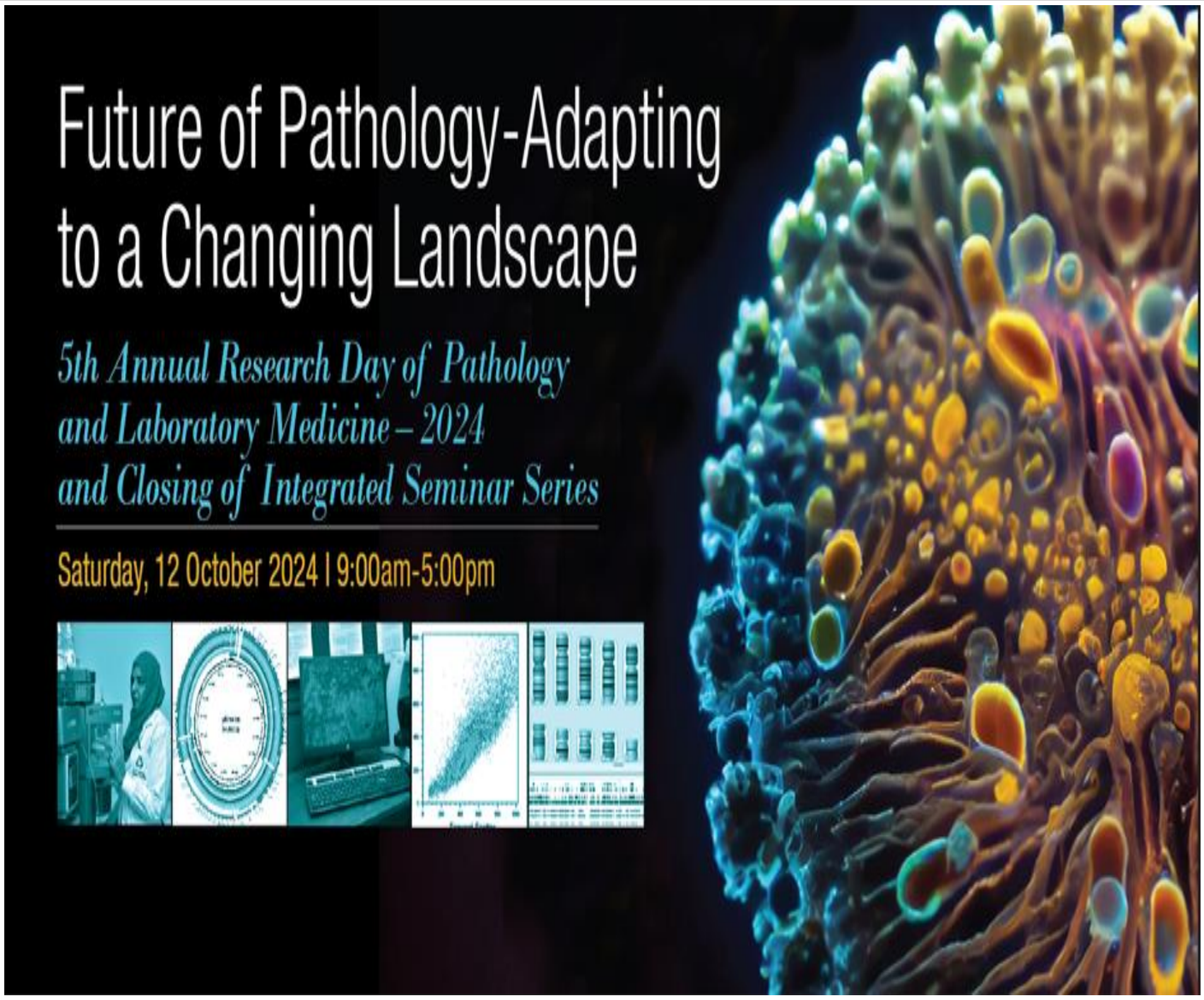

Abstract Book

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آغا خان یونیورسٹی
THE AGA KHAN UNIVERSITY

Foreword

We are pleased to present this abstract book, published in conjunction with the **5th Annual Research Day of Pathology and Laboratory Medicine** – 2024 and the Closing of the Integrated Seminar Series "**Future of Pathology - Adapting to a Changing Landscape**."

This year's event marks a significant milestone for our department, celebrating our research progress and achievements. As the field of pathology continues to evolve, it's essential to embrace innovation and adapt to emerging challenges. The theme of this year's event reflects our commitment to exploring new technologies, methodologies, and approaches that will shape the future of pathology.

We are honored to host distinguished guests, renowned experts, and talented researchers. Their contributions will undoubtedly enrich our understanding and inspire further advancements in pathology research.

We have invited both local and international speakers to discuss emerging topics like climate change, big data, and the role of multi-omics in research. These sessions aim to provide valuable insights, foster interdisciplinary collaborations, and showcase innovative research in pathology and laboratory medicine.

I would like to express my sincere gratitude to our organizing committee, faculty, staff, and residents for their dedication and hard work in making this event a success.

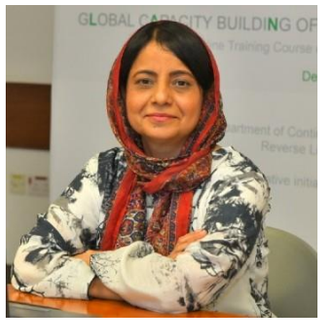
We hope this abstract book serves as a valuable resource, highlighting the outstanding research conducted by our department members. We look forward to welcoming you all and encourage your active participation in the Research Day.

Thank you for your participation and support.

Dr. Hafsa Majid

Chair, Scientific Committee

Message from Chair, Pathology & Laboratory Medicine



Welcome to the 5th Annual Research Day of the Department of Pathology and Laboratory Medicine – 2024, and the Closing of the Integrated Seminar Series, titled *Future of Pathology: Adapting to a Changing Landscape*. This event is a wonderful opportunity to celebrate the research accomplishments and innovative research activities of our department.

This year's theme, "Future of Pathology – Adapting to a Changing Landscape," is apt considering the revolution AI is bringing into pathology practices. As we stand at the intersection of rapid technological advancements, evolving healthcare models, and an ever-increasing need for precision medicine, the role of pathology and laboratory medicine is expanding and transforming. Our responsibility is to not only keep pace with these changes but to lead the way in shaping the future of diagnostics, research, and patient care in Pakistan.

Our mission is to reflect the diverse and impactful work being conducted by our faculty, researchers, and trainees. From cutting-edge discoveries to innovative applications of digital pathology, each contribution demonstrates the vigor and dedication of our team. I am particularly proud of our students and trainees, whose passion and creativity are driving the future of our discipline.

We hope this event will foster collaboration and idea-sharing across our academic community, inspiring further learning, research, and leadership. I encourage everyone to participate actively and support our young researchers as they share their innovative work. It is vital to acknowledge and celebrate their contributions to the university and society.

In closing, I extend my deepest gratitude to everyone who has contributed to the planning and organization of this event. Your dedication and hard work have made this day possible, and I look forward to an engaging and inspiring Research Day.

Thank you.

Dr. Erum Khan
Professor & Chair
Pathology & Laboratory Medicine

Message from Vise Chair Research, Pathology & Laboratory Medicine

The Department of Pathology and Laboratory Medicine boasts a rich history of groundbreaking research and discovery in basic, clinical, and translational sciences. Currently, the Department is at the forefront of personalized medicine, thanks to our exceptional researchers who excel in translating bench research to bedside applications and evaluating new drugs and devices in clinical settings. Our missions are supported by outstanding faculty and trainees.

The 5th Annual Research Day of Pathology and Laboratory Medicine – 2024 will be held in conjunction with the integrated VBD training seminars, marking a significant milestone of progress, growth, and success. This event is an opportunity to reflect on past achievements, celebrate current advancements, and look forward to a promising future.

We believe that the Pathology research showcase will offer a unique platform for scientists, researchers, and medical professionals to share their knowledge and expertise, contributing to the advancement of Pathology. The theme, “Future of Pathology – Adapting to a Changing Landscape,” underscores the importance of cooperation and a multidisciplinary approach in combating diseases and achieving the common goal of disease elimination. Our aim is to foster collaboration and networking among professionals in pathology and laboratory medicine.

We have curated an exciting and comprehensive program covering a wide range of topics related to Pathology, including the latest research developments, practical solutions, and best practices. We look forward to welcoming you and providing a memorable and enriching experience.

