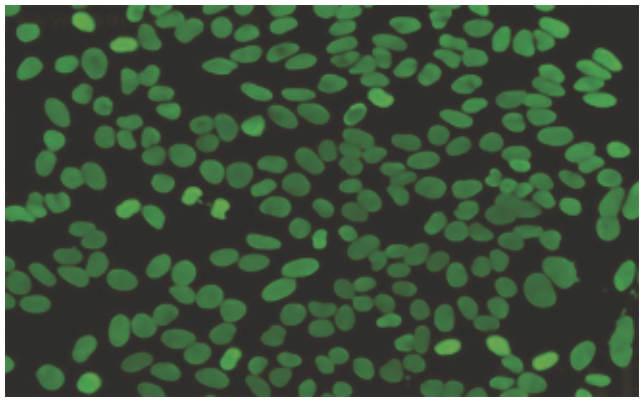
IFA processing, image capturing and result confirmation

The HELIOS processor is capable of performing all IFA processing and reading automatically.

Once the processing is complete and the slides are ready, the microscope fitted within the HELIOS system focuses on 3 different areas on each slide and captures the images for review and verification. The software then runs the images through a default library of images to match the pattern of positivity. These patterns are what helps the clinicians either in reaching a diagnosis or getting additional testing for disease identification. A detailed classification with high magnification images is available on the website which is free to access for everyone and serves as an essential tool for assisting pathologists as well as clinicians in identifying and understanding a sample result (website given under references).

Most commonly encountered patterns and their clinical significance

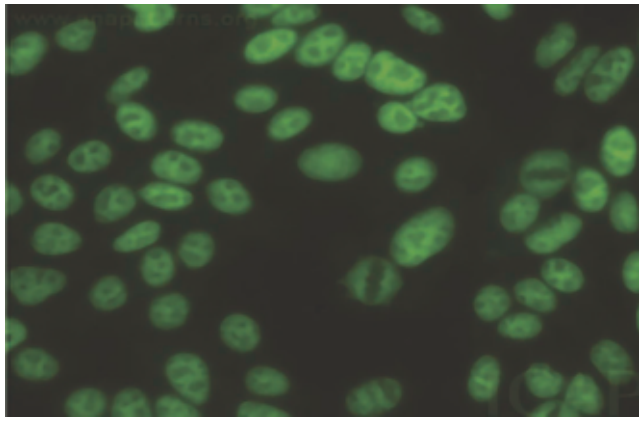
Homogeneous (Diffuse nuclear staining) AC-1.



Defined as "Homogeneous and regular fluorescence across all nucleoplasm. The nucleoli maybe stained or not stained depending on cell substrate. Mitotic cells (metaphase, anaphase, and telophase) have the chromatin mass intensely stained in a homogeneous hyaline fashion."

This pattern is mostly associated with Systemic Lupus Erythematosus (SLE). Confirmation requires testing for anti dsDNA. It is also the most prevalent pattern of positivity seen in chronic autoimmune hepatitis. Such cases do not require further testing if symptoms are already suggestive of the disease.

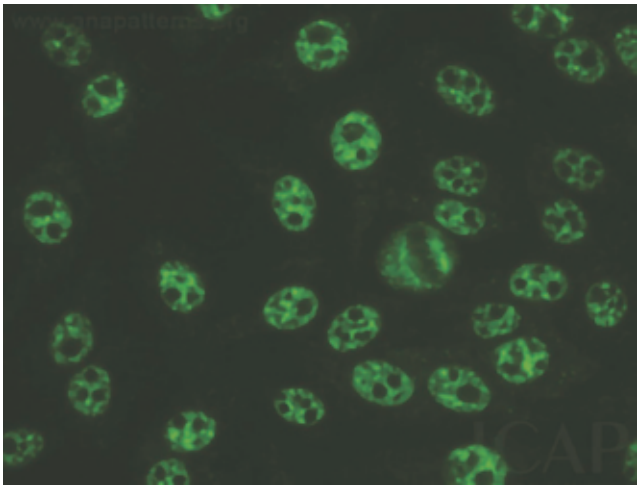
Nuclear fine speckled AC-4



Defined as "Fine tiny speckles across all nucleoplasm. The nucleoli may be stained or not stained. Mitotic cells (metaphase, anaphase, and telophase) have the chromatin mass not stained."

This pattern can be positive to variable degrees in systemic autoimmune rheumatic diseases, including Sjogren syndrome, different clinical forms of Lupus, diabetes mellitus, congenital heart block and systemic sclerosis. In diabetic patients this can give a false positive results. The target antigens include Mi-2 and TIF, the latter is strongly associated with malignancy in old patients.

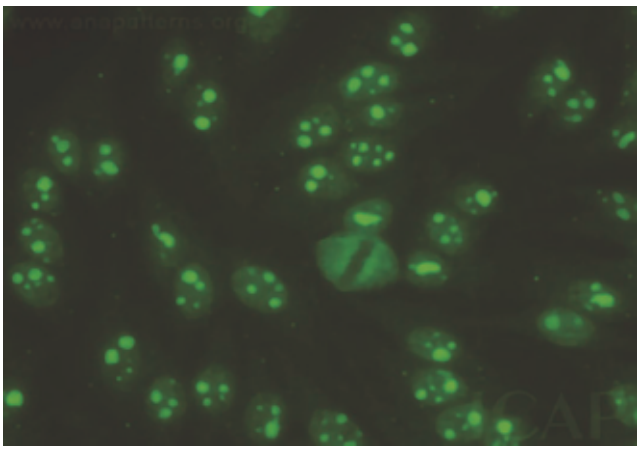
Nuclear large/coarse speckled AC-5



Defined as "Coarse speckles across all nucleoplasm. The nucleoli may be stained or not stained. Mitotic cells (metaphase, anaphase, and telophase) have the chromatin mass not stained."

Coarse speckled positivity is seen in patients of mixed and undifferentiated connective tissue disorders in addition to different clinical forms of lupus and systemic sclerosis. False positive results can be seen in non-diseased individuals in low titers. Therefore, clinical input is essential in all such cases. Patients without symptoms and/or negative ENA results may be spared the immunomodulation therapies.

