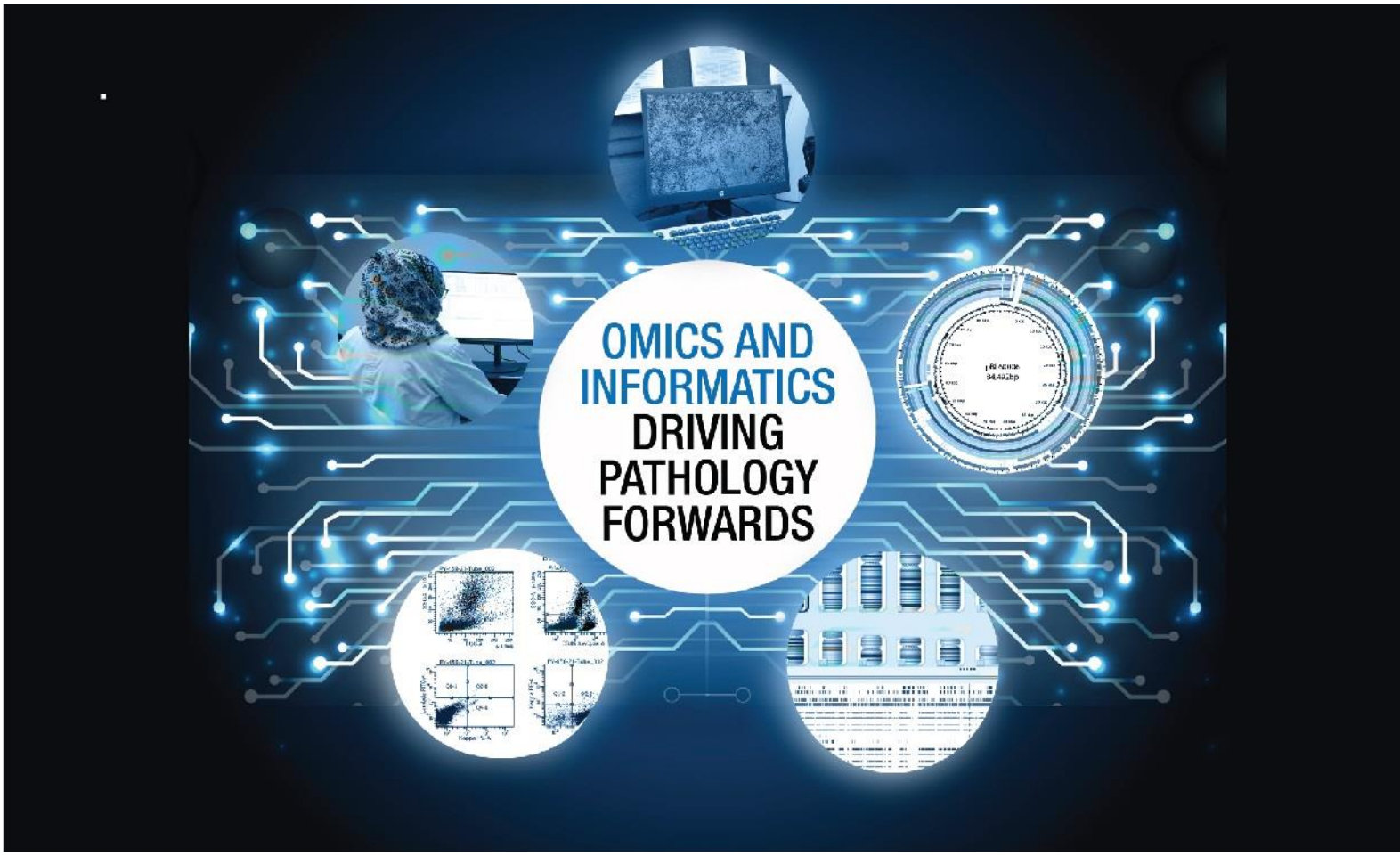


# Pathology and Laboratory Medicine Research Day 2021

Friday, Oct 15, 2021 | 10:00 am - 5:30 pm

# Abstract Book

ACCME ACCREDITED

**Activity Code: AKU-DCPE-CD-0045**

TOPIC: RESEARCH DAY 2021: OMICS AND INFORMATICS - DRIVING PATHOLOGY FORWARDS

The Aga Khan University is accredited by the Accreditation Council for Continuing Medical Education (ACCME) to provide continuing medical education for physicians."

The ACCME designates this live educational activity for a maximum of 5.00 credit hours of AMA PRA category 1 credit™

*The faculty, staff and any individual connected to this activity confirms that this activity do not have any financial relationship with proprietary entities, producing, marketing, re-selling or distributing healthcare goods or services consumed or used on patients*

**Contact:**
Pathology.labmed@aku.edu | 0213 486 4513

Please note: The CME certificate fee of Rps 315/- (300 + SST@ Rps. 15) would be charged. For AKU & outsider both participants Attendance and post event evaluation form is mandatory to get certificate.

آغا خان یونیورسٹی
THE AGA KHAN UNIVERSITY

# Message

We welcome all the attendees to the Department of Pathology and Laboratory Medicine Research Day 2021. This event gives us an opportunity to celebrate research related activities in the department. Our aim is to provide outstanding diagnostic services, achieve excellence in teaching & research, and promote the growth of faculty members, staff, and postgraduate students.

The dynamics of health and education has changed with Covid-19 pandemic. The department of pathology was at the forefront of the fight against the pandemic and its faculty and staff played a major role in the diagnosis and management of Covid, Research related to the pandemic was and is being conducted throughout the world and our department also contributed. A number of research studies on Covid-19 were published by various sections of the department and more are in process. To change in the norms of life such as social distancing during this pandemic has created an urgent need for acquiring digital technology more than ever. Currently most meetings and conferences are being held online. Artificial intelligence (AI) is an emerging field and already facilitating physicians in many ways. Pathology Informatics is the study and practice of data acquisition, storage, processing, retrieval, analysis, and presentation to facilitate pathology workflow and increase the accuracy and value of the information contained within the data.