Najia Ghanchi

Vice. Chair Research
Associate Professor, Microbiology
Department of Pathology and Laboratory Medicine

ORGANIZING TEAM:

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Chair, Organizing Committee
Assistant Professor & S.Head
Chemical Path
Pathology and Laboratory Med
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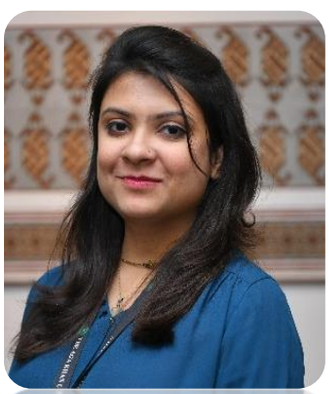

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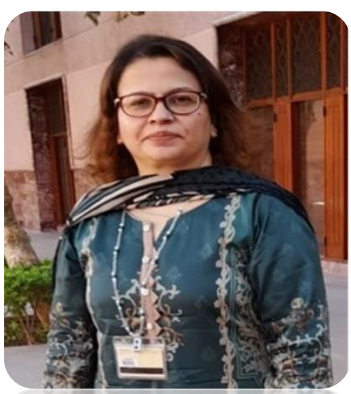

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5th Annual Research Day of Pathology and Laboratory Medicine – 2024 and Closing of Integrated Seminar Series

Future of Pathology-Adapting to a changing Landscape

Date: 12th October 2024 | Venue: Movenpick Hotel | 09:00 am to 5:00 pm

Program	
8:30 – 9:00 am	**Registration**
9:00 – 9:05 am	**Tilawat** Dr Muhammad Bilal Arian *Senior Instructor translational unit,* *Department of Pathology & Laboratory Medicine* *Aga Khan University*
9:05 – 9:10 am	**Program Introduction** Dr Hafsa Majid *Chair Organizing Committee* *Department of Pathology & Laboratory Medicine* *Aga Khan University*
9:10 - 9:15 am	**Mapping Progress-Research Milestones of Dept. of Pathology & Laboratory Medicine** Dr Najia Ghanji *Associate Professor, Vice-Chair Research* *Department of Pathology & Laboratory Medicine* *Aga Khan University*
9:15 – 9:25 am	**Address by Guest of Honor –** Prof. Syed Asad Ali *Chair, Department of Community Health Sciences* *Associate Dean Research,* *Medical College Aga Khan University, Karachi, Pakistan*
9:25 – 9:35 am	**Address by Guest of Honor** – Dr. Asim Belgaumi *Professor of Pediatric Oncology, Dept. of Oncology* *Associate Dean for Clinical Affairs, AKU Medical College* *Chief Medical Officer, Aga Khan University Hospital System*
9:35 – 10:25 am	**Global Health - Emerging Infections and Climate Change** Wes Van Voorhis MD PhD *Professor, Allergy and Infectious Diseases* *Director, Center for Emerging and Re-emerging Infectious Diseases (CERID)* *University of Washington, USA* **Emerging Infections in Pakistan and its impact on global health** Dr. Kausar Jabeen *Assistant Professor and Section Head Microbiology* *Department of Pathology & Laboratory Medicine* *Aga Khan University*
10:25 – 11:10 am	**Tea Break & Poster Presentation**

11:10 – 12:00 noon	**What is One Health, and Why Does it Matter for Vector Borne Diseases?** Julianne Meisner *Assistant Professor, Epidemiology* *Assistant Professor, Global Health* *Adjunct Assistant Professor, Env. and Occ. Health Sciences* *University of Washington, US* **Data to Discovery-Analyzing the Impact of ATRC** Dr Muhammad Abbas Abid *S. Instructor Department of Pathology & Laboratory Medicine* *Aga Khan University, Karachi, Pakistan* Ms Rabiya Owais *Manager Data Analytics,* *Department of Pathology & Laboratory Medicine* *Aga Khan University, Karachi, Pakistan*
12:00 – 12:50 pm	**From Genomics to Proteomics: How Multi-Omics is Transforming Diagnostics** Dr Fyezah Jehan *Associate Professor & Chair, Department of Paediatrics & Child Health* *Aga Khan University, Karachi, Pakistan* **Transforming Healthcare: The Impact of Metabolomics** Prof. Dr Aysha Habib Khan *Department of Pathology & Laboratory Medicine* *Aga Khan University, Karachi, Pakistan*
12:50 – 1:00 pm	Group Photograph
1:00 – 2:00 pm	**Lunch, Prayer and Networking Break**
2:00 – 2:50 pm	**Smart Pathology: AI and Digital Innovations in Diagnostic Accuracy (Zoom)** Dr Anil Parwani *Donald A. Senhauser Chair, Department of Pathology* *Chief of Pathology Services for the Health System* *Distinguished Professor of Pathology and Biomedical Informatics* *Wexner Medical Center - Department of Pathology* *The Ohio State University*, USA **Epidemiologic Surveillance - Utilizing Postmortem Minimally Invasive Tissue Sampling** Dr Zeeshan Uddin *Assistant Professor Histopathology* *Department of Pathology & Laboratory Medicine* *Aga Khan University, Karachi, Pakistan*
2:50 – 3:40 pm	**Precision Medicine - Advancing Treatment for Genetic Diseases** Dr. Salman Kirmani *Associate Professor & Chair* *Division of Women and Child Health* *Aga Khan University, Karachi, Pakistan* **AI meets Haematology: From Data to Diagnosis** Dr. Natasha Ali *Professor and Associate Dean* *Department of Pathology and Laboratory Medicine/ Oncology* *Aga Khan University*

3:40-3:55 pm	**Tea Break**
3:55 – 4:30 pm	**Paper Presentation** **Dr. Ali. Nasir:** Azole resistance and cyp51A gene mutations in clinical and environmental isolates of Aspergillus fumigatus from Pakistan **Dr. Umer. Naeem:** Unveiling the Thrombotic Spectrum: Exploring the Role of Anti-B2GPI Antibodies in Antiphospholipid Syndrome Suspects **Dr. Sarosh Moeen:** Rethinking Histology Slide Digitization Workflows for Low-Resource Settings **Dr. Natasha Ali:** The Effect of ABO Incompatibility on the Outcome of Stem Cell Transplant **Dr. Sabiha:** Phylogenetic analysis of Crimean Congo Hemorrhagic Fever Virus (CCHFV) strains circulating in Pakistan (2023)"
4:30 – 4:35 pm	**Closing Remarks** Prof Dr. Erum Khan *Chair Department of Pathology & Laboratory Medicine* *Aga Khan University, Karachi, Pakistan*
4:35 – 4:55 pm	**Certificates and Awards Distribution**
4:55 – 5:00 pm	**Vote of Thanks & Closing** Dr Najia Ghanji *Associate Professor, Vice-Chair Research* *Department of Pathology & Laboratory Medicine* *Aga Khan University*

PLENARY SESSION ABSTRACTS

O-01

Global Health - Emerging Infections and Climate Change

Dr. Wes Van Voorhis,

Center for Emerging and Re-emerging Infectious Diseases (CERID),
University of Washington, Seattle, WA USA

The world is experiencing emerging infections at an increasing rate. Part of the reason for this are catastrophic weather events and increasing temperatures, likely caused by climate change. The effects of climate change and emerging infections can be observed in Pakistan. For instance, in 2022, massive and unprecedented rainfall led to an enormously increased burden of vector-borne disease, especially Dengue and Malaria. Emerging infections are further exacerbated by travel and migration of humans. An example is the emergence of Zika virus, that originated in Brazil, now found in Pakistan. Zika virus in Pakistan was first detected by Aga Khan researcher Professor Najeeha Iqbal Talat working with Michael Gale of the University of Washington. Wes Van Voorhis will discuss the interaction of Climate Change and Emerging Infections, using examples from the United World Antiviral Research Network (UWARN), in which Aga Khan University is participating.

O-02

AI and Digital Innovations in Diagnostic Accuracy

Dr. Anil V Parwani,

The Ohio State University

In recent years, the field of pathology has undergone a remarkable transformation, driven by advancements in technologies and our growing understanding of diseases at a molecular level. One such technology is digital pathology which enables the digitization of histopathology slides, creating vast repositories of digital images that can be analysed, shared, and utilised for research, education and diagnostic purposes. Advanced image analysis algorithms and machine learning techniques are being employed to extract valuable information from these images, aiding pathologists in making more accurate and efficient diagnoses.

Precision pathology represents a shift in the way we diagnose, classify, and treat diseases. A key area of focus in precision pathology, is the utility of digital pathology and artificial intelligence as well as integration of genomic information into diagnostic and treatment decisions. By analysing the DNA and RNA profiles of tumours, researchers can identify specific genetic mutations, gene expression patterns, and molecular signatures that guide personalised treatment strategies. This can be effectively combined with AI-powered algorithms to create a “PRECISE” pathology diagnosis leading to customized treatments for individual patients, these insights allow for targeted therapies, resulting in better patient outcomes and reduced side effects.