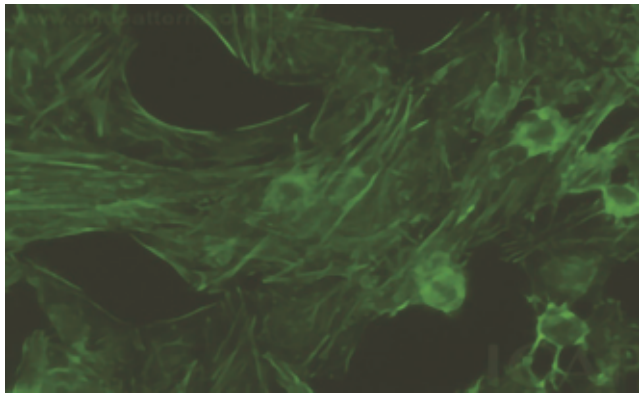
Homogeneous nucleolar AC-8



Defined as "Diffuse fluorescence of the entire nucleolus, while the metaphase plate shows no staining."

Commonly seen in patients of systemic sclerosis and less so in other systemic autoimmune rheumatic diseases.

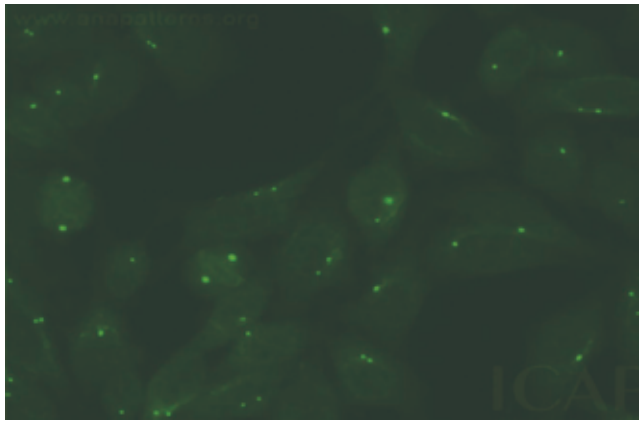
Cytoplasmic fibrillar/Linear (actin-like) AC-15



This pattern is characterized by decorated cytoskeletal fibers, sometimes with small, discontinuous granular deposits. Typical staining show striated actin cables spanning the long axis of the cells.

Positivity is seen in Autoimmune hepatitis type 1, chronic Hepatitis C infection and celiac disease (IgA isotype). If AIH type one is clinically suspected, it is recommended to confirm reactivity with smooth muscle antibodies (IgG isotype), typically detected by IIFA on rodent tissue (liver, stomach, kidney); anti-smooth muscle antibodies are included in the international criteria for AIH type one. F-actin is the main target antigen and more clinically significant than G-actin.

Centrosome (pole positivity) AC-24



This pattern identifies and stains Distinct centrioles (1-2/cell) in cytoplasm and at the poles of mitotic spindle.

Within the spectrum of the SARD, the AC-24 pattern is found in patients with Raynaud's phenomenon, localized scleroderma, SSc, SLE and RA, either alone or in combination with other SSc-associated antibodies. This pattern has low positive predictive value for any disease.

Insights into CCHF and Other Viral Hemorrhagic Fevers.

Drs Sobia Khan and Mohammad Zeeshan
Microbiology

What is CCHF?
Crimean Congo hemorrhagic fever (CCHF) is one of the severe forms of hemorrhagic fever endemic in Africa, Asia, Eastern Europe and the Middle East. This zoonotic viral disease is caused by tick-borne virus Nairovirus (family Bunyaviridae) which is transmitted by Hyalomma tick.

Mode of transmission:
The ticks primarily infest livestock animals; however, human infections can occur through contact with the blood, bodily fluids, and tissues of infected animals or through the bite of an infected tick. Moreover, human to human transmission via percutaneous or per mucosal exposure to blood and body fluids containing the virus is frequently seen, which has important public health implications.

Clinical presentations:
The disease is generally asymptomatic in infected animals but highly fatal in humans with a case fatality rate of 10–40 percent (1,2).

The onset of CCHF is sudden, with initial signs and symptoms including headache, high fever, back pain, joint pain, stomach pain, and vomiting. Red eyes, a flushed face, a red throat, and petechiae (red spots) on the palate are common. If disease progresses, large areas of severe bruising, severe nosebleeds, and uncontrolled bleeding at injection sites can be seen, beginning on about the fourth day of illness and lasting for about two weeks.

CCHF and Pakistan:
Sporadic CCHF cases observe through the year in Pakistan, however, numbers increase during Eid Zuha session due to high exposure with the cattle. From January 2014 to May 2020, up to 356 verified CCHF cases have been reported by the National Institute of Health, Islamabad, Pakistan. The majority of these patients were reported from Baluchistan (38%), followed by Punjab (23 percent), Khyber Pakhtunkhwa (19 percent), Sindh (14 percent) and Islamabad (6 percent). Seroprevalence of CCHF in Pakistan is around 2.7 percent, with rural inhabitants having a higher prevalence.

Viral hemorrhagic fever (VHF):
Although VHFs are caused by several families of viruses, these viruses share some common characteristics:

- They are RNA viruses and cause of emerging diseases because RNA changes over time at a high rate.
- They are enveloped in a lipoprotein outer layer, easier to destroy with physical (heat, sunlight, gamma rays) and chemical (bleach, detergents, solvents) methods.
- Naturally exist in animal or insect populations, referred to as host populations, and are generally restricted to the geographical areas where the host species live.
- They spread to people when a person encounters an infected animal or insect host. After the initial spread into the human population, some VHF viruses can continue to spread from person-to-person.

VHF and VHF Diseases:

Table 1: List of VHF agents and VHF diseases.

VHF	VHF Diseases
Arenavirus (order Bunyavirales)	• Chapare Hemorrhagic Fever (CHHF) • Lassa Fever • Lujo Hemorrhagic Fever (LUHF)

Flavivirus	• Alkhurma Hemorrhagic Fever (AHF) • Kyasanur Forest Disease (KFD) • Omsk Hemorrhagic Fever (OHF) • Severe Dengue • Yellow Fever
Filovirus	• Ebola Disease • Marburg virus disease (MVD)

Hantavirus (order Bunyavirales)	• Hantavirus
	  ○ Hantavirus Pulmonary Syndrome (HPS)
	  ○ Hemorrhagic Fever with Renal Syndrome (HFRS)

Nairovirus (order Bunyavirales)	Crimean-Congo Hemorrhagic Fever (CCHF)
Phenuivirus (order Bunyavirales)	Rift Valley Fever (RVF)