Realizing the great importance and need for Informatics, Department of Pathology & Laboratory Medicine, Research Committee decided the theme of Research Day 2021 as "Omics and Informatics: Driving Pathology Forwards" to create awareness among pathologists & clinical colleagues. The four International Speakers will update us on Omics and Artificial Intelligence in practice, ethics and training in Informatics and Digital Pathology. Our Chief Information officer will talk about Electronic Health records followed by a presentation on tracking system in pathology by Section Head, Histopathology.

An important segment of our program is a Round Table Discussion with all Section Heads and Chair of the Department and Service Line Chief to determine future directions. This will be followed by Turbo Talks covering omics and AI by local speakers.

We hope that this activity will serve as a platform for the entire AKU academic community to connect, collaborate and share ideas for further learning, research and leadership. We invite everyone to join and encourage young enthusiastic researchers and offer them suggestions. It is important to acknowledge their contributions to the university and society.

In the end, we would like to thank all those who participated in the planning phase and worked tirelessly to organize this event and make it a success.

Thank you. Please enjoy the program.

**Dr. Nasir Ud Din**
Associate Professor & Chair,
Departmental Research Committee

**Dr. Afia Zafar**
Professor & Chair,
Department of Pathology and Laboratory Medicine

# Members of Organizing Committee

## Research Day 2021

## Chair, Organizing Committee

| | |
|---|---|
| Dr. Nasir Ud Din | Histopathology |

## Resource Person

| | |
|---|---|
| Dr. Rumina Hasan | Microbiology |
| Dr. Bushra Moiz | Haematology Transfusion and Medicine |
| Dr. Erum Khan | Microbiology |

## Members

| | |
|---|---|
| Dr. Bushra Moiz | Haematology & Transfusion Medicine |
| Dr. Anila Rashid | Haematology & Transfusion Medicine |
| Dr. Joveria Farooqi | Microbiology |
| Dr. Najia Ghanchi | Molecular Pathology |
| Dr. Kiran Iqbal | Molecular Pathology |
| Dr. Qurratulain Chundriger | Histopathology |
| Dr. Hafsa Majid | Chemical Pathology |
| Dr. Mustafa Aslam | Forensic Medicine |
| Ms. Shamsha Punjwani | Pathology and Laboratory Medicine Office |
| Ms. Amna Nasir | Research Office |
| Ms. Gulnar Zafar Ali | Pathology and Laboratory Medicine Office |
| Ms. Mahek Anwar Ali | Pathology and Laboratory Medicine Office |
| Ms. Sarah Baber | Research Office |
| Ms. Shahnila Alidina | Research Office |
| Ms. Asia Khan | Research Office |
| Ms. Bushra Fordil | Research Office |

# Research Day 2021

**Department of Pathology and Laboratory Medicine**

**Friday, October 15, 2021| 10:00AM-5:00PM | Hybrid, Aga Khan University, Karachi**

## Theme: Omics and Informatics - Driving Pathology Forwards

**Friday, Oct 15, 2021, | Hybrid, Aga Khan University, Karachi**

## Programme at a Glance

| Time | Programme |
|---|---|
| 10:00 – 10:05 | **Tilawat and National Anthem** |
| **Welcome Address** | |
| 10:05 – 10:15 | **Opening Remarks & Program Introduction**<br>***Dr. Nasir Ud Din***<br>Chair Departmental Ethical and Research Committee<br>Department of Pathology and Laboratory Medicine |
| 10:15 – 10:20 | **Welcome Address**<br>***Prof. Mushtaq Ahmed***<br>Vice Dean Medical College, Aga Khan University |
| **Keynote Addresses** | |
| 10:25 – 11:10 | **Covid-19 Pandemic Emphasized the Emerging Role of Omics, Artificial Intelligence, and Digital Laboratory Medicine**<br>***Prof. Dr. Sergio Bernardini***<br>University of Tor Vergata, Rome, Italy |
| 11:10 – 11:40 | **Pathology Education, advocating for the Pathology pipeline and digital communications**<br>***Dr. Kamran Mirza***<br>Loyola University Chicago |
| 11:40 – 12:10 | **Validating Artificial intelligence (AI) for Pathology Practice**<br>***Dr. Liron Pantanowitz***<br>University of Michigan |
| 12:10 – 12:40 | **Ethics and training in informatics**<br>***Ulysses G J Balis***<br>***University of Michigan*** |
| 12:40 – 2:00 | **Lunch and Prayer Break** |
| 2:00 – 2:20 | **Electronic Health Record System (EHRS)**<br>***Dr. K. Nadeem Ahmed***<br>***Chief Medical Information Officer – Global***<br>***The Aga Khan University & Hospitals*** |
| 2:20 – 2:40 | **Where do we stand in Pathology Informatics: Our Experience!**<br>***Dr. Arsalan Ahmed***<br>***Associate Professor – Histopathology***<br>***The Aga Khan University & Hospital*** |