This talk will describe where we are in the journey towards utilizing these digital pathology and AI tools for routine pathology practice and what are the possibilities, once fully implemented.

O-03

Epidemiologic Surveillance-Utilizing Postmortem Minimally Invasive Tissue Sampling

Dr Zeeshan Uddin,

Aga Khan University Hospital, Karachi

Complete diagnostic autopsies, considered the gold standard, are rarely done in low resource settings to determine a person's cause of death. Many health centers lack the resources and infrastructure needed to carry out the complex procedure, which can also be considered culturally unacceptable in some societies like Pakistan.

Minimally Invasive Tissue Sampling (MITS) is an innovative post-mortem examination approach for understanding the causes of death by providing improved health data. MITS is carried out by trained pathologists and/or technicians. The process starts with collecting small amounts of tissue and fluid from key organs by inserting fine needles into the body. These samples are then subjected to histopathological, microbiological and molecular analysis to find out the disease process, key pathogens, infectious diseases etc. It is being utilized in various mortality surveillances across the globe.

Child Health and Mortality Prevention Surveillance (CHAMPS) is one of such surveillance programs created to gather better mortality data in children under five years, in sub-Saharan Africa and South Asia. CHAMPS is a collaborative, multicenter effort led by The Emory Global Health Institute (EGHI) with funding from the Bill & Melinda Gates Foundation. Karachi, Pakistan is one of the latest sites for CHAMPS project, launched in 2023, led by the department of Pediatrics and Child Health, Aga Khan University, Karachi.

O-04

Machine learning: background and applications in clinical care

Dr. Julianne Meisner

University of Washington

The advent of Big Data has accelerated the use of statistical models for prediction, however high-dimensional data has unique features and challenges. Machine learning methods are one of several approaches suitable to handling high-dimensional data while optimizing the bias vs. variance tradeoff. In this talk, Dr. Meisner will introduce the basics of statistical prediction and machine learning, and how they are used for prediction. She will then briefly summarize key applications in clinical practice.

O-05

AI Meets Haematology: From Data to Diagnosis

Dr Natasha Ali

Aga Khan University Hospital

The future practice in haematology will involuntarily involve the integration of artificial intelligence-based systems in routine day-day working. Several studies in literature have already reported AI based algorithms to identify benign vs malignant cell populations, pre-process and analyse digital medical images and interpret data to provide clinical diagnosis. The objective of these approaches is to allow informed interventions, provide enhanced treatment, improve accuracy and precision of results and to minimize analytical errors. However, these tools become purposeless if applied incorrectly. It is imperative that haematologists understand the various techniques, implementations, and challenges of machine learning. The potential for AI-based applications in haematology are endless but where do we stand and what is the limit to our reach?

ORAL PRESENTATION ABSTRACTS

O-01

Azole resistance and *cyp*51A gene mutations in clinical and environmental isolates of *Aspergillus fumigatus* from Pakistan

Syed Ali Raza Nasir, Sadaf Zaka, Joveria Farooqi, Najia Ghanchi, Kauser Jabeen*

Affiliation: Aga Khan University Hospital

**Corresponding author. Email: alir.nasir@aku.edu*

Objective:

Azole resistance in clinical and environmental *Aspergillus fumigatus* isolates has been reported globally. We aimed to detect azole resistance in *A. fumigatus* from clinical and environmental sources in Pakistan. Mutations in *cyp*51A gene that confer resistance to azoles in *A. fumigatus* were also determined.

Material Ansd Methods :

144 clinical and 25 environmental A. fumigatus from across Pakistan were screened for azole resistance using EUCAST protocol. Minimum inhibitory concentrations (MICs) were determined for posaconazole, voriconazole and itraconazole by broth microdilution for 144 clinical and 25 environmental isolates using CLSI standards. Mutations in the cyp51A gene of A. fumigatus were determined by sequencing.

Results:

On agar screening, none of the isolates screened positive for resistance. Among the clinical isolates, 13 (9%) had non-wild-type MICs for posaconazole, 1 isolate was resistant to and 17 (11.8%) were intermediate to voriconazole, and all isolates had wild-type MICs for itraconazole. The isolate resistant to voriconazole had an MIC of 2 µg/ml. Of the environmental A. fumigatus isolates, 3 (12%) had non-wild-type MICs to posaconazole, while all isolates were sensitive to voriconazole and had wild-type MICs for itraconazole. Amongst the 27 isolates sequenced, all the voriconazole non-susceptible clinical isolates (n=4) had different combinations of multiple single nucleotide polymorphisms (SNPs) in the cyp51A gene while the

voriconazole susceptible clinical isolates had none. All 4 of these patients had prior azole exposure.

Conclusion:

While no known mutations in the *cyp*51A gene were found, since this is the first genotypic study from our country, the existence of multiple SNPs exclusively in voriconazole non-susceptible clinical isolates suggests genotypic evidence of resistance.

Keywords: azole resistance; *Aspergillus fumigatus*; *cyp*51A; Single Nucleotide Polymorphisms
Presentation type: Oral

O-02

Unveiling the Thrombotic Spectrum: Exploring the Role of Anti-B2GPI Antibodies in Antiphospholipid Syndrome Suspects

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Abstract count: 245

Abstract

Introduction: The antiphospholipid syndrome (APS) is characterized by thrombotic or obstetric complications. The study aimed to assess the prevalence of anti-B2GPI antibodies in patients suspected of APS and explore their correlation with thrombotic events.

Material and Methods: The study took place in the sections of Chemical Pathology and Hematology, Department of Pathology and Laboratory Medicine, The Aga Khan University, Karachi, Pakistan. Ethical approval was obtained, and the study involved 133 participants aged 18-60 tested for anti-cardiolipin antibodies and provided telephonic consent. Demographic, clinical, and laboratory data were collected using a structured questionnaire. Serum samples were analyzed for anti-B2GPI antibodies and the association between antiphospholipid (aPL) antibodies and clinical features was observed.

Results: The study included 120 females (90.2%) and 13 males (9.8%) with a mean age of 31.3±8.8 years. Predominant clinical manifestations included unexplained miscarriages at >10 weeks of gestation (n=77/120 female, 64.2%), while deep venous thrombosis (DVT) was a common non-obstetric clinical feature (n=18/133, 13.5%).

The median level of Anti-B2GPI antibodies was 2.12 U/ml (1.34-7.04) and 7.5% (n=10) were positive only. Two of the ten positive patients displayed positive anti-B2GPI antibodies while concurrently testing negative for other aPL antibodies. A

significant association was identified between the presence of anti-B2GPI antibodies and DVT and other venous thromboembolic events.

Conclusion: The study highlights the importance of anti-B2GPI testing in the diagnostic workup of APS. The anti-B2GPI antibodies were notably associated with thromboembolic events, highlighting their potential implications for risk assessment in this population.

Keywords: Antiphospholipid syndrome, antibodies, vascular thrombosis, obstetric complications

O-03

Abstract:

The Effect of ABO Incompatibility on the Outcome of Stem Cell Transplant

Natasha Ali, Mohammad Usman Shaikh and Salman Adil

Introduction: The impact of ABO mismatch on the outcome of allogeneic haematopoietic stem cell transplant (HSCT) remains disputed. Approximately 50% HSCTs are performed across the ABO blood group barrier. The aim of this study was to determine the effect of ABO mismatch on engraftment, graft versus host disease (GVHD) and outcome in patients undergoing allogeneic HSCT at our centre.

Subjects & Methods: We performed a retrospective chart review from 2004 till 2019. Variables analysed included age, gender, diagnosis, stem cell source, type of mismatch, frequency of acute and chronic GVHD, ABO mismatch related complications (engraftment, pure red cell aplasia, haemolysis) and overall survival.

Results: Total transplants performed during the study period were 351 of which evaluable allogeneic stem cell transplant cases were 200. Main indications were acute leukemia (87), β-thalassemia major (45) and aplastic anaemia (68). The mean age ± SD was 20.3 ± 12.7 years (range: 2-54 years). Stem cell source was peripheral blood (PB) in 87 patients, bone marrow (BM) in 51 patients and both (PB and BM) in 62 patients. One hundred and thirty-five donor-patient pairs (68%) were ABO matched while 65 were ABO mismatched (32%). Of these 65 pairs, 18 were major mismatched, 39 were minor ABO mismatched while 8 were bidirectionally

mismatched. There was no difference in neutrophil engraftment between the two groups (p-value: 0.57). Of the 65 ABO mismatched pairs, all patients with minor and bidirectional mismatch achieved engraftment while 89% with major ABO mismatch engrafted. In major and bidirectional group, acute transfusion reactions (febrile non-haemolytic and haemolytic) occurred frequently. No patient with ABO mismatched transplant developed pure red cell aplasia or delayed haemolytic reaction. The cumulative incidence of acute GVHD was more in the ABO mismatched group (p value: 0.03) while that of chronic GVHD was comparable. The was no difference in overall survival between the two groups.