Approach to a patient with viral hemorrhagic fever:
Viral hemorrhagic fever (VHF) is a term used to describe severe systemic febrile illnesses caused by viruses of arenavirus, filovirus, bunyavirus and flavivirus families. These viruses are geographically restricted to the areas of their host species. Numerous studies have looked at common laboratory parameters to distinguish VHF from other diseases. These laboratory markers are not highly specific but may offer guidance for identifying patients with VHF where direct diagnostic techniques for VHF are not readily available (5). Findings from these studies are summarized below:

Table 2: General laboratory tests for suspected VHF

Viruses	Laboratory parameters
Hantaan virus and Seoul virus	Elevated blood urea nitrogen and creatinine
Huaiyangshan hemorrhagic fever virus	Leukopenia Elevated creatine kinase Elevated alanine aminotransferase (ALT)
Dengue hemorrhagic fever	Thrombocytopenia (<100 × 109/L) Prolonged APTT Normal prothrombin time Elevated AST and ALT
CCHFV, Sudan virus disease, Ebola Virus disease	Leukopenia Thrombocytopenia Elevated ALT and AST

Risk assessment of patients:
Patients could be divided into two groups:

1. At risk with No high-risk exposure: (low risk exposure category i.e., they have not had any direct exposure to blood or body fluids from a known VHF case, or other high-risk exposure).

2. High risk exposure (i.e., they have had direct exposure to blood or body fluids from a known VHF case, or other high-risk exposure). Such patients are considered to have a significant risk of having VHF, though the majority will not have VHF.

Following risk assessment, the below laboratory investigations should be carried out for VHF screen.

Recommended Laboratory Tests

- A thin blood smear (EDTA blood specimen) to look for malaria parasites on at least two occasions
  - Thick films should not be prepared
- Two sets of blood cultures, using routine blood culture bottles, from separate vein puncture sites taken at least 30 minutes apart
  - 20 to 30ml per set (5-10ml volumes are appropriate for children)
- White blood cell and differential count and either haemoglobin or haematocrit
- Renal profile (urea & electrolytes)
- Urine culture, if urinalysis results suggest an infection
- Glucose measurements
- Liver functions tests
- Prothrombin time (PT) and Activated Partial Thromboplastin Time (APTT)

"No high-risk exposure" category, after VHF screen is negative, should be maintained until an alternative diagnosis is confirmed.

"High risk exposure" category, VHF specific diagnostic tests should be carried out concurrently with the VHF screen tests listed above.

Diagnostic techniques:

1. **Nucleic Acid Detection:**

- **Reverse transcription PCR (RT-PCR)** is widely used for molecular diagnosis of most VHF-associated viruses. Multiplex assays for simultaneous detection of more than one causative viruses of VHF were first described in 2002, with limit of detection as low as 1 × 10−2 to 1 × 105 copies/mL .
- **Microarrays:** specialized type of multiplex PCR that uses a solid matrix spotted with oligonucleotides that represent specific genes of different organisms. An unknown fluorescently labeled DNA anneals and releases a positive signal that is compared with the intensity of known positive signals. FilmArray (Biofire, Salt Lake City, Utah) has developed a qualitative biodefense panel for detecting EBOV and MARV, however it has some limitations including a short shelf life of test pouches.
- **Loop-mediated isothermal amplification (LAMP):** This method amplifies nucleic acids using 2 nested primers and omits the need for expensive thermocyclers as the reaction takes place at a single temperature (65°C). The product can be measured in real time by turbidometry or through an intercalating dye. LAMP assays have been developed for detection of severe fever and thrombocytopenia syndrome virus and is a promising technology for field diagnostics with a rapid turnaround time.

2. **Immunochromatography:** antigen detection by immunochromatography requires little training, minimal amounts of patient samples, and does not require electricity. An immunofiltration test is available for EBOV and can detect viral proteins from urine within 30 minutes. A rapid diagnostic kit for DENV that can detect both the NS1 antigen and antibodies to DENV by immunochromatography is commercially available. These modalities have played an important role in field diagnosis and outbreak investigations in endemic regions.

3. **Serological diagnosis:** Serology is not useful in diagnosing acute VHF illness, as the presence of IgM antibodies can represent different stages of disease or a prior symptomatic or asymptomatic infection. Furthermore, it lacks specificity due to cross-reacting antibodies.

4. **Viral culture:** No longer used owing to prolonged cultivation time as well as expertise of laboratory personnel. Moreover, for most hemorrhagic viruses a highly advanced BSL three or four lab is required.

Management:

1. **Infection control precautions in institutes:**

a) Health care worker training about CCHF transmission, exposure prevention, and use of personal protective equipment.
b) Contact precautions include appropriate personal protective equipment (an impervious gown, gloves, mask, and eye/face protection)
c) Respiratory protection (N95 mask or FFP3 respirator) is required during aerosol-generating procedures.
d) Shoe covers are warranted when there is significant environmental contamination.
e) Precautions may be discontinued for patients with no signs and symptoms of disease for at least three days, with a platelet count >50,000/mm3 and normal coagulation tests. If possible, a

negative blood polymerase chain reaction for viral hemorrhagic fever should also be documented

2. Postexposure management:

a) The exposed individual should undergo a two-week period of monitoring for symptoms or signs of CCHF, including daily temperature measurement and weekly assessment of complete blood count measurement; no quarantine is required.

b) In case of a temperature above 38 °C or other clinical symptoms of VHF (diarrhoea, etc.), hospitalize immediately in strict isolation.

c) **Post-exposure prophylaxis with ribavirin** (35 mg/kg loading dose followed by 15 mg/kg (maximum 1 g) every eight hours for 10 days) should be considered for high-risk exposures to Lassa fever or CCHF.
High risk exposure being defined as one of the following i.e., (1) Penetration of skin by a contaminated sharp instrument (for example, needle stick injury); (2) Exposure of mucous membranes or broken skin to blood or bodily secretions (for example, blood splashing in the eyes or mouth); (3) Participation in emergency procedures without appropriate personal protective equipment (4) Prolonged (hours) and continuous contact in an enclosed space without appropriate personal protective equipment.

3. Environmental cleaning:

a) CCHF virus (CCHFV) can be inactivated by disinfectants including one percent sodium hypochlorite (household bleach), 70 percent alcohol, two percent glutaraldehyde, hydrogen peroxide, and peracetic acid.

b) The virus is susceptible to high temperature at 56°C (133°F) for 30 minutes or 60°C (140°F) for 15 minutes.

c) Housekeeping staff should use personal protective equipment when cleaning.