| | |
|---|---|
| 2:40 – 3:45 | **Advancements in Aga Khan Clinical Laboratory: Challenges and Success Factors**<br>Roundtable Discussion with Chair, Service Line Chief and Section Heads of Chemical Pathology, Hematology and transfusion Medicine, Histopathology, Microbiology and Molecular Pathology<br>***Moderator: Dr. Joveria Farooqi*** |
| 3:45 – 4:00 | **Break** |
| 4:00 – 4:05 | **Turbo Talk 1:**<br>Ups and Down of COVID-19: Can We Predict the Future? Utility of Google Trends to Forecast the Burden of COVID-19 in Pakistan<br>***Dr. Muhammad Abbas - Pathology & Laboratory Medicine*** |
| 4:05 – 4:10 | **Turbo Talk 2:**<br>Use of Artificial intelligence in health diagnostics - A validation study on chorionic villi<br>***Dr Zehra Talat – Jinnah Sindh Medical University*** |
| 4:10 – 4:15 | **Turbo Talk 3:**<br>Urinary metabolomics using Gas Chromatography Mass Spectrometry as potential biomarkers for autism spectrum disorder<br>***Dr Zaib Un Nisa – Pathology & Laboratory Medicine*** |
| 4:15 – 4:20 | **Turbo Talk 4:**<br>Chikungunya outbreak in Karachi Pakistan 2016-2017: An analysis of viral isolates<br>***Dr. Erum Khan - Pathology & Laboratory Medicine*** |
| 4:20 – 4:25 | **Turbo Talk 5:**<br>Systematic Identification and Differential Expression Profiling of Preterm Birth-Associated Plasma ncRNAs in LMICs Cohort of Pregnant Women<br>***Dr Waqasuddin Khan - Pediatrics and Child Health*** |
| 4:25 – 4:30 | **Turbo Talk 6**<br>Introduction of SARS-CoV-2 variant B.1.1.7 in Pakistan through travelers arriving from UK<br>***Dr Asghar Nasir - Pathology & Laboratory Medicine*** |
| 4:30 – 4:35 | **Turbo Talk 7**<br>Upregulated type I interferon responses with downregulated anti-inflammatory genes in transcriptomes of individuals with Asymptomatic COVID-19 in Pakistan<br>***Dr Kiran Iqbal-- Pathology & Laboratory Medicine*** |
| 4:35 – 4:40 | **Turbo Talk 8**<br>Lipid A-Ara4N as an alternate pathway for (colistin) resistance in Klebsiella pneumonia isolates in Pakistan<br>***Dr Seema Umar – Ex Resident – Pathology & laboratory Medicine*** |
| 4:40 – 4:45 | **Turbo Talk 9**<br>Increasing IgG antibodies to SARS-CoV-2 in asymptomatic blood donors through the second COVID-19 wave in Karachi associated with exposure and immunity in the population<br>***Dr Hassan Hayat - Pathology & Laboratory Medicine*** |
| 4:45 – 4:50 | **Closing and Vote of Thanks**<br>***Dr. Najia Ghanchi*** |

# Keynote Speaker

***Sergio Bernardini***
***Professor in Clinical Biochemistry, MD, PhD***
***University of Tor Vergata, Rome***

## ***Covid-19 Pandemic emphasized the emerging role of artificial intelligence, mobile health, and digital laboratory medicine.***

SARS-CoV-2, the new coronavirus causing COVID-19, is one of the most contagious disease of past decades. COVID-19 is different only in that everyone is encountering it for the first time during this pandemic. The foremost challenge that the scientific community faces is to understand the growth and transmission capability of the virus. As the world grapples with the global pandemic, people are spending more time than ever before living and working in the digital milieu, propelled to an unprecedented level especially as AI has already proven to play an important role in counteracting COVID-19. AI and Data Science are rapidly becoming important tools in clinical research, precision medicine, biomedical discovery, and medical diagnostics. Machine learning (ML) and their subsets, such as deep learning, are also referred to as cognitive computing due to their foundational basis and relationship to cognition. To date, AI based techniques are helping epidemiologists in projecting the spread of virus, contact tracing, early detection, monitoring, social distancing, compiling data and training of healthcare workers. Beside AI, the use of telemedicine, mobile health or mHealth and the Internet of Things (IOT) is also emerging. These techniques have proven to be powerful tools in fighting against the pandemic because they provide strong support in pandemic prevention and control. This presentation highlights applications and evaluations of these technologies, practices, and health delivery services as well as regulatory and ethical challenges regarding AI/ML-based medical products.

***Kamran M. Mirza,***
***Associate Professor of Pathology and Laboratory Medicine***
***Loyola University Chicago***

## ***Pathology Education, Advocating for the Pathology Pipeline, Digital Communications and Informatics of Pathology***

Dr. Mirza will highlight the state of pathology education and how digital communications can help promote the dwindling pipeline to this field. He will discuss novel pedagogical tools such as pathelective.com and the creation of the digital communications fellowship in pathology.

***Liron Pantanowitz***
***Director, Division of Anatomic Pathology A. James French Professor of Pathology***
***University of Michigan***

## ***Artificial intelligence in practice***

Recently there has been much hype regarding the application of Artificial intelligence (AI) tools in healthcare. Efforts that couple whole slide imaging (WSI) with advanced computation and deep learning methods have incited much interest in applying AI to pathology. A plethora of studies about AI in pathology have been published. These AI algorithms have been used to detect rare events, automatically quantify features in digital images, diagnose diseases from WSI, and even make prognostic predictions by directly analyzing pixels. Despite these advances there are currently very few laboratories using these AI tools in routine pathology practice. The objectives of this presentation are to:

- Provide an overview of machine and deep learning methods relevant to pathology.
- Discuss the applications, benefits, and barriers of using AI in Anatomical Pathology.
- Share lessons learned from developing, deploying, and validating such AI algorithms

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# Research Day Abstracts

## 1.0 Turbo Talks | Omics & Artificial Intelligence

### 1.1 Ups & downs of COVID-19: Can We Predict the Future? Utility of Google Trends to Forecast the Burden of COVID-19 in Pakistan

*Muhammad Abbas Abid, Sibtain Ahmed, Maria Helena Santos de Olivera, Zeeshan Ansar Ahmed, Imran Siddiqui, Lena Jafri*
**Section of Chemical Pathology, Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi, Pakistan*

**Aim:** The ability to forecast changing trends of COVID-19 can help drive efforts to sustain the increasing burden on healthcare system. We aim to study the utility of Google trends search history data and demonstrate if a correlation exists between search data and actual COVID-19 cases and if the data can be used to forecast patterns of testing and disease spikes.

**Methods:** Weekly data of COVID-19 tests performed and positive cases for Pakistan was retrieved from online COVID-19 data banks for a period of 60 weeks. Search history related to COVID-19, corona virus and the most common symptoms of the disease was acquired from Google trends for the same period. Statistical analysis was performed to analyze the cross-correlation between the two data sets. Search terms were adjusted for time lag in weeks to find the highest cross-correlation for each search term.