Conclusion: Acute transfusion reactions were frequently seen in major and bidirectional ABO mismatched groups. Increased risk of acute GVHD was observed in ABO mismatched pairs. The overall survival in both groups was comparable.

Key words: ABO incompatibility, HSCT, outcome, GVHD

O-04

Rethinking Histology Slide Digitization Workflows for Low-Resource Settings

Sarosh Moeen[1], Talat Zehra[2], Romana Idrees[1], Saad Nadeem[3]

1 Aga Khan University Hospital, Karachi, Pakistan, 2 Jinnah Sindh Medical University, Karachi, Pakistan, 3 Memorial Sloan Kettering Cancer Centre, New York, USA

Introduction

Histology slide digitization is becoming essential for telepathology (remote consultation), knowledge sharing (education), and using the state-of-the-art artificial intelligence algorithms (augmented/automated end-to-end clinical workflows). However, the cumulative costs of digital multi-slide high-speed brightfield scanners, cloud/on-premises storage, and personnel (IT and technicians) make the current slide digitization workflows out-of-reach for limited-resource settings, further widening the health equity gap; even single-slide manual scanning

commercial solutions are costly due to hardware requirements (high-resolution cameras, high-spec PC/workstation, and support for only high-end microscopes).

Methods

In this work, we present a new cloud slide digitization workflow for creating scanner-quality whole-slide images (WSIs) from uploaded low-quality videos, acquired from cheap and inexpensive microscopes with built-in cameras. Specifically, we present a pipeline to create stitched WSIs while automatically deblurring out-of-focus regions, up sampling input 10X images to 40X resolution, and reducing brightness/contrast and light-source illumination variations.

Results

We demonstrate the WSI creation efficacy from our workflow on World Health Organization-declared neglected tropical disease, Cutaneous Leishmaniasis (prevalent only in the poorest regions of the world and only diagnosed by sub-specialist dermatopathologists, rare in poor countries), as well as other common pathologies on core biopsies of breast, liver, duodenum, stomach and lymph node.

Conclusion

We will release our code, pretrained models, and cloud platform for uploading microscope videos and downloading/viewing WSIs with shareable links (no sign-in required) for telepathology and knowledge sharing.

POSTER PRESENTATION ABSTRACTS

P-01

Antifungal susceptibility of clinically significant Mucorales against amphotericin, posaconazole and isavuconazole from Pakistan

Muhammad Farooq, Tooba Rahim, Sadaf Zaka, Afia Zafar, Kauser Jabeen, Joveria Farooqi, Najia Ghanchi

Aga Khan University, Karachi, Pakistan

Background:

Antifungal susceptibility profiles vary among various genera and species of Mucorales, therefore accurate species identification and antifungal susceptibility testing is crucial for mucormycosis surveillance and treatment. A recent study evaluating antifungal activity of 52 clinical Mucorales isolates from Europe, USA and Asia Pacific reported amphotericin B to be the most active antifungal agent followed by posaconazole and isavuconazole. Such studies are not available from Pakistan.

Objectives:

In this study, we determined isavuconazole, amphotericin B and posaconazole minimum inhibitory concentration (MICs) in clinically significant Mucorales using broth microdilution.

Methods:

The study was conducted at the Aga Khan University Hospital laboratory, Karachi, Pakistan from April-December 2023. All clinically significant mucorales isolates from patients of all ages, both male and female were included. Susceptibility testing was performed by reference broth microdilution method according to clinical laboratory standards institute (CLSI) M38-A2. Although clinical breakpoints for

Mucorales isolates are undefined, epidemiological cutoff values (amphotericin B: 2 ug/ml; posaconazole: 0.5 ug/ml; isavuconazole: 1 ug/ml were used to categorize strains into wild-type and non-wild type.

Results:

A total of 52 Mucorales were identified during the study period. Patients were of ages between 12-90 years and 22/52 patients were female. Source of isolation of Mucorales were predominantly from pulmonary specimens 26/52, followed by wound and surgical tissues 16/52 and then nasal 10/52. The most common mucoraceous mold was Rhizopus species 32/52 followed by Apophysomyces species 11/52 and Mucor species 9/52. Susceptibility testing so far has been performed on 31 strains. MIC values of tested strains of Rhizopus species ranged from 0.12-1 ug/ml for amphotericin B, 0.5-4 ug/ml for isavuconazole and 0.12-2 ug/ml for posaconazole. Nine isolates had non-wild type MIC for isavuconazole, and eighteen isolates had non-wild type MIC for posaconazole. MIC determination of rest of the isolates is in process.

Conclusions:

We are reporting preliminary results of susceptibility testing of Mucorales from Pakistan. Continued surveillance of antifungal susceptibility is needed to determine any emerging resistance to commonly used antifungals for mucoraceous molds.

O-02

Abstract Title: Identification of fungi from histopathology positive formalin fixed paraffin embedded tissue by panfungal PCR

Syeda Rija Zehra1 Kauser Jabeen1 Sadaf Zaka1 Joveria Farooqi1, Najia Ghanchi1

Aga Khan University, Karachi, Pakistan

Introduction: Fungal infections affect over a billion people globally with 1.5 million deaths per year. According to a study around 1.78% of total population in

Pakistan is affected by a serious fungal infection. In clinical practice invasive tissue specimens are either not sent or a very small quantity is submitted for cultures and histopathological diagnosis is based on morphology. So we aim to successfully optimize ITS sequencing for diagnosis of fungal infections from formalin-fixed paraffin embedded tissue and determine the proportion of samples that can be reliably identified to genus level and assess its agreement with culture.

Methods: This is an ongoing cross-sectional study where achieved tissue biopsies, sent to Aga Khan University Hospital laboratory, from 2020 with histopathology proven fungal elements were chosen using laboratory database. FFPE blocks 20 micron-thick sections were collected in eppendorfs. DNA extraction was done by using QIAamp FFPE Kit (Qiagen, Hombrechtikon, Switzerland) according to manufacturers' protocol. Conventional PCR was done with positive and negative controls by using forward primers ITS1 and reverse primer ITS4 to amplify ITS1, ITS2 and 5.8S region. Gel electrophoresis done to obtain amplification bands. PCR product was re-purified and sent for sequencing. Sequencing results were read using BLAST.

Results: Until now we have tested a total of 57 samples out of which DNA was amplified in 17 (29.82%) samples. 6 (35%) histopathology proven fungal infection samples were reliably identified to genus level by ITS sequencing and showed agreement to culture as well. Selected tissues included both sterile and non-sterile body sites. Organism showing agreement included *Aspergillus flavus, Aspergillus penicillioides, Aspergillus niger/tubingensis* and *Cryptococcus neoformans. Other amplified organisms were classified as potential contaminants.*

Conclusion: Our results were in accordance with previously conducted studies and in combination with conventional laboratory methods panfungal PCR can increase diagnostic yield, especially in culture-negative samples.

O-03

Candida auris fungemia outcomes in hospitalized patients: a retrospective single center study from Pakistan

Hafsa Khan Tareen[1], Samavia Mehmood[2], Joveria Farooqi[2], Sadaf Zaka[2], Kauser Jabeen[2]

1 Medical College, 2 Dept. Pathology & Laboratory Medicine , Aga Khan University,

Introduction: *Candida auris* is a recognized clinically devastating multi-drug-resistant nosocomial organism. Our center was one of the first in the world to report the emergence of *C. auris* as an organism with outbreak potential. We now present clinical factors that affect the outcomes of patients with *C. auris* candidemia during 2017 to 2022, after the initial outbreak.

Methods: This retrospective study was conducted on hospitalized patients at the Aga Khan University Hospital, Pakistan from 2017 to 2022. Frequencies for different risk factors were computed in patients with *C. auris* fungemia. Odds ratio for different age groups, comorbid conditions, ICU stay, and antifungal treatment were compared, keeping death as the main outcome.

Results: Over the study period, 154 patients with *C. auris* fungemia were identified from the laboratory database, of whom 33.11% died. Range of age was 1 month to 89 years, mean length of hospital stay was 17 days (±17.75), mean ICU stay was 4 days (±10.09), mean duration of antifungal treatment was 12 days (±28.57), and mean days for microbiological clearance was 6 days (±8.50). The rate of resistance against fluconazole was 90% in 76 patients whose MIC interpretation was available, and no resistance was found against amphotericin and anidulafungin.