4. Prevention:

a) Avoiding tick exposure and contact with animal bodily fluids.

b) Wearing light-colored clothing and tucking shirts into pants and pants into socks is helpful to minimize exposure.

c) The use of a 20 to 30 percent N,N-diethyl-m-toluamide (DEET) repellant for skin and permethrin-treated clothes also minimizes tick bites.

d) Acaricides are effective against ticks in livestock and should be applied to animals prior to entering slaughterhouses.

e) Surveillance in animals: one health approach is of utmost importance to combat CCHF. A review reported that only 12 of 25 countries reporting CCHF cases in humans since 2006, reported concomitant cases in animals, pointing out the lack of surveillance capacity in animal populations.

f) Vaccination:

Bombay Blood Group

Drs Shayan Ashfaq and Muhammad Hasan
Hematology and Transfusion Medicine

In Bombay, India in 1952, an individual was discovered to have an interesting blood type that reacted to other blood types in a way that had not been seen before. Serum from this individual contained antibodies that reacted with all RBCs from normal ABO phenotypes (i.e., groups O, A, B, and AB) and the RBCs appeared to lack all of the ABO blood group antigens.

The Hh blood group contains one antigen, the H antigen, which is found on virtually all RBCs and is the building block for the production of the antigens within the ABO blood group.

The H antigen is produced by a specific fucosy ltransferase. Depending upon a person's ABO blood type, the H antigen is converted into either the A antigen, B antigen, or both. If a person has blood group O, the H antigen remains unmodified. Therefore, the H antigen is present in the highest amounts in blood type O and in the least amounts in blood type AB.

In India, where H deficiency was first discovered, the frequency of both phenotypes combined is one in 10,000. H deficiency is slightly more common in Taiwan, affecting one of 8,000 people.

Antibodies produced against the H antigen
Anti-H type IgM is more common than IgG
Anti-H is naturally occurring in people with H antigen deficiency.
Anti-H reactivity Capable of hemolysis
Anti-H can activate the complement cascade which lyses RBCs while they are still in the circulation (intravascular hemolysis).
Transfusion reaction Yes—can cause an acute hemolytic transfusion reaction
Hemolytic disease of the newborn Possible
HDN could arise in mothers with the Bombay phenotype (Oh, h/h)

If patients with anti-H in their circulation receive transfusions of blood that contains the H antigen (e.g., blood group O), they are at risk of suffering an acute hemolytic transfusion reaction.

The maternal production of anti-H during pregnancy could cause hemolytic disease in a fetus who did not inherit the mother's Bombay phenotype.

The H blood group locus (containing FUT1) and the secretor locus (containing FUT2) are located on chromosome 19 at q.13.3. FUT1 and FUT2 are tightly linked, being only 35 kb apart. Because they are highly homologous, they are likely to have been the result of a gene duplication of a common gene ancestor. Likewise, a number of mutations have been reported to underlie the para-Bombay phenotype.

As this blood group is rare, we can search for Bombay blood group by following these steps like Get all the family members and relatives of the patients tested for the blood group. It's very likely that one or the other relative has this group, put up a request for the requirement in the leading newspapers, be open to get blood from other cities and the most effective way is visiting all blood banks of the city as well as neighboring big cities.

Role and Application of Cytogenetics in Multiple Myeloma

Muneba Sharif, Nazneen Islam and Dr Zeeshan Ansar
Molecular Pathology

Introduction
Multiple Myeloma (MM), a malignancy arising from plasma cells, is the second most common haematological cancer worldwide . Despite significant progress in its diagnosis and treatment, MM remains a complex and heterogeneous disease with variable clinical outcomes. Cytogenetics, a branch of genetics focusing on the study of chromosome structure and function, has emerged as a vital tool in understanding the underlying genetic alterations that drive MM pathogenesis. By providing crucial insights into the genetic makeup of tumor cells, cytogenetics has revolutionized the way we approach the diagnosis, prognosis, and treatment of this challenging disease.

Role of Cytogenetics in Diagnosis
The diagnosis of MM has undergone remarkable transformation due to advances in cytogenetic techniques. Traditionally, MM was classified based on morphological characteristics and the presence of monoclonal proteins. However, cytogenetic studies have demonstrated that MM is a genetically heterogeneous disease, with various chromosomal abnormalities contributing to disease development and progression. These genetic changes can be detected using methods such as conventional karyotyping, fluorescence in situ hybridization [FISH], and more recently, high-throughput technologies like array-based comparative genomic hybridization [aCGH] and next-generation sequencing [NGS]. By identifying specific chromosomal aberrations, cytogenetics helps refine the diagnostic criteria, leading to improved accuracy and tailored therapeutic approaches. Karyotyping and FISH have been pivotal techniques in the clinical diagnosis of MM for decades. Research, including our own findings, indicates that FISH exhibits greater sensitivity than Karyotyping in identifying genetic abnormalities associated with MM. This heightened sensitivity can be attributed, at least in part, to FISH's ability to detect subtle chromosome changes, such as microdeletions, which might not be easily discerned

through Karyotyping alone. Notably, the application of plasma cell enrichment in FISH analysis has demonstrated even higher rates of detection for MM-related genetic anomalies compared to direct FISH. Consequently, FISH offers distinct advantages over Karyotyping in the diagnosis of MM. Nevertheless, FISH's diagnostic efficacy remains constrained by its reliance on probes targeting known gene mutations, limiting its ability to identify all genetic variations detectable via Karyotyping. As such, both these approaches possess unique strengths and can synergistically complement each other.

Test	Features
Karyotype	**Hypodiploidy/Hyper diploidy**
FISH	**t(4;14)** **t(11;14)** **t(14;16)** **Del(17/17p)** **t(4;20)** **Gain(1q)**