**Results:** A total of 10,066,255 SARS-CoV-2 diagnostic tests were analyzed. Search terms of 'fever' and 'cough' were the most searched online, followed by coronavirus and covid. The highest peak cross-correlations with the weekly case series, at a lag of 1 week was noted for loss of smell and loss of taste. The combined model also yielded a modest performance for forecasting positive cases. The linear regression model revealed loss of smell (adjusted R2 of 0.7) with the significant 1-week, 2-week and 3-week– lagged time series, as the best predictor of weekly positive case counts. Search terms of fever, headache and shortness of breath displayed statistically significant correlation with total number of tests performed with a 1-week time lag

**Conclusion:** Google trends can serve as a vital tool for predicting pandemic pattern and pre hand preparedness in such unprecedented crisis. The information can be used to for careful planning and arrangements to meet increased diagnostic and healthcare demands.

### 1.2 Use of Artificial intelligence in health diagnostics- A validation study on chorionic villi

*Talat Zehra, Asma shaikh, Nighat Jamal, Asma Shabbir,* Binis*h Arif, Nausheen Ferozuddin*
**Section Histopathology, Department of Pathology and Laboratory Medicine, Jinnah Sindh Medical University, Karachi,Pakistan*

**Introduction:** The ever-increasing use of digitization and artificial intelligence has left its impact on every field of life. Since the last two years, due to Covid-19 pandemic, the world has witnessed an exponential digital adoption in every field of life. With the aid of Artificial intelligence, the work of pathologist can become much easier, as these techniques can accurately quantify, measure and picked up small pathologies which can be missed by pathologist if he is overworked. In this study we validated the diagnosis of chorionic villi by the help of image analysis software and compare it with manual diagnosis.

**Material & Method:** We took 60 slides of previously diagnosed cases of products of conception according to College of American Pathologist guidelines. Glass slides were scanned into digital slides using microscope camera at 4x objective (0.1 um pixel). Digital slides were imported into Aiforia Oy software for all subsequent steps, including annotation, training, and analysis of digital slides. The software was trained for various size and shapes chorionic villi.

**Result:** Out of 60 cases of previously diagnosed cases, the software was able to diagnose 50 cases correctly. The concordance was 83.33%.

**Conclusion:** Though, digital pathology and use of artificial intelligence in image analysis are relatively newer and novel techniques but now there is growing adoption of these techniques worldwide. So it is the right time for the people of developing world to adopt these novel techniques as it can overcome the scarcity of pathologists all over the world in general and in developing world particularly. The results of this study were encouraging. We did some pilot projects on malarial parasites and blast cells identification on hematology cases. We have certain limitations because of the unavailability of digital microscope or a pathology slide scanner.

### 1.3 Urinary Metabolomics using Gas Chromatography Mass Spectrometry as potential biomarkers for autism spectrum disorder

*Zaib un Nisa, Prem Chand, Hafsa Majid, Sibtain Ahmed, Aysha H Khan, Azeema Jamil, Lena Jafri*
**Section Chemical Pathology, Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi,Pakistan*

**Background:** Diagnosis of autism spectrum disorder (ASD) is generally made phenotypically and hunt for ASD-biomarkers continues. The purpose of this study was to compare urine organic acids profiles of ASD versus typically developing (TD) children to identify potential biomarkers for diagnosis and exploration of ASD etiology.

**Methods:** This descriptive cross-sectional study was performed in the Section of Chemical Pathology, Department of Pathology and Laboratory Medicine in collaboration with Department of Pediatrics and Child Health, Aga Khan University, Pakistan. Random urine samples were collected from children with ASD diagnosed by pediatric neurologist based on DSM-5 criteria and TD healthy controls from August 2019 to June 2021. The urine organic acids were analyzed by Gas Chromatography Mass Spectrometry. To identify potential urinary biomarkers for ASD canonical linear discriminant analysis was carried out for the organic acids, quantified in comparison to internal standard.

**Results:** A total of eighty-five subjects were enrolled in the current study. The mean age of the ASD (n=65) and TD groups (n=20) were 4.5±2.3 and 6.4±2.2 years respectively with 72.3% males in ASD group and 50% males in TD group. Parental consanguinity was 47.7% and 30% in ASD and TD groups, respectively. The common clinical signs noted in children with ASD were developmental delay (70.8%), delayed language skills (66.2%) and inability to articulate sentences (56.9%). Discriminant analysis showed that 3-hydroxyisovalericc, homovanillic acid, adipic acid, suberic acid and indole acetic were significantly different between ASD and TD groups. The biochemical classification results reveal that 88.2% cases were classified correctly into 'ASD', or 'TD' groups based on the urine organic acid profiles

**Conclusions:** The urine organic acids that were good discriminators between ASD and TD groups were 3-hydroxy isovaleric acid, homovanillic acid, adipic acid, suberic acid and indole acetic. The discovered potential biomarkers could be valuable for future research in children with ASD/

### 1.4 Chikungunya outbreak in Karachi Pakistan 2016-2017: An analysis of viral isolates

*Erum Khan, Dhani Prakoso, Kelli Barr, Kehkashan Imtiaz, Joveria Farooqi, Maureen Long*
**Section Microbiology, Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi, Pakistan*

**Introduction:** Chikungunya virus (CHIKV) is a mosquito-borne alphavirus that produces severe polyarthralgia, joint pains, and rash. Over the past 10 years CHIKV has emerged in many new regions such as Europe, the Western Hemisphere, and the Pacific Islands. Between December 19, 2016 and February 22, 2017, an outbreak of CHIKV has been reported in Pakistan including Karachi with 818-suspected cases, while 107 confirmed cases were reported in July 2017. CHIKV was subsequently isolated from three patients presenting with headache, arthralgia, myalgia, and vomiting. Prior to this outbreak, there were no data for sequences of CHIKV from Pakistan in GenBank.

**Objective**: This study was performed to identify which arboviruses (DENV, WNV, Japanese Encephalitis virus (JEV), and CHIKV) were the cause of acute undifferentiated febrile illness in the Sindh region of Pakistan.

**Study Design:** A cross-sectional study was performed which targeted 1000 patients (250/year), from April 2015 to July 2017, which included male and female patients between the ages of 10 to 86 years meeting the enrollment criteria.