Older adults and elderly were two times more likely to die than young adults (ages 18-34). The odds of death were three times higher with ICU stay (3.26, CI:1.52-7.02) and malignancy (3.18, CI:1.29-7.88). The odds of death though higher, did not achieve significance with diabetes (1.72, CI:0.86-3.42), antifungal

commencement after a positive culture (2.01, CI: 0.98-4.12), hypertension (1.35, CI: 068-2.65), and CLD (1.48, CI:0.53-4.15). Prior surgery, immunosuppressants and indwelling catheters or lines were equivocal for outcomes.

Conclusion: Nosocomial *Candida auris* fungemia was found to be associated with death in older age groups. Patients admitted to the ICU and with underlying malignancy are 3 times more likely to have death as an outcome. Diabetes, malignancy and chronic liver disease may predispose to an increased risk of death. Starting antifungals after waiting for culture to turn positive may also increase the probability of death.

Keywords: *Candida auris*; candidemia; death; ICU, malignancy.

O-04

Establishing a Rare Registry for Inherited Metabolic Disorders at the Biochemical Genetic Lab in Pakistan: A Decade of Data

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Dept. of Pathology and Laboratory Medicine, Aga Khan University

Introduction: Metabolomics is the study of unique chemical fingerprints that specific cellular processes leave behind. Generally, metabolomics includes study of small molecules (<1.5 Kda) and is also called “Small Molecule Profiling”. Metabolomics provides analysis of both “endogenous” e.g., amino acids, lipids, cofactors, nucleotides, sugars, hormones, etc. as well as “exogenous” metabolites e.g., drugs, toxins, environmental contaminants, pesticides, herbicides, etc. The commonly used samples are biofluids like saliva, blood, urine and faeces. Metabolic analyses at first relied upon nuclear magnetic resonance (NMR), although recent improvement in the field of mass spectrometry (MS) and Tandem MS has opened broader horizons for research, service and education. In this overview, the research domain of Mass spectrometry was explored with Chemical Pathologists perspective from Pakistan.

Methods As a baseline, search engine *Pakmedinet* was searched using key words "Metabolomics", "Mass spectrometry", "Pakistan" with no date and time restrictions and the search was further refined and only articles having Chemical Pathologists as authors were included. Review articles, abstracts only, case reports, case series and frequency reports were excluded.

Results: The literature review yielded a total of 1167 articles of which 1155 non-relevant articles were excluded and 12 were included. Majority of the publications were case series and retrospective lab data with no significant efforts focused on high yield exploratory research using MS.

Conclusion: The review revealed MS utilization for method development and biomarkers evaluation from the basic biological sciences group and pharmaceutical industry in Pakistan, but scattered efforts in silos lacking clinical utilization in liaison with Chemical Pathologists. It is high time for Pakistan to utilize MS advances for research and development, as it is the driving force behind service and education. As a way forward, newborn screening, inherited metabolic disorders diagnostics, heavy metals analysis and toxicology are the domains to pursue research for Chemical Pathologists in the country. To serve the purpose, formulation of working groups, establishing liaisons with institutes having expertise and provision of funding opportunities under the umbrella of PSCP is required.

Keywords: Metabolomics, Mass spectrometry, Pakistan

O-05

Aspergillus flavus and azole resistance in clinical and environmental isolates from Pakistan

Adan Zubair, Sadaf Zaka, Tooba Muhammad Rahim, Amna Irfan, Joveria Farooqi, Najia Ghanchi, Kauser Jabeen

Aga Khan University Hospital, Karachi, Pakistan

Introduction:

Determine itraconazole, voriconazole and posaconazole MICs in A. flavus isolated from clinical and environmental specimens using broth microdilution technique. Detection of mutations in CYP 51 gene in clinical and environmental strains of A. flavus.

Methods:

EUCAST recommendations were followed for agar screening of isolates to VOR, ITR and POS. MICs of 233 (159 clinical and 74 environmental) *A. flavus* strains from different regions of Pakistan were determined by BMD using the Clinical and Laboratory Standards Institute (CLSI) M38 guidelines. The results were interpreted according to the epidemiological cut-off values (ECVs) established in 2022 by CLSI. The *cyp51A* gene of 36 samples (22 clinical and 14 environmental) was amplified and sequenced.

Results:

Upon Agar Screening, 169/177 (95%) showed no growth on any azoles, while 2/177 (1.1%) showed growth with posaconazole, 6/177 (3.3%) with itraconazole. The MICs determined by BMD, showed all MICs below ECVs in Itraconazole, Voriconazole, and Posaconazole (except 4 isolates). Several synonymous and non-synonymous point mutations were found in our strains. Among the synonymous mutations found in *cyp51A*, C132T, C165T, C342T, G390A, T723C, T907C, T927C, C1065T, T1368C, G13671, have been reported in the literature. These mutations are reported in both sensitive and resistant strains, and their role in azole resistance is still unknown. Among the non-synonymous mutations identified in cyp51A, only K322N has been reported in previous studies, although it is not associated with resistance.

Conclusion:

Overall sensitivity in antifungal susceptibility profile was observed to azoles among the tested isolates, as evident from both agar screening and MIC determination by BMD. Although several synonymous and non-synonymous mutations were

identified in the *cyp51A* gene, the majority including those reported in the literature displayed no clear association with azole resistance. Overall, our findings necessitate further investigation to elucidate the role of identified mutations in antifungal response and future antifungal resistance surveillance studies are needed to monitor the emergence of azole resistance in *A. flavus*.

O-06

The Antifungal Susceptibility Testing of Dermatophytes from Pakistan by Broth Microdilution Method

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Introduction:

Terbinafine resistance in dermatophytes has been recently reported in many countries. Similarly higher itraconazole and voriconazole minimum inhibitory concentrations in dermatophytes have also emerged. Antifungal susceptibility profile data of dermatophytes from Pakistan is limited and needs evaluation. Aim was to determine the antifungal susceptibility patterns of dermatophytes against terbinafine, itraconazole, and voriconazole with broth microdilution test according to EUCAST method E.Def 11.0.

Method:

This study was conducted in Aga Khan University Hospital, Karachi, Pakistan. Dermatophyte isolates were identified using phenotypic methods. Antifungal susceptibility testing for terbinafine, voriconazole, and itraconazole was performed using broth microdilution method by modified EUCAST methodology. Chloramphenicol with a stock concentration of 100ug/ml, was supplemented in the inoculum suspension to avoid bacterial contamination. All antifungal drugs were prepared with ranges of 8-0.03 ug/ml. Plates were incubated for 5-7 days at 25^{o}C without agitation. Spectrophotometric readings at 450nm using 50% inhibition were compared with visual readings of the plates. The results were interpreted to

distinguish wild-type (WT) isolates from non-WT strains, by using ECOFF values as per EUCAST standards.

Results:

Isolates were yielded from skin scrapings predominantly 14/15, followed by skin biopsy 1/15. Patients' ages ranged from 20-71 years. Isolates were identified as "*Trichophytontonsurans"* (46.66%), "*Trichphyton mentagrophytes"* (33.33%), "*Trichophyton rubrum"* (13.33%) and "*Microsporum gypseum"* (6.66%). For the "*Trichophyton tonsurans,"* ECOFF values are not established therefore ECOFF values of "*Trichophyton indotinea"* for its interpretation were used. The MICs ranges and mean Optical Density (OD) using 50% reduction for terbinafine was 0.12->8 μg/ml and 0.353, for itraconazole was 0.06-2 μg/ml and 0.353 and for voriconazole was 0.06-1 μg/ml and 0.338. Most of the isolates (86.66%) had nonwild type against terbinafine. Higher MICs (>8ug/ml) of terbinafine were noted in 6 (40%) isolates. Most isolates had wild-type MICs for itraconazole except 5 isolates (33.3%) that had non-wild-type MICs. All isolates had wild-type MICs for voriconazole.

CONCLUSION:

We are reporting preliminary results of susceptibility testing of dermatophyte species from Pakistan. Molecular identification of isolates through ITS and resistance determination through SQLE genotyping is in process.

O-07

Detection of Acute Tropical Fever Pathogens in Blood Samples Using Multiplex PCR Diagnostic Kit.