Table: 1
Prognostic Implications of Cytogenetics

The prognostic implications of cytogenetics in MM play a crucial role in understanding the disease's behaviour and tailoring treatment approaches. Cytogenetic abnormalities are genetic changes in the chromosomes of MM cells that can provide valuable insights into disease prognosis and guide therapeutic decisions. MM is a complex and heterogeneous plasma cell neoplasm that arises from the bone marrow. Cytogenetic abnormalities are common in MM and have been extensively studied for their impact on disease progression, treatment response, and overall survival. These genetic alterations can encompass various structural changes, such as translocations, deletions, and amplifications, affecting key genes and pathways involved in cell growth, survival, and immune response. One of the most well-known cytogenetic abnormalities in MM is the t (4; 14) translocation, which leads to the dysregulation of the fibroblast growth factor receptor three (FGFR3) and multiple myeloma SET domain [MMSET] genes. This alteration has been associated with poorer prognosis and resistance to certain treatments, making it a significant marker for risk stratification. Conversely, the t (11; 14) translocation involving cyclin D1 [CCND1] and immunoglobulin heavy chain (IGH) enhancer regions is generally linked to a more favourable outcome and better response to therapies. Other cytogenetic abnormalities, such as del (17p) involving the tumor suppressor gene TP53, are associated with high-risk MM. Patients with del (17p) often exhibit aggressive disease behaviour, resistance to treatment, and shorter survival. Detection of these high-risk markers through cytogenetic analysis enables clinicians to consider alternative therapeutic strategies, including novel targeted agents and combination therapies. Advancements in technology, such as FISH and NGS, have improved the ability to detect cytogenetic abnormalities with higher precision and sensitivity. This has led to a deeper understanding of the genetic landscape of MM and the identification of new prognostic markers. For instance, the presence of specific chromosomal abnormalities, like gain (1q) and del (1p), has been associated with adverse outcomes and therapeutic challenges. In recent years, the integration of cytogenetic information into risk stratification models, alongside clinical and biochemical parameters, has enhanced treatment decision-making. Tailoring therapies based on a patient's cytogenetic profile can optimize outcomes by selecting the most appropriate treatment regimen. Additionally, ongoing research aims to decipher the intricate interactions between genetic alterations and the bone marrow microenvironment, shedding light on the mechanisms driving disease progression and treatment resistance. The prognostic implications of cytogenetics in MM have greatly deepened our understanding of the disease's heterogeneity and its impact on patient outcomes. Identifying specific cytogenetic abnormalities enables clinicians to personalize treatment strategies, offering patients the best chance of a favourable response and prolonged survival. As technology continues to evolve, the integration of genetic information into clinical practice will undoubtedly shape the future of MM management.

Conclusion:
Disease biology in MM is best reflected based on the molecular subtype of the disease and the presence or absence of specific cytogenetic abnormalities.30,31 For example, abnormalities such as t(4;14), t(14;16), t(14;20), gain (1q), del(1p), and del(17p) influence disease course, response to therapy, and prognosis in MM

Low-grade Nasopharyngeal Papillary Adenocarcinoma in a Deceivingly "blue" Biopsy

Dr Qurratulain Chundriger
Histopathology

Nasopharyngeal carcinomas, albeit rare, are mostly of non-keratinizing Squamous cell type, arising in the younger population. Adenocarcinomas arising in this location are extremely rare and less recognized by both the Clinicians as well as Pathologists. This articles touches upon one such case that I came across a few months back.

Case Presentation: A fragmented biopsy from nasopharyngeal mass of a 25-year-old male patient was received, measuring 1.4 x 0.6 cm in aggregates. After processing, the H&E-stained microscopic slide showed predominantly respiratory mucosa covered lymphoid tissue fragments, with marked "blueness" on low power (figure A), showing dense lymphoid sheets with prominent secondary lymphoid follicles showing germinal centers. Scattered among these fragments, were two tiny pieces of stratified squamous epithelium lined tissue (Figure A red circles, Figure B showing low power and Figure C showing high power views), with underlying population of cuboidal to columnar cells forming interconnected cystic spaces, with admixed thickened and hyalinized blood vessels. The cystic spaces were clearly connected to and originating from the surface epithelium. These cells having moderate amount of eosinophilic cytoplasm with elongated and grooved coffee bean like nuclei (Figure D), mimicked papillary carcinoma of thyroid, to such an extent, that immunohistochemical markers for the same were requested on the tissue.

The markers included **TTF-1** (Figure E, thyroid transcription factor-1, commonly positive in tumors arising in lung and thyroid), **PAX-8** (Figure F, another transcription factor positive in thyroid and female genital tract tumors) and **Thyroglobulin** (Figure G, as the name implies, positive in thyroid origin tumors).

The lesional cells stained positively for **CKAE1/AE3** (Pan-keratin cocktail, confirming the epithelial nature/origin of these cells, not shown), **TTF-1** in the nuclei (figure E) and negatively for the other two markers mentioned above. Another set of stains was also applied including **GFAP** (Glial fibrillary acidic protein, not shown) for a possible minor salivary gland origin, and **CDX2** (transcription factor (not shown) positive in gastrointestinal tract tumors and intestinal type adenocarcinoma of nasopharyngeal origin). Both turned out to be negative. **Ki-67** Proliferative index was also quite low (Figure H, less than 10%) in these fragments.

For the "blue" fragments in the biopsy, a panel of stains (none of them shown here) including **CD20** (Pan B-cell marker), **CD3** (Pan T-cell marker), **Bcl-2** (anti-apoptotic marker, usually stains all lymphoid cells except germinal centers of secondary lymphoid follicles) and **Tdt** (Terminal deoxynucleotidyl transferase, a marker of hematogones and blasts), which showed mixed reactive staining with negative **Tdt** in all lymphoid cells. **Bcl-2** negativity paired with high proliferative index in germinal centers confirmed the reactive nature of the "blueness" caused by hyperplastic lymphoid tissue.

In this way, after excluding a possibility of secondary involvement by *Papillary thyroid carcinoma, Carcinoma of salivary gland origin* and *intestinal type adenocarcinoma of nasopharynx* (although the morphology did not support this one, it is more common than the concluded diagnosis), a final diagnosis of **Low grade Nasopharyngeal Papillary Adenocarcinoma** was made.