**Result:** PCR for sequencing was performed using Three RT PCR positive serum samples (Karachi-1, Karachi-2, and Karachi-3) were chosen for DNA sequencing of the envelope (E) 1 gene of the CHIKV. Sequence analysis indicated that the CHIKV isolated from three patients in Karachi were of the East Central South African (ECSA) lineage. This corresponds to other sequences of CHIKV isolated during Pakistan and India outbreaks in 2016. Based on part of the E1 region sequenced (567 bp), sequences Karachi-1 (Genebank: MG516709) and Karachi-2 (Genebank: MG516710) had one nucleotide difference (99.8% similarity) compared with other CHIKV isolates from Pakistan, with the exception of CHIKV strain 01/2016 (Genebank: MF7746113) which had two nucleotides' differences (99.6% similarity).

**Conclusion:** The study showed that the CHIKV isolates collected from Karachi were from the ECSA lineage and it is most likely that CHIKV outbreak in Pakistan 2016 came from India.

### 1.5 Systematic Identification and Differential Expression Profiling of Preterm Birth-Associated Plas-ma ncRNAs in LMICs Cohort of Pregnant Women.

*Waqasuddin Khan, Samia Kanwar, Javairia Khalid, Farah Khalid, M. Imran Nisar, Fyezah Jehan.*
**Department of Pediatrics and Child Health, Aga Khan University, Karachi, Pakistan*

**Introduction:** Preterm birth (PTB), defined as delivery before 37 weeks of gestation, occurs in 11.1% of pregnancies worldwide, and is associated with significant neonatal morbidity and mortality. LMICs bear a disproportionate burden of PTB. An estimated 1 million preterm infants die in the neonatal peri-od each year and many who survive face lifelong disability. Several biomarkers have been identified that are involved in the early detection of PTB. Less than 2% of the human genome is transcribed and translated into proteins. Non-coding RNAs (ncRNAs) are a class of RNA molecules that are not trans-lated into protein. In pregnant women with PTB, some ncRNAs can be aberrantly expressed in the pla-centa or maternal serum.

**Objective:** The aim of this study is to identify the differential expression of ncRNAs by analyzing cell-free transcriptomics done on plasma from first trimester (8th - 19th weeks of gestation) on 90 pregnant women enrolled in the AMANHI biorepository cohort (2014 – 2018) compared with full-term controls (>37 week of gestation) to the active contraction (24-34 weeks of gestation).

**Method:** Sequencing data was pre-processed using RNA analysis pipeline, sRNAnalyzer and aligned to multiple human ncRNAs databases. Significantly associated differentially expressed ncRNAs with PTB were identified by DESeq2 if they exhibited a p value of 0.05 and a log2 fold change of ≤ 1 (down-regulated) or ≥1 (up-regulated). Genes nearby or overlapped with differentially expressed ncRNAs were considered for enrichment analysis.

**Result:** 17 ncRNAs were highlighted that could play a role in the identification of pregnant women who deliver prematurely. Further validation via the TaqMan Array is currently underway.

### 1.6 Introduction of SARS-CoV-2 variant B.1.1.7 in Pakistan through international travelers

*Asghar Nasir, Ali Raza Bukhari, Nídia S. Trovão, Peter M. Thielen, Akbar Kanji[1], Syed Faisal Mahmood, Najia Karim Ghanchi, Zeeshan Ansar, Brian Merritt, Thomas Mehoke, Safina Abdul Razzak, Muhammed Asif Syed, Suhail Raza Shaikh, Mansoor Wassan, Uzma Bashir Aamir, Guy Baele, Zeba Rasmussen, David Spiro, Rumina Hasan and Zahra Hasan.*
**Section Molecular, Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi, Pakistan*

**Background:** The SARS-CoV-2, B.1.1.7 variant (Alpha) first became predominant in the United Kingdom (UK) at the end of 2020. Here we identify the introduction of B.1.1.7 strains in Pakistan and propose rapid diagnostics for its surveillance.

**Methods: Inbound travelers arriving in** Karachi from the UK between December 2020 and February 2021 were tested for SARS-CoV-2 infection. We screened a subset of respiratory samples from COVID-19 cases for S-Gene Target Failure (SGTF) on TaqPathTM COVID-19 (Thermo Fisher Scientific) and GSD NovaType SARS-CoV-2 (Eurofins Technologies) assays. We conducted sequencing on the MinION platform (Oxford Nanopore Technologies (ONT) and performed Bayesian phylogeographic inference while integrating the patients' travel history information.

**Result:** Of the thirty-five SARS-CoV-2 positive cases screened, thirteen were found to have SGTF strains. The presence of B.1.1.7 lineage variants in thirteen samples was confirmed using a combination of genomic sequencing and targeted mutation-detection PCR assays. Phylogenetic analysis of sequence data available for six cases included four B.1.1.7 strains and one B.1.36 and B.1.1.212 lineage isolate, respectively. Phylogeographic modeling estimated at least three independent B.1.1.7 introductions into Karachi, Pakistan, originating from the UK. B.1.1.212 and B.1.36 were inferred to be introduced either from the UK or the travelers' layover location.

**Conclusion:** We report the introduction of SARS-CoV-2 B.1.1.7 and other lineages in Karachi by international travelers, via several international flight routes. The study highlights the ease of SARS-CoV-2 transmission through travel and the importance of testing and quarantine post-travel to prevent transmission of new strains and subsequent local spread, as well as recording detailed patients' metadata. Such results can help inform policies on restricting travel from destinations where new highly transmissible variants are prevalent.

### 1.7 Upregulated type I interferon responses with downregulated anti-inflammatory genes in transcriptomes of individuals with Asymptomatic COVID-19 in Pakistan

*Kiran Iqbal, Ali Raza Bukhari, Nídia S. Trovão, Natasha Ali, Hassan Hayat, Syed Faisal Mahmood.*
**Section Microbiology, Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi, Pakistan*

**Background**: Pakistan has thus far experienced relatively low COVID-19 related morbidity and mortality. Both host and pathogen related factors likely impact COVID-19 outcomes. It is important to understand protective mechanisms that restrict SARS-CoV-2, assisting disease control.

**Methods:** We used a blood transcriptome approach to investigate early immune biomarkers focusing on asymptomatic COVID-19, SARS-CoV-2 positive cases. These were compared with symptomatic COVID-19 cases and, healthy controls un-infected with the virus.