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INTRODUCTION:

Fever is a common presentation of infections in low-income countries often linked to seasonal variations and tropical infections. Diagnosing tropical infectious diseases relies on clinical assessment, posing challenges due to nonspecific symptoms. Pakistan has witnessed seasonal outbreaks of dengue, chikungunya, extensively drug-resistant Salmonella Typhi,also accounts for 98% of the region's total malaria burden among seven Eastern Mediterranean Region countries. Accurate diagnosis is crucial to avoid excessive use of antibiotics and ensure timely treatment. The development of commercial panel-based testing has led to significant improvements in targeted therapy and antimicrobial stewardship. The introduction of a commercial panel Fast Track Diagnostics Luxembourg S.à.r.l. detects dengue virus, *Rickettsia* spp., Salmonella Typhi and Para Typhi, West Nile virus, *Plasmodium* spp. , chikungunya virus and *Leptospira* spp. on whole blood claim the early diagnosis and management of common tropical infections in Pakistan. The aim was to validate the use of this rapid test for diagnosing common tropical infections i.e. dengue, malaria, and typhoid in Pakistan, and to understand the demographic characteristics of patients with these infections.

Method:

Patients with fever presenting to Aga Khan University clinical laboratories were recruited for the study. Nucleic acid was extracted using the Qiagen protocol and Multiplex real-time PCR using Fast Track tropical fever kits. Validated tests for malaria dengue and typhoid were used as comparisons.

Results:

Study revealed male patients under 2-10 years old had fever as their main symptom. They mostly had short-lived fever and took antimicrobials before testing. Of 1123 samples collected over one year, 400 blood samples were randomly selected to perform PCR. Of these 400 PCR tests, 211 were positive. Sensitivity and specificity

of malaria, dengue, and salmonella were 92.40% 98.41%, 35.29% 94.57%, 25% 93% respectively.

Conclusion:

Our study found promising results for tropical diseases like malaria. We correlated our tests with clinical symptoms, showing the relevance of positive PCR tests in symptomatic patients. This highlights the need for syndromic panel testing for timely diagnosis and management. Our results demonstrated the highest predictive values for malaria, followed by dengue and salmonella. These findings provide useful insights for healthcare practitioners in the region.

Keywords: Tropical fever, syndromic testing, malaria,dengue

O-08

Title: Chronic Myeloid Leukemia in a Patient with Beta Thalassemia Major– A Rare Presentation

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Abstract:

Introduction:

β-thalassemia major is a genetic hemoglobinopathy characterized by point mutations of β-globin chain resulting in accumulation and deposition of unpaired β-globin chains in red blood cells(RBC). Longer life expectancy in thalassemia patients due to availability of better treatment options has resulted in the emergence of various health issues, including different forms of malignancies. We report a case

of Chronic Myeloid Leukemia occurring in a patient with beta thalassemia which is a very rare presentation.

Case Report:

A 24-year-old girl presented in Hematology Clinic with complains of weakness and lethargy. She was diagnosed with β-thalassemia major at the age of 6 months and was on regular blood transfusions since then, in addition to oral iron chelation therapy. Complete Blood Count (CBC) done showed raised white blood cell count . Peripheral film showed hyperleukocytosis, nucleated RBCs, low platelets, and 3% blast cells. Ultrasound abdomen showed Hepatosplenomegaly with a spleen of 17.6cm.

Thus, Bone Marrow biopsy was performed which was suggestive of Chronic Myeloid Leukemia in chronic phase. Bone marrow chromosome testing revealed a translocation between chromosomes 9q34 and 22q11.2, resulting in the Philadelphia chromosome, found in all cells. A FISH assay for BCR-ABL1 translocation was conducted, and BCR-ABL1 translocation was detected in 98% of the 200 nuclei examined.

She was managed on lines of Chronic Myeloid Leukemia initially with .Imatinib 400mg once daily. However, Early Molecular Response was not achieved with Imatinib and she also developed also developed severe thrombocytopenia with it. She was then switched to Ponatinib, a third-generation tyrosine kinase inhibitor. Upon which she responded very well and also achieved Early Molecular Response.

Conclusion:

In conclusion, thalassemia patients are at increased risk of developing hematological malignancies. This emphasizes the need to create screening, diagnostic, and treatment plans to avoid additional health problems in thalassemia patients. Therefore, it is crucial not to overlook patients presenting with worsening symptoms.

Key Words: Beta Thalassemia, Hematological Malignancy, Leukemia

O-09

Comparative Analysis of HbA1c Proficiency Testing: A Three-Year Evaluation of Method Performance Using Advia 1800 and Cobas 503 Systems

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Background

The HbA1c proficiency survey (GH5I) by the College of American Pathologists (CAP) is a critical tool for evaluating the performance of clinical laboratories in HbA1c testing. From 2022 to 2024, three surveys were conducted annually, each consisting of five specimens. The study aimed to compare the Standard Deviation Index (SDI) and Coefficient of Variation (CV) for all specimens across these years, particularly focusing on the transition from the Advia 1800 to the Cobas 503 system.

Method

In this study, we analyzed the SDI and CV across all surveys conducted from 2022 to 2024. Initially, from 2022 to 2023, specimens were processed using the Advia 1800 system. Due to the lack of a specific CAP peer group for this system, the results were compared against all methods reported in the CAP survey. In 2023, the laboratory transitioned to the Cobas 503 system, which included access to a dedicated peer group.

Results

The analysis revealed distinct differences in the performance metrics between the two systems. For the Advia 1800 system, the Standard Deviation Index (SDI) ranged from -2.06 to -0.28, indicating a wider variability and potential bias compared to the peer group average. In contrast, the Roche Cobas 503 system

demonstrated an SDI range of 0.04 to 0.88, reflecting a closer alignment with the peer group and improved accuracy. Regarding the Coefficient of Variation (CV), the Advia 1800 system exhibited a CV range of 3.06% to 4.56%, suggesting greater variability in test results. The Roche Cobas 503 system, however, showed a reduced CV range of 2.58% to 3.00%, indicating more consistent and reliable performance.

Conclusion

The study underscores the importance of method selection and the availability of peer group comparisons in proficiency testing. The findings suggest that the transition to the Cobas 503 system enhanced the accuracy and consistency of HbA1c testing in our laboratory, as reflected in improved SDI and CV values. This work contributes to ongoing efforts to improve the quality of HbA1c testing in clinical laboratories.

O-10

An Introduction to Homocystinuria with Reference to the Prevalence and General Pathophysiology of the Disease

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Abstract

Background: This study was done to determine the frequency of Homocystinuria disorders diagnosed on plasma amino acid (PAA) analysis and identify the clinical spectrum and biochemical findings of patients presenting with homocystinuria.

Methods: It was a Cross-sectional study, conducted by Departments of Pathology and Laboratory Medicine and Department of Paediatric and Child Health, Aga Khan University Hospital from Jan 2013-Dec 2023.

Patients with Homocystinuria identified on PAA were stratified into five groups; (a) High Methionine group (Cystathionine beta synthase deficiency, GNMT, SAHH, MAT, ADK): high methionine and low cystine and high serum total homocysteine (tHcy), normal serum B12 and folic acid (FA), (b) High Methionine group (Cobalamin-related remethylating disorders): elevation in urine methylmalonic acid, high tHcy, normal serum B12 & FA. Data was analyzed by Microsoft Excel 2021.

Results: There were 174 patients of which 93 (53%) were males. Mean age was 4.68 years. All of them had HCU due to some underlying cause.

The main clinical features we looked at were mental retardation, seizures, lethargy, developmental delay and failure to thrive. The data for the aforementioned pathologies was recorded from the patients' medical forms.

During the study period, 1910 PAA and UOA were analysed and 43 patients with homocystinuria were detected. Median age was 11.4 months (interquartile range-IQR 6-41.5) and 56% were female. On stratification into type of disorders, 37%(n=16) had homocystinuria due to CBS, 30%(n=13) had Cb1-RD, nonspecific Homocystinuria in 27%(n=12), while B12 deficiency was noted in 5%(n=2) respectively.

Nineteen patients (44%) out of 43 were followed by metabolic physician while the rests were outside referrals, followed by general paediatricians. The mortality rate in patients followed by paediatricians was high [43% (9/22) vs. 5% (1/19)] patients followed by metabolic physician.

Conclusion: Screening tests including PAA, UOA, tHcy, B12 and FA provide valuable clues to the etiology of Homocystinuria.

K**eywords** Cystathionin beta synthase disorder, Methylmalonic Aciduria, Cobalamin related remethylation disorders, Vitamin B 12 Deficiency.

O-11

Infection with Rhodotorula species, an emerging threat!