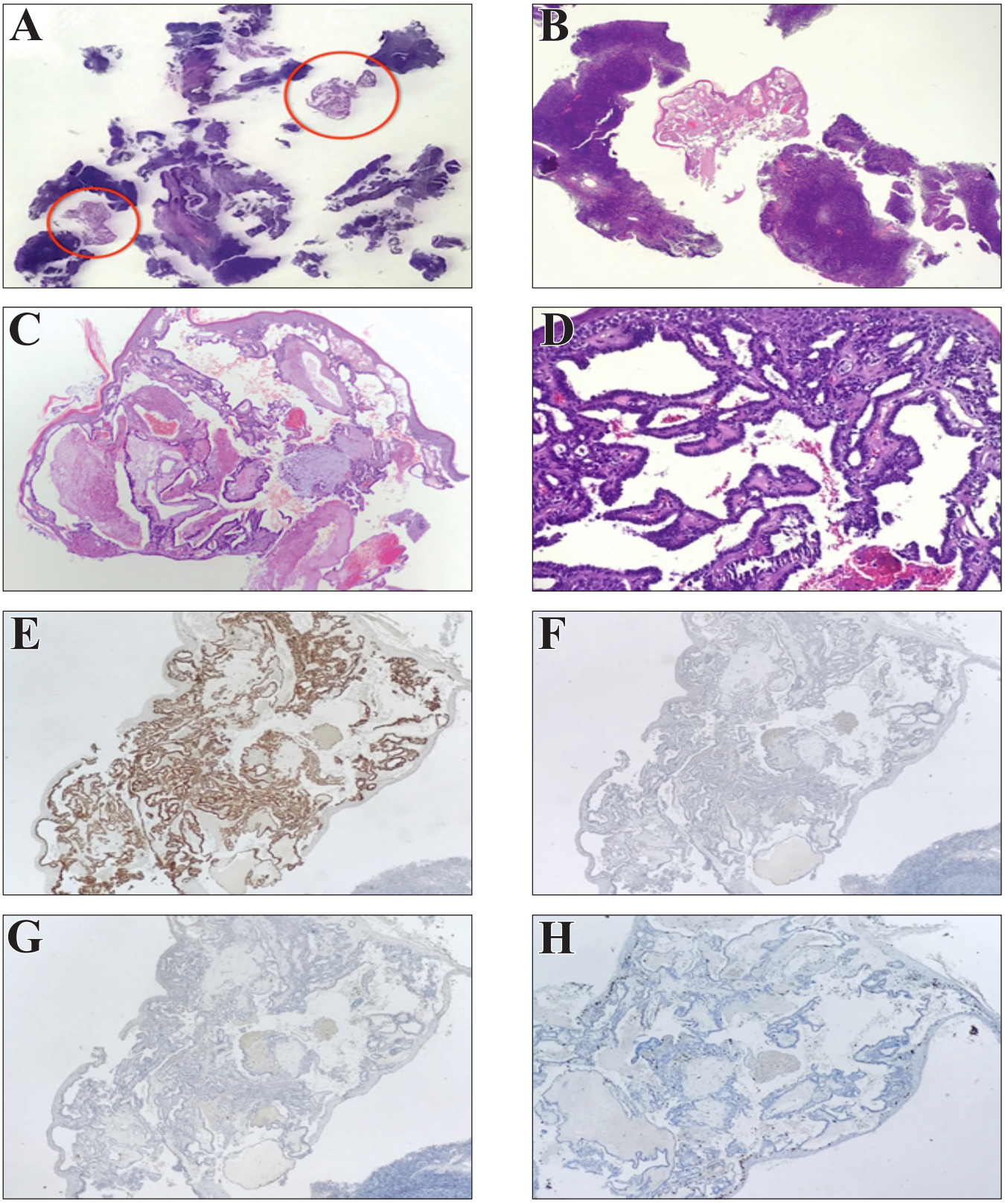
Discussion: Low-grade Nasopharyngeal Papillary Adenocarcinoma is an extremely rare, low-grade tumor of nasopharyngeal origin, that arises most commonly from the epithelium of posterior nasopharyngeal wall. Unlike the commonly encountered squamous cell carcinoma of nasopharynx, these are not associated with Ebstein Barr Virus infection as testing for Epstein-Barr virus (EBV)-encoded small RNAs (EBERs) has been found to be negative in these lesions. P16 (surrogate for Human Papilloma Virus infection) is also negative. These tumors may arise in any age group and do not show any gender predilection. Single case report by Wang et al. has described **ROS1::GOPC** fusion *(*PMID: 33546077, free to access article, *DOI: 10.1097/MD.0000000000024377).*

The **WHO blue book on head and neck tumors** describes the **essential diagnostic criteria** for this entity as **"complex papillary and glandular growth with infiltration of the submucosa; low-grade cytology"** and desirable criteria as "transition from

surface epithelium to tumour; TTF-1 positive and absence of thyroglobulin and PAX8". The features in this case fulfilled these criteria.

Complete surgical removal is the treatment of choice.

Local recurrence is rare, only when incompletely excised. No known reports of metastasis from this tumor have been described so far in the English literature.



Transplant Immunology – Diagnostics

Sabira Sharif, Drs Asghar Nasir and Zeeshan Ansar
Molecular Pathology

Transplantation the process of moving cells, tissues, or organ from one site to another for the purpose of replacing or repairing damaged or diseased organs and tissues. It saves thousands of lives each year; However, the immune system poses a significant barrier to successful organ transplantation when tissues/organs are transferred from one individual to another. Rejection is caused by the immune system identifying the transplant as foreign, triggering a response which ultimately destroy the transplanted organ or tissue. Donor and recipient are carefully matched before transplantation to minimize the risk of rejection. They are matched based on their blood group, tissue typing and how the recipient's blood serum reacts to donor cells. The better matched the donor and recipient the more successful the transplantation is.

Compatibility between donor and recipient is accessed using combination of tests including:
HLA typing
The human leukocyte antigen (HLA) system is a cluster of gene complex encoding the major histocompatibility complex (MHC) proteins known as antigens, located on the cell surface of leukocytes. Success of organ transplant is, in large part determined by compatibility of MHC genes of donor and recipient. Any cell displaying some other HLA type is "non-self" and is seen as an invader by the body's immune system, resulting in the rejection of the tissue bearing those cells. The more alike the HLA alleles of the Donor and Patient are the more likely the transplant will be successful. The HLA alleles of the Donor the Patient are determined by the isolation of Genomic DNA and amplified by primers specific HLA alleles (SSP PCR). Subsequently, amplicons are detected by gel electrophoresis and analyzed by Score software.

Panel Reactive Antibodies – Mixed
Class I and class II antibodies in our blood play a crucial role in how your immune system recognizes and reacts to foreign tissues. Class I antibodies recognize antigens on virtually all cells in the body, while class II antibodies specifically target cells involved in the immune response. Panel Reactive Antibody (PRA) Screening by Luminex, for both Class I and Class II, is a diagnostic test that detects and quantifies antibodies in a recipient's blood against a panel of potential donor antigens. By assessing the level of sensitization, the PRA screening provides crucial information for donor compatibility and the risk of immune-mediated complications in organ transplantation. This comprehensive and sensitive approach helps guide decisions regarding donor selection, cross-matching strategies, and personalized immunosuppressive regimens, ensuring optimal transplant outcomes and minimizing the risk of antibody-mediated rejection. Single Bead Antigens - Class I and Class II
The solid phase assays like (single antigen bead assay) SAB, also known as virtual crossmatch, detect anti-HLA antibodies against the donor. In single antigen bead assay, the main advantage is increased sensitivity and specificity of the results. In SAB assay, each bead detects antibody directed against a single HLA antigen. Median Fluorescence Intensity (MFI) values of SAB assay quantifies the intensity of each anti HLA antibody present and helps in detecting significant DSAs in recipient.