**Results:** Asymptomatic COVID-19 cases displayed lower levels of inflammatory immune response transcripts, apoptosis and metabolic pathway related genes. However, they displayed an up-regulation of genes involved in antigen presentation as compared to symptomatic COVID-19 cases. Plasma of asymptomatic COVID-19 cases showed increased titers of inflammatory cytokines as compared with controls. Importantly, there was upregulation immune response genes including type I interferon (IFN) responses but downregulation of apoptosis- and allergy-related gene transcripts, in asymptomatic COVID-19 cases as compared with healthy controls. Running a population comparison, we found such an upregulated type I IFN response to be absent in transcriptomic data of mild COVID-19 in a study from Germany.

**Conclusion**: Early SARS-CoV-2 infection-induced type I IFN responses observed in the asymptomatic COVID-19 Pakistani population likely to contribute to reduced disease associated morbidity and mortality observed.

### 1.8 Lipid A-Ara4N as an alternate pathway for (colistin) resistance in Klebsiella pneumonia isolates in Pakistan

*Kiran Iqbal Masood, Seema Umar, Zahra Hasan, Joveria Farooqi, Safina Abdul Razzak, Nazish Jabeen, Jason Rao, Sadia Shakoor, Rumina Hasan.*
**Section Microbiology, Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi, Pakistan*

**Objectives**: This study aimed to explore mechanism of colistin resistance amongst Klebsiella pneumoniae isolates through plasmid mediated mcr-1 gene in Pakistan. Carbapenem and Colistin resistant K. pneumoniae isolates (n=34) stored at -80oC as part of the Aga Khan University Clinical Laboratory strain bank were randomly selected and subjected to mcr-1 gene PCR. To investigate mechanisms of resistance, other than plasmid mediated mcr-1 gene, eight clinical isolates, including six with colistin resistance (MIC >4 μg/ml) and 2 with intermediate colistin resistance (MIC >2 μg/ml) were sent for whole genome sequencing to Eurofins (Germany).

**Results:** RT-PCR conducted revealed absence of mcr-1 gene in all isolates tested. Whole genome sequencing results revealed modifications in Lipid A-Ara4N pathway. Modifications in Lipid A-Ara4N pathway were detected in ArnA_ DH/FT, UgdH, ArnC and ArnT genes. Mutation in ArnA_ DH/FT gene were detected in S3, S5, S6 and S7 isolates. UgdH gene modifications was found in all isolates except S3, mutations in ArnC were present in all except S1, S2 and S8 and ArnT were detected in all except S4 and S7.

**Conclusion:** In the absence of known mutations linked with colistin resistance, lipid pathway modifications may possibly explain the phenotype, but this needs further exploration.

### 1.9 Increasing IgG antibodies to SARS-CoV-2 in asymptomatic blood donors through the second COVID-19 wave in Karachi associated with exposure and immunity in the population

*Muhammad Hasan, Bushra Moiz, Shama Qiaser, Zara Ghous, Areeba Hussain, Kiran Iqbal Masood, Natasha Ali, Pedro Simas, Marc Veldohen, Paula Alves, Syed Hani Abidi, Kulsoom Ghias, Erum Khan and Zahra Hasan.*
**Section of Haematology & transfusion Medicine, Department of Pathology & Laboratory Medicine, Aga Khan University, Karachi, Pakistan*

**Introduction:** One million cases of COVID-19 have been reported in Pakistan until August 1, 2021. However, due to the limited testing capacity in the country, the true level of SARS-CoV-2 infections is unknown. Most individuals with COVID-19 have asymptomatic or mild disease and diagnostic testing is not done. Volunteer healthy blood donors can be a control population for assessment of underlying infections. We tested this cohort for antibodies against SARS-CoV-2 at the time of the second pandemic wave in Karachi, Pakistan.

**Methods:** Between December 2020 and February 2021, 558 healthy blood donors were enrolled at the Aga Khan University Hospital blood bank. Serum IgG levels were determined to spike and receptor binding domain (RBD) of SARS-CoV-2. Data from IgG positive donors was collected to assess their prior history of exposure to SARS-CoV-2.

**Result:** During the study period, 558 blood donors including 553 (99.1%) males and 5 (0.9%) females with a mean (±SD) age of 29.0±7.4 years (range 17-53 years) were enrolled. IgG to spike protein was detected in 298/558 blood donors (53.4%) and furthermore, 93/298 individuals (31.2%) had antibodies to RBD. Only 190 /298 individuals (63.7%) could be contacted and flue like symptoms, domestic/international travel history, contact with individuals who were either suspected or confirmed for COVID-19 PCR were observed in 71/190 (37.4%), 47/190 (24.7%), 34 /190 (17.9%), 28/190 (14.7%) blood donors, respectively. It was observed that 11/44 (25%) donors were RT-PCR positive while 4/12 (33.3%) had IgG to SARS-CoV-2 prior to blood donation.

**Conclusion:** The results indicated a high seroprevalence of 53.4% of IgG antibodies to spike protein and 31.2% of IgG to RBD of SARS-CoV-2 in asymptomatic individuals. The study indicated a high level of asymptomatic infection in the community and gives insights into the exposure and protective immunity in the population.

## 2.0 Digital Poster Abstracts

### 2.1 Indirect Estimation of Reference Interval for Serum TSH in Pakistani Neonates from Mixed Distributions using Truncation Points and the Kolmogorov-Smirnov Distance

*Sibtain Ahmed, Aysha H Khan, Lena Jafri, Hafsa Majid, Farooq Ghani, Imran Siddiqui*
**Section of Chemical Pathology, Department of Pathology & Laboratory Medicine, Aga Khan University, Karachi, Pakistan*

**Introduction:** Serum TSH reference intervals (RIs) are methodology, population and age specific. However, the ethical and practical challenges restrict the establishment of pediatric RIs using conventional approaches advocates the use of indirect data mining-based algorithms. This study was carried out to estimate the reference interval of neonatal serum TSH in Pakistani population using an indirect approach.