Sobia Muhammad Asad Khan, Joveria Farooqi, Faheem Naqvi, Naeem Aziz, Rumina Hasan, Kauser Jabeen

Introduction:

The genus *Rhodotorula* includes at least 50 species, of which *R. mucilaginosa*, *R. glutinis, and R. minuta* are known to cause disease in humans. Globally, *Rhodotorula* species have been increasingly identified from clinical specimens rendering it as the 4th most common non-candidal yeasts. In a global review, a total of 204 cases were identified from 24 countries, including China (N=43), Brazil (N=34), Spain (N=30), India (N=16), Italy (N=12), USA (N=13), and Taiwan (N=10). No clinically relevant cases have been reported from Pakistan to date. Thus, we aim to describe patients with invasive *Rhodotorula* infection from Pakistan.

Materials and methods:

Integrated laboratory management system was used to identify *Rhodotorula* species isolated from all samples received at the Aga Khan University Hospital Laboratories, Karachi, Pakistan between January 2010 - March 2024. Demographic

data was collected for all samples and detailed clinical history was evaluated for 9 patients that were admitted to our hospital and deemed clinically relevant. All samples were processed as per ASM (American Society of Microbiology) guidelines and phenotypic identification of yeast was done using conventional methods, API 20C AUX and Vitek 2 Yeast ID card (*bioMerieux*, France). Susceptibilities were performed according to CLSI guidelines by broth microdilution using YeastOne Sensititer plates (Thermo Fisher Scientific).

Results:

A total of 65 cases were identified between January 2010-March 2024, of which 53 (81.5%) were from invasive sites. Invasive infections were mostly blood stream N=36/53 (67.9%) followed by pleural N=6/53 (11.3%), skin and soft tissue N=5/53 (9.4%), CNS N=4/53 (7.5%), abdominal N=1/53 (1.8%), and source could not be determined for one sample. Contamination could not be ruled out in 11.1% (4/36) of blood-stream infections and 88.2% (15/17) of non-blood stream infections with growth of multiple environmental organisms simultaneously and were excluded from analysis. Male to female ratio was 24:10 and 76.4% of the studied population was less than 1 year of age. Detailed clinical findings were evaluated for 9 patients that were admitted at Aga Khan Hospital. Assessment of underlying risk factors revealed that 6/9 patients had central lines, 3/9 received total parenteral nutrition, 2/9 had intestinal perforation, 2/9 had chronic liver disease and diabetes. Three patients received I/V amphotericin for Rhodotorula fungemia and other patients were not treated. The mean duration of treatment was 14.3 days, 7/9 patients were discharged in stable condition and the outcome could not be assessed for 2 patients as they were shifted to another hospital. Serum BDG level was raised in two patients (mean BDG: 417.09). MICs by broth microdilution were performed for 5 isolates and all of them had low MICs to amphotericin (0.12ug/ml).

Conclusion:

Rhodotorula species is one of the rare yeasts and no case has been reported from Pakistan to date. Since it is ubiquitously present in the environment, careful assessment of the clinical evidence and underlying risk factors is crucial for diagnosis. Thus, we present the first clinically relevant Rhodotorula fungemia case series from our region.

O-12

Diagnostic Dilemma of Patients with Homocystinuria: Experience from A Tertiary Care Centre in Pakistan

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Abstract

Background: To determine the frequency of Homocystinuria disorders diagnosed on plasma amino acid analysis.

Methods:

Patients with Homocystinuria 2013 to Dec 2021 results of plasma amino acids. Subjects were stratified into groups; (a) Cystathionine beta synthase deficiency (CBS): high methionine and low cystine and high serum total homocysteine (tHcy), normal serum B12 and folic acid (FA), (b) Cobalamin-related remethylating disorders (Cb1-RD): elevation in urine methylmalonic acid, high tHcy, normal serum B12 & high FA, (c) B12 deficiency (B12 def): serum B12 <200pg/ml, high tHcy, normal urine methylmalonic acid, (d) nonspecific Homocystinuria: high tHcy with normal B12, methionine, and urine methylmalonic acid.

Results:

High risk subjects suspected of having inherited metabolic disorders were screened in BGL. Out of the total 43 cases of homocystinuria were detected. Median age of the cases was 11.4 months (interquartile range-IQR 6-41.5);56% being female. On stratification into type of disorders, 37% (n=16) had homocystinuria due to CBS, 30%(n=13) had Cb1-RD, nonspecific Homocystinuria in 27%(n=12), while B12 deficiency was noted in 5%(n=2) respectively. The mortality rate in patients followed by paediatricians was high [43% (9/22) vs. 5% (1/19)] patients followed by metabolic physician.

Conclusion: Screening tests including PAA, UOA, tHcy, B12 and FA provide valuable clues to the etiology of Homocystinuria.

Keywords :CBS disorder, MMA, Cobalamin related remethylation disorders,B12 Deficiency.

O-13

Frequency of Fluconazole resistant candida species in High Vaginal swabs"

Authors: Ayman Shahid, Kausar Jabeen, Joveria farooqi, Saeeda Chandio

Introduction: Vulvovaginal candidiasis (VVC) is a prevalent issue among healthy women, with over 90% of cases caused by *Candida albicans*. Traditionally, systemic and topical azole therapies, including fluconazole, have been recommended. However, recent observations suggest a decline in the efficacy of these treatments due to emerging resistance. This resistance is potentially attributed to the routine use of azole therapies and over-the-counter antifungal medications.

Objectives: This study aims to determine the frequency of fluconazole resistance in *Candida* species isolated from high vaginal swabs (HVS) and to emphasize the importance of antifungal susceptibility testing for these specimens.

Methods: A prospective observational study was conducted from April 23 to May 16, 2024, at the Microbiology Laboratory of Aga Khan University Hospital, Karachi, Pakistan. A total of 192 HVS specimens from outpatients were analyzed. Candida species were identified using conventional methods, including the Germ Tube test, Chrom Agar, SDA, BIGGY, MYCOSEL Agar, and Urease test. Fluconazole susceptibility was assessed by disk diffusion.

Results: Among the 192 specimens, 134 were identified as *Candida albicans* (69.7%), 45 as *Candida glabrata* (23.4%), 5 as *Candida krusei* (2.6%), and 3 as *Candida tropicalis* (1.5%). Fluconazole resistance was observed in 4 of 134 *C. albicans* isolates (2.98%). For *C. glabrata*, 41 isolates (91.1%) were dose-dependent and 4 (8.8%) were resistant. *C. krusei* showed inherent resistance, while all *C. tropicalis* isolates were sensitive to fluconazole.

Conclusion: The study highlights a notable incidence of fluconazole resistance among *Candida* species, particularly *C. albicans* and *C. glabrata*. This emerging resistance underscores the necessity for antifungal susceptibility testing in patients experiencing recurrent or persistent VVC despite azole therapy. Enhanced diagnostic and therapeutic strategies are essential to manage and mitigate resistance effectively.

Keywords: *C.auris,* Fluconazole, anti-microbial resistance.

O-14

Title: Is there an upsurge of window period blood donations at Pakistan?

Authors: Bushra Moiz, Hasan Hayat, Fatima Farhan, Zeeshan Ansar

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Introduction

According to WHO, 12 million people in Pakistan are suffering from hepatitis B or C, with 150,000 new cases annually. Most infections are acquired through contaminated needles, surgical procedures, or infected blood transfusions. Studies have shown that while HIV seropositivity remains low (<0.2%), hepatitis B and C prevalence is significant at 3% and 4%, respectively. Nucleic acid amplification testing (NAT) is a key method for detecting these infections, but most studies in Pakistan rely on mini pooled samples (MP-NAT), which may miss low viral load cases. More sensitive individual sample testing (ID-NAT) could offer better detection rates.

Methods

This observational descriptive study was conducted at Aga Khan University Hospital, Karachi, and Shifa International Hospital, Islamabad. Data on seroreactivity and NAT-positivity for HIV-1, HBV, and HCV from non-remunerated blood donors over the past 10 years were collected. Comparisons between MP-NAT and ID-NAT for viral detection were made.

Results

The anticipated findings are expected to highlight a rising trend in viral hepatitis and HIV among blood donors, mirroring the broader community's infection rates. These results could demonstrate the superiority of ID-NAT over MP-NAT, particularly in detecting low viral loads of HBV.

Conclusion

This study may provide crucial insights into viral hepatitis trends in Pakistan, emphasizing the need for more stringent blood screening protocols and broader hepatitis B vaccination policies to reduce transmission risks.

Keywords

Hepatitis, NAT, Blood Donors, Pakistan, MP-NAT, ID-NAT

Presentation type: Poster

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O-15

"Yield of Blood Cultures in Children with Febrile Neutropenia at Tertiary Care Hospital, Karachi"

Authors: *Muhammad Mustafa, Ali Faisal Saleem*

Introduction

Febrile neutropenia (FN) poses a significant threat in pediatric oncology, often leading to severe complications due to infections. While historically gram-negative bacteria were the primary culprits, recent trends indicate a shift towards gram-positive cocci. This study aims to evaluate the frequency of positive blood cultures and antibiotic susceptibility patterns among pediatric cancer patients with FN at Aga Khan University Hospital Karachi, aligning with global epidemiological shifts.