Complement-dependent cytotoxicity
Complement dependent cytotoxicity (CDC) cross match test was first standardized by Terasaki in 1960's. It is used to detect the donor specific antibodies against the recipient. If the result of the CDC cross match is positive, then transplantation with that donor is not advised. CDC detects complement dependent anti HLA specific antibodies. CDC test is based on donor lymphocytotoxicity testing, where Donor lymphocytes T cells and B cells are Isolated from fresh blood sample and cross matched with patient's serum. If the recipient's antibodies attack the donor cells, they are considered a positive match and transplantation will not be suitable due to increased risk of hyper-acute rejection. CDC is traditionally considered gold standard method for donor and recipient compatibility for transplantation.

THE BEST OF THE PAST

Radiologist # Paedsradiology #Followtheirlead

Interviewee: Associate Prof Waseem Akhter
Interview Recorded by Dr Shayan Anwar

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

All my time spent at AKU is worth narrating, either it is as resident working under supervision or supervising new generation or growing my department as leading academic center in the country. However, the most AHAA moments were when I worked with the AKU multi-disciplinary team in villages to train midwives about basic ultrasound to improve maternal and neonatal outcomes and the results were promising. It is one of the best examples where radiology can be incorporated at community level for betterment of society especially in resource poor country.

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

Immediately after my house job and FCPS-1, I was fortunate to get selected as a resident at AKU in 2001. I was so naïve to radiology that for the first time I saw fluoroscopic machine at AKU, thanks to residency program and AKU who made me what I am today. University is very supportive, I was encouraged to excel not only as a clinician but also as a researcher, I got selected in clinical scholar program in 2007 and did diploma in clinical epidemiology and biostatistics which groomed and opened my new avenues of academic radiology career in Pakistan. I enjoy being an academic pediatric radiologist at AKU.

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?

Teamwork, discipline, and desire to learn new things. But most important thing which I acquired is "How I can help or do value addition to my patients and colleagues."

4. As a senior paediatric radiologist of the country, please share your experience of development of paediatric radiology practices in Pakistan and its future in next 10 years.
We pioneered the introduction of this 'subspecialty' in Pakistan, setting the stage for others to follow suit. Our vision extends further, aspiring to launch the first pediatric radiology fellowship program in the country. This program aims to establish a formal academic foundation for this specialized field, contributing significantly to its growth and recognition.

5. As a senior paediatric radiologist of the country, please share your experience of development of paediatric radiology practices in Pakistan and its future in next 10 years.

We pioneered the introduction of this 'subspecialty' in Pakistan, setting the stage for others to follow suit. Our vision extends further, aspiring to launch the first pediatric radiology fellowship program in the country. This program aims to establish a formal academic foundation for this specialized field, contributing significantly to its growth and recognition.

FAREWELL CHRONICLES: CELEBRATING DR ANILA RASHID'S JOURNEY

It was with mixed emotions that we bid farewell to Dr. Anila Rashid, a distinguished hematologist. Her journey encompasses a residency, instructional roles, and, most recently, as an Assistant Professor since 2017.

Dr. Rashid's dedication to advancing hematology and her notable accomplishments, included multiple publications and a prestigious award such as the ISTH 2015 'Reach the World Award Grant'. Her research interests in Haemostasis and coagulation, coupled with her commitment to excellence, have significantly enriched the field. She has authored more than 27 publication in both National and International journals. Few of her colleagues have expressed their thoughts regarding Dr. Anila.

Dr Muhammad Shariq, Section Head Hematology shared "Dr. Anila Rashid commenced her residency training alongside me in November 2008, demonstrating an insatiable thirst for knowledge and a remarkable aptitude for the intricacies of hematology. Upon transitioning to a faculty role within our institute, her impact has been nothing short of profound, especially in her contributions to the coagulation subsection of hematology. Her journey from a resident to a respected faculty member has been marked by an unwavering commitment to excellence, an unparalleled passion for hematology, and a relentless pursuit of advancement in the coagulation subsection. Her contributions have been invaluable to our institute, and her dedication serves as an inspiration to us all."

Dr Natasha Ali, Associate Professor, Hematology shared "Apart from her stellar contributions to the service mandate of the section, Anila was dedicated to the teaching of our post graduate trainees, and she brought a new flavor in the teaching and learning strategies. Dr Anila was and will always remain a prominent member of the Haematology team and her contributions to the section will always be cherished."

Dr Muhammad Hasan, Assistant Professor, Hematology shared " Dr. Anila has been a wonderful senior colleague, providing support and valuable academic guidance to me since 2013, when I joined residency at AKU. She is an accomplished hematologist, a good teacher, and a kind person. I wish her all the best for her future endeavors!

As Dr. Rashid embarks on a new chapter beyond AKU, we extend our heartfelt gratitude for her invaluable contributions and wish her continued success and fulfillment in all her future endeavors. May the next phase of her journey be as rewarding and impactful as her time with us. Farewell, Dr. Rashid, and best wishes for the exciting adventures that lie ahead!

POLAROID

Chemical Pathology

In the Biochemical Genetics Laboratory, LC-MS/MS experts meticulously dissect a tuning report for Acylcarnitine, navigating the intricate realms of mass spectrometry precision. Meanwhile, another skilled technologist, analyzing the DBS specimen. Here, scientific expertise converges with cutting-edge technology, forging the path to diagnostic excellence.

Precision in Practice: Technologist entering results of Anti-Cardiolipin antibodies analyzed on the ELISA based instrument EPI-Max. Lab medicine is revolutionizing autoimmune disorders diagnostics, employing advanced technologies like ELISA and molecular testing to unveil precise biomarkers, enhancing early detection and personalized treatment strategies for improved patient outcomes.

Radiology

20th Health Asia International Conference, 7th Annual Seminar on Radiology-held on 21st October 2023- Speakers from from left to right- Dr. Muhammad Azeemuddin, Dr. Zafar Sajjad, Dr. Naila Nadeem, Dr Shayan Sirat, Mr. Asif Bilal, Ms. Shirin Bhimani.