**Materials and Method:** A data mining of serum TSH results of neonates (≤1month of age) from 2013-2018 was done. Two subgroups on the basis of age from birth to 5 days and 6-30 days were assessed. The German Society of Clinical Chemistry and Laboratory Medicine's Working Group on Guide Limits (DGKL) validated indirect algorithm was utilized for the statistical analysis.

**Results:** A total of non-duplicate 82299 neonatal serum TSH tests were retrieved over a period of 6 years, including 88 % (n=70788) aged 0-5 days and 12 % (n=11511) ranging from 6 days to 1 month. The estimated RIs for the first age partition was 0.7 (90% CI 0.6-0.8) to 15.5 (90% CI 12.9-16.2) and for the second group 0.7 (90% CI 0.5-0.9) to 7.8 (90% CI 6.1-9.9) uIu/mL.

**Conclusions:** This study revealed age related trends in serum TSH. The study advocates the need for population specific RIs owing to the significant variations noted on comparison with previously published literature. Precise RIs becomes vital particularly when serum TSH is undertaken as a confirmatory test for presumptive positive results on newborn screening for congenital hypothyroidism.

### 2.2 Frequency of Heparin Induced Thrombocytopenia (HIT) in patients at a Tertiary Care Hospital

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**Background & Introduction:** Heparin-induced thrombocytopenia (HIT) is a life-threatening complication of exposure to heparin (e.g. unfractionated heparin, low molecular weight [LMW] heparin) that occurs in a small percentage of patients exposed, regardless of the dose, schedule, or route of administration.
HIT results from an autoantibody directed against endogenous platelet factor 4 (PF4) in complex with heparin. This antibody activates platelets and can cause catastrophic arterial and venous thrombosis. Untreated HIT has a mortality rate as high as 20 percent; although with improved recognition and early intervention, mortality rates have been reported as below 2 percent.

**Rationale of Study:** This study was planned to see the ratio (in percentage) of HIT positive patients with respect to gender, age and platelet count in a Tertiary Care Hospital.

**Material & Method:** The study was conducted at AKUH Coagulation section for 7 months period from Jan 2021 to July 2021. The specimen was collected in yellow cap top separate gel clot activator blood sample test tube. ID-PaGIA Heparin/PF4 Antibody Test KIT (gel agglutination assay) used for testing.222 specimen was included in this study.

**Result:** Total of 222 patients were included in our study from 1st January 2021 to July 31st, 2021.
Among these 222 patients 21[9.5%] cases were HIT positive.
Out of these 21 cases 57.14% [12 cases] were Male while 42.86% [9 cases] were Female.
Age wise 90.5% [19 cases] were above 40 years of age while 9.5% [2 cases] were below 40 years of age.
History wise 47.62% [10 cases] were COVID positive previously.
Median platelet count was 53.5 x 109/L ranging from lowest 16 x 109/L to highest 99 x 109/L.

**Conclusion:** Positive cases of HIT were 9.5% in period between June 2021 to July 2021 out of which Male were 57.14% while Female were 42.86%.
90.5% HIT positive cases were above age of 40 years.
47.62% HIT positive cases were previously COVID positive.
Median platelet count was 53.5 x 109/L ranging from lowest 16 x 109/L to highest 99 x 109/L.

**2.3 Machine learning approach to classify subtypes of breast cancer disease**

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**Background:** Breast Cancer is one of the most exquisite and internecine diseases that cause the number of deaths every year among females all over the world, early detection, and diagnosis of such type of disease is a challenging task to reduce the mortality rate. The prognosis and treatment vary between subtypes; Hence, it is essential to distinguish subtypes for tailored follow-ups. Meanwhile, the development of artificial intelligence algorithms provides an effective way to extract more useful information from complex databases and classification. Since a diverse and vast amount of cancer datasets are already in the public domain, gene expression microarray datasets have yet to be widely accepted for the diagnosis and classification of cancers. Machine learning has proven potential to address mining cancer-specific patterns in cancer prediction and prognosis, cancer diagnosis, and personalized medicine.

**Objective:** In the current study, we aim to determine suitable biological markers for classifying breast cancer subtypes with Unsupervised and Supervised learning methods.

**Method:** We use high-throughput gene expression microarray datasets of healthy and breast cancer samples from the public repository for models training and validation. We utilized unsupervised and supervised machine learning algorithms to classify groups and select optimal subset biomarkers for early diagnosis. Model performance was analyzed by Sensitivity, Specificity, Area under curve and Receiver operator curve

**Result:** Our Purposed method, including Unsupervised and Supervised learning, has demonstrated great potency to classify groups based on their genomic profile and select appropriate biomarkers that can aid in the early diagnosis of breast cancer disease subtypes.

**Conclusion:** The current study demonstrates the ability of machine learning to classify groups based on their genomic profile, which is essential for the determination of breast cancer subtypes, a factor that affects prognosis and treatment.

***2.4*** **Intestinal Spirochetosis that presented with sign and symptoms of Acute Appendicitis- *A Case Report***

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**Introduction:** Many patients present to emergency department in day-to-day practice of surgeons and general physicians with classic signs and symptoms of acute appendicitis. They undergo appendectomy, after clinical diagnosis of acute appendicitis is made, that is supported by laboratory and radiological investigations. The usual histopathological findings in these biopsies are concurrent with the clinical diagnosis. However, we came across a rare case in a young man, who was clinically suspected of acute appendicitis. But histopathologically, it came out to be a case of Intestinal Spirochetosis.

**Microscopic Examination:** There were filamentous organisms carpeting the surface epithelium forming brush border, which was also highlighted on special stain GMS. Rest of appendiceal wall showed moderate lymphocytic and neutrophilic infiltrates

**Discussion & conclusion:** Though is rarely encountered in our day-to-day practice, it could be a cause of acute appendicitis. The points that are unique in our case, which can differentiate it from usual cases of AA are, that this patient presented with vague symptoms of mild to moderate intensity of pain that lasted for a week and was on and off that increased on exertion. Usually, acute appendicitis patients present early due to severe pain, nausea and vomiting and clinically deteriorate with time, which was not in our case.