Objective

To determine the frequency of positive blood cultures and their antimicrobial susceptibility pattern Among Pediatric Cancer patients with FN at Aga Khan University Hospital Karachi

Methods

Study Design: Cross-sectional Prospective study
Setting: The Aga Khan University Hospital, Karachi
Duration: Six months from 1st October 2022 to 31st March 2023
Subjects: Pediatric oncology patients of either gender less than 18 years admitted with febrile neutropenia were noted.

Results

The study involved 101 pediatric patients with a mean age of 7.94 ±4.18 years, of which 65.3% were males. Blood borne malignancies (primarily leukemias) were present in 76.2% of patients, while 23.8% had solid tumors (various tumors). The

mean time since diagnosis was 7.75 ±8.38 months. The mean ANC count was 488, with 58.4% classified as severe ANC. Positive blood cultures were found in 13.9% of patients, with a higher mean length of hospital stay (7.64 days) compared to those without growth. 57.1% of total positive cultures were gram positive organism, The most prevalent among gram positive organism was staphylococcus not aureus. Antibiotic resistance patterns showed high resistance to Oxacillin, Penicillin, and Cotrimoxazole, while Gentamicin, Amikacin, Piperacillin/Tazobactam and Meropenem exhibited high sensitivity.

Conclusion

This study highlights the changing microbial landscape and antibiotic resistance patterns in pediatric oncology, emphasizing the importance of antibiotic stewardship. By aligning local findings with global trends, it provides valuable insights for optimizing patient care and addressing infectious complications in pediatric cancer patients with febrile neutropenia.

Keywords

Children, Febrile Neutropenia, Blood Culture

O-16

Abstract Dept of Pathology seminar

Temporal mapping of SARS-CoV-2 variants during the pandemic reveals similar genomic divergence in unvaccinated and vaccinated cohorts

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Introduction

Vaccinations against SARS-COV-2 have been critical in preventing death or severe disease during a COVID-19 infection. However, it is not clear whether the spectrum of infecting variants differed between vaccinated and unvaccinated individuals. Here we compared SARS-CoV-2 genomes of COVID-19 positive individuals in 2021 and 2022, studying phylogenetic variations during the period.

Objective

To investigate whether vaccination status affected the type of SARS-COV-2 variants in patients.

Methods

A total of 569 SARS-CoV-2 genomes and corresponding metadata of sequences submitted from Pakistan between May 2021-October 2022 were downloaded from GISAID (www.gisaid.org). Phylogenetic analysis was carried out on Augur pipeline and visualized on Auspice (auspice.us). The pipeline was used to prepare datasets for phylogenetic analysis then aligned with respect to the reference sequence (NC 045,512.2) using the MAFFT alignment tool. An alignment maximum-likelihood tree was generated using iqtree2 tool by applying the substitution model General Time Reversible (GTR). Downstream analysis was done by applying visualization filters based on variables such as vaccination status, country of SARS-CoV-2 exposure and demographics.

Results

A total of 569 genomes with known vaccination status were included in the analysis, of which 167 were from vaccinated and 402 were from unvaccinated individuals. Females were 268 (47.1%). Majority (71%) of the cases were less than 18 years old. 308 (54.1%) and 261 (45.9%) samples were collected in the year 2021 and 2022 respectively.

Overall the Delta variant (B.1.617.2) was the most identified at 24.3% of all the lineages, followed by Omicron (BA.5.2) (15.8%) and BA.2 (11.8%) respectively. The predominant lineage identified in 2021 was B.1.617.2 (44.5% of all samples collected in 2021) and BA.5.2 (33.3%) in 2022.

Comparison analysis shows a similar pattern of divergence in both unvaccinated and vaccinated groups

Conclusion

Our study showed a similar pattern of genomic divergence in SARS-CoV-2 infections in unvaccinated and vaccinated groups. It is possible that COVID-19 severity may have varied between groups and this needs to be studied further. Importantly, we observed a similar spread of SARS-CoV-2 variants regardless of vaccination status.

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Human Leucocyte Antigen Alleles Diversity and Haplotypes Analysis in Related Patients: A Pakistani Retrospective Study

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Abstract

Background

Pakistan is the 5th most populous country globally comprising of 220 million from mainly 12 different South Asian ancestries. HLA typing is used to identify

individuals prior to transplantation. These data can be used to understand genetic variation in the population

Objective

To investigate the spectrum of HLA types and haplotypes in the case subjects in Pakistani population (Aga Khan Hospital, Karachi).

Methods

A retrospective review of HLA typing testing (January 2020 to October 2023) at The Aga Khan University Hospital, Karachi, Pakistan was conducted. The total cohort comprised n= 576 unlinked cases received for HLA typing of which, 28.13 % were females. HLA typing was performed using the Olerup sequence-specific primers HLA-typing kit. Statistical was performed using the Chi-squared analysis.

Results

We observed a high frequency of HLA alleles for Class I; -A*01 (13.11 %), -A*02 (18.75 %), -A*03 (07.73 %), -A*11 (14.76 %), -B*35 (13.11 %), -B*40 (11.02 %), -C*07 (23.52 %), and Class II; -DR*03 (23.87 %), -DR*04 (6.68 %), -DR*07 (09.81 %), -DR*15 (18.32 %), -DQ*02 (29.60 %), -DQ*03 (24.57 %), -DQ*05 (22.14 %), and -DQ*06 (22.22 %). Across the population, the most frequent haplotypes were; A*26 B*8 C*7 at 2.00 % and HLA DR*3-DQ*2 at 23.95 %.

Conclusions

Our analyses revealed the HLA allele and Haplotype frequencies for Class I and Class II and compared with previous studies. Furthermore, various alleles and haplotypes explained the diversity of HLA genetics from the Pakistan population. This highlights a need to fully explore the diversity of genetics in the Pakistani population, through non-related cohort analysis to examine this further.

Keywords: Human Leucocyte Antigen, HLA, Genotyping, Pakistan.

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Decreased frequency of Latent Tuberculosis in patients with increasing COVID-19 disease severity reflects a pan T cell suppression after SARS-CoV-2 infection.

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ABSTRACT

Background

Pakistan ranks 5th in terms of burden of TB with high rates of transmission and latent TB infections (LTBi). LTBi is characterized by the presence of Mtb specific activated T cells in circulation and is considered to be a protective form of tuberculosis. How it impacts other infectious diseases is not well known. Both *Mycobacterium tuberculosis* (Mtb) and Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) are respiratory pathogens. The impact of coexistence of both infections simultaneously is not well understood. Here we analyze the presence of LTBi and its impact on COVID-19 disease outcome.

Methods

Age and sex matched healthy controls (HC, n=147) and COVID-19 patients (n=128) were recruited in either ambulatory (n=103) or hospitalized (n=25) categories. LTBi was determined using X.DOT-TB, an ELISpot assay (figure 1). IgG antibodies to SARS-CoV-2 RBD protein and to Rubella virus were measured

by ELISA and ECLIA respectively. mRNA levels of immune related genes including IFN- γ, as well as proinflammatory IL-6, anti-viral biomarkers; IFN-α, OAS-1, MAVS and, cytokine regulators, SOCS-1 and SOCS-3 in PBMCs of study subjects were measured through RT-PCR. Nonparametric test including Pearson's Chi square and Mann-Witney test was applied to determine the association between parameters tested in both groups in presence and absence of LTBi.

Results

We found lower frequencies of IFN-g secreting T cells in COVID-19 cases (18%) as compared with HC groups (32%; $p<0.0001$). Interestingly, no hospitalized COVID-19 cases showed LTBi positivity. Enumeration of IFN-γ counts to Mtb antigens showed lower responses in COVID-19. The anti-SARS-CoV-2 RBD IgG levels did not show any difference between LTBi positive or negative individuals. We also did not find any difference in IgG levels against Rubella among the study groups. mRNA expression levels of SOCS3- a negative regulator of immune response gene was higher in COVID-19 as compared with HC.

Conclusions

Our results show there is no impact of LTBi on protective responses against SARS-CoV-2. However, lower detection of LTBi among COVID-19 strongly suggests a graded pan T cell suppression with increasing disease severity in COVID 19 as indicated by downregulation of T cell response against a COVID-19 unrelated (Mtb) antigen. Furthermore, it was confirmed by higher expression of SOCS3- a negative regulator of immune response, among COVID-19 patients.

Keywords: COVID-19, Latent tuberculosis, coinfection, T cell downregulation.