20th Health Asia International Conference, 7th Annual Seminar on Radiology-held on 21st October 2023- Technologist section.

20th Health Asia International Conference, 7th Annual Seminar on Radiology-held on 21st October 2023- Inauguration.

Hematology

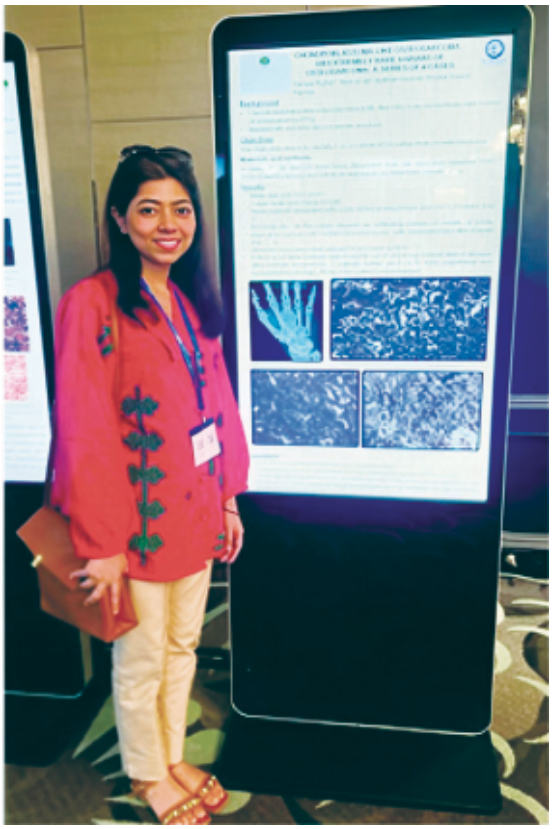
Hematology resident analyzing daily quality control charts of coagulation tests and exploring the intricate world of blood cells under the microscope lens

Section of Hematology & Transfusion medicine celebrating World Blood Donor Day 2023, with gratitude for every life-saving drop

Histopathology

A picture collage of departmental consultation conference, where histopathologic review slides of complex cases on a multi-head microscope. This holds profound significance in enhancing the accuracy and thoroughness of biopsy specimen reporting. This collaborative approach fosters a collective expertise, promoting in-depth discussions among pathologists, ultimately leading to more comprehensive and precise diagnostic conclusions. Through shared insights and diverse perspectives, this conference greatly improves the quality of histopathological assessments, ensuring optimal patient care and diagnostic excellence.

Dr. Zoonish, Senior Instructor Histopathology attended and presented her work at the International Association of Pathology Conference in Thailand in November 2023. The picture on left depicts a fun activity at the end, where these letters were hidden randomly under the seats and Dr. Zoonish Ashfaq (extreme left) participated in it.



Dr. Tamana Asghari also attended International Association of Pathology conference in Thailand in November 2023 where she presented her research work.

Microbiology

Dr Ali Raza Nasir (Resident III- Microbiology) received 1st position in Oral Presentation

Dr Rumina Hasan (Microbiology) received Lifetime Achievement Award at PAP Conference

Molecular Pathology

Dr. Zeeshan Ansar has been selected to receive the 2023 Florabel G. Mullick Honorary Award at USCAP's 2023 Annual Meeting in New Orleans, LA.

Global Pathology Education Award

This award was created to improve global health by increasing the exchange of information on the science of pathology. It provides international pathologists practicing in medically under-resourced areas with complimentary registration to attend the CAP23 virtual annual meeting. Awardess gain access to outstanding continuing education in all pathology specialities taught by world-class experts.

2023 Global Pathology Education Award Recipients

Melaku Abay, MD
Arsi University

Shaimaa Abdelmalik Hassan Pessar, MD

Zeeshan Ahmed, MBBS
Aga Khan University Hospital

Javeria Aijaz, MBBS, PhD
Indus Hospital & Health Network

CAP 2023 Global Pathology Education Award
Dr. Zeeshan Ansar has received two international awards from the world renown pathology societies (USCAP and CAP) in 2023.

Test in Focus

آغا خان یونیورسٹی ہسپتال، کراچی
The Aga Khan University Hospital, Karachi

Department of Pathology and Laboratory Medicine

Serum Biotinidase (BTD)

UPDATE NO:06, VOL :XXIX,2023

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

JUNE, 2023

What's New

The Clinical laboratory is already performing a screening test for Biotinidase deficiency on dried blood spot specimens. Initiating quantification of serum biotinidase enzyme activity is a step forward for confirmation of Biotinidase deficiency.

Introduction:

Biotinidase is responsible for the recycling of biotin (also known as Vitamin-H), by cleaving biocytin and biotinyl-peptides as a hydrolase, thereby liberating biotin for reutilization. Biotin is a cofactor required for the optimal activity of four biotin responsive carboxylase enzymes: propionic CoA Carboxylase and Methyl crotonyl CoA carboxylase involved in branched chain amino acid metabolism; acetyl CoA carboxylase involved in Kreb's cycle and pyruvate CoA Carboxylase involved in ketone metabolism.

Biotinidase deficiency is an autosomal recessive disorder caused by absent or markedly deficient activity of enzyme Biotinidase during the normal proteolytic turnover of holocarboxylase and other biotinylated protein, associated with secondary alterations in amino acid, carbohydrate, and fatty acid metabolism. The disorder is categorized into profound and partial Biotinidase deficiency. Patients with profound biotinidase deficiency have less than 10% of mean normal serum activity, while patients with the partial biotinidase deficiency variant have 10–30% of mean normal serum activity.

Intent of Use:

This test is used for:

- Evaluation of patients with suspected biotinidase deficiency
- Patients with low enzyme activity on Dried blood spot Biotinidase testing

Important Note:

- Serum samples should be immediately frozen and sent to laboratory as early as possible.
- Serum samples may not be suitable for individuals who have undergone transfusion
- Moderate to gross hemolysis causes falsely elevated biotinidase values
- Lipemic samples must be ultracentrifuged before assaying
- Certain sulfa drugs may cause interference

Principle:

Colorimetry.
Enzyme linked immunosorbent assay (ELISA)

Specimen Type:

5 cc clotted blood or 3-5 cc serum

Charges:

Rs.5000.0
*Revisions may apply

Schedule:

The test is performed on 2nd and 4th Monday every month and reported after two days.

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

CAP ACCREDITED
COLLEGE of AMERICAN PATHOLOGISTS

hospitals.aku.edu/Karachi/clinical-laboratories