**2.5 MORPHOLOGICAL DIVERSITY OF ACINIC CELL CARCINOMA AT A TERTIARY CARE HOSPITAL IN PAKISTAN**

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**Background:** Acinic cell carcinoma (ACC) is a rare clinical entity, comprising 1-3% of salivary gland tumors and second most common salivary gland cancer in children. ACC is usually a slowly progressing tumor, however, a proportion of them may have aggressive behavior with nodal & lung metastasis & recurrence. The current study will describe clinicopathological data of ACC in local population.

**Aim:** The aim of our study was to determine frequency of ACC presenting at a tertiary care hospital in Pakistan and to describe their clinicopathological features.

**Methods:** A retrospective study was conducted. All cases of ACC diagnosed in our section during 18-year period (2002-2018) was included. Patient's demographics, clinical and histological features were noted from the patient's pathology reports available on Integrated Laboratory Management System (ILMS).

**Results:** D In present study out of 40 cases, mean age was 44 years and 24(60%) were female. Parotid gland was involved in 80%. Average tumor size was 4.1 cm. Diverse morphological patterns included micro cystic (80%), followed by tubular (30%), follicular (27.5%), papillary cystic (12.5%) and dedifferentiated (5%). Associated lymphoid stroma was present in (37.5%) of cases along with hemorrhage (50%), cystic degeneration (35%) necrosis (25%). Tumors were staged as T1 in (17.5%), T2 in (40%), and T3 in (42.5%) cases. Average follow-up was of 8 years with recurrence in 15%, metastases 10%, and death in15% of patients.

**Conclusion:** ACC may show histological overlap with other carcinomas of salivary gland as it has diverse patterns. Correct diagnosis is possible if pathologists are aware of morphological variation. De-differentiation should be searched in all cases.

### 2.6 Upgradation of Gallstone Analysis to Metabolomics: Development of Pakistani Library for Gallstone Analysis Using Fourier Transform Infrared Spectroscopy – Attenuated Total Reflectance (FTIR–ATR)

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**Background:** The Corona-Score is one of the first and most widely used predictive model for coronavirus 2 (SARS-CoV-2) infection. The purpose of this study was to validate the performance of Corona-Score in a cohort of Pakistani patients pursuing care for suspected infection.

**Methods:** After seeking institution's ethical committee exemption, results of serum lactate dehydrogenase (LDH), C-reactive protein (CRP), ferritin, absolute lymphocyte and neutrophil counts, chest x-ray findings and demographics of suspected COVID-19 cases with respiratory symptoms were recouped from electronic medical record. The pre-validated score as proposed by Kurstjens S, et al., was calculated. The subjects were divided into SARS-CoV-2 positive and negative based on reverse transcription-polymerase chain reaction (RT-PCR) findings. Median and interquartile range (IQR) was calculated for the score in the two groups and the difference was assessed using the independent sample median test. Receiver operating characteristics (ROC) curve analysis was plotted. Statistical analyses were carried out using SPSS 26, with statistical significance set at p value <0.05.

**Results**: A total of sixty cases, 30 (50%) RT-PCR positive and 30 (50%) negatives with a median Corona Score of 3.5 (IQR: 0-6) and 1.5 (IQR: 0-4) respectively, were evaluated. A p-value of 0.61 showing no statistically significant between group differences was observed. The area under the curve of Corona-Score in our population of patients was 0.59 (95% CI: 0.45–0.74). Using the cut-off values of four originally identified by Kurstjens et al. the model displayed 43.3% sensitivity and 70% specificity with an overall accuracy of 56.67 %.

**Conclusion:** Corona-Score displayed a lower diagnostic accuracy which may be attributable to the different genetic framework, viral strain and severity of the disease in Pakistanis compared to the population where this score was originally validated. However, large multi-center studies across the country are dire need of time to evaluate the score in overly exhausted health care setup and limited availability of PCR testing.

### 2.7 Upgradation of Gallstone Analysis to Metabolomics: Development of Pakistani Library for Gallstone Analysis Using Fourier Transform Infrared Spectroscopy – Attenuated Total Reflectance (FTIR–ATR)

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**Introduction**: In Pakistan, the diagnostic facilities for assessing the composition of gallstones are primitive. Fourier Transform Infra-Red (FTIR) Spectroscopy is the method used in the developed countries for quick, cheap and accurate analysis of gallstones. The FTIR method determines the composition of the stone, and the results are compared to an already developed and validated library for confirmation and categorization. Unfortunately, no commercially available library is present for gallstones neither does any such library exist for Pakistan. We aim to develop and validate a Gallstone Standard Library (GSL) for the analysis of gallstones using FTIR Spectroscopy.

**Methods:** The study was conducted at the Department of Pathology & Laboratory Medicine, Aga Khan University, Pakistan. Pure standards (cholesterol, calcium carbonate, bilirubin, and bile salts) and gallstone specimens were analyzed using FTIR Nicolet iS-5 Spectrometer from Thermo Fisher Scientific, USA. Thermo Scientific™ QCheck™ algorithm, embedded within the OMNIC™ software, was used to identify the unique spectral fingerprint of the patient samples to match with known, standard material. Matching of >75% was considered acceptable. Validation for accuracy of the library was performed for twenty analyzed gallstones at an international reference lab.

**Results**: Concerted search analysis was performed against the developed GSL consisting of 71 "pure component" spectrum divided into 5 types to generate the library. For the Gallstone Real Patient Library (GRPL), 117 patient samples were analyzed. Ninety-eight gall stones (83.8%) out of 117 stones matched with the developed GSL. Majority stones were mixed stones (95.92%), with cholesterol being the primary component (91.83%). Results of the developed library were 100% in agreement with the reports received from the external reference lab.

**Conclusion:** The library developed displayed good consistency and can be used for detection of gallstone composition in Pakistan and replace the traditional labor- and time-intensive chemical method of gallstone analysis.

## 2.8 CD34 EXPRESSION IN MYXOFIBROSARCOMA AND ITS ASSOCIATION WITH CLINICO-PATHOLOGICAL PARAMETERS

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**Background:** Myxofibrosarcoma(MFS) is a malignant fibroblastic neoplasm that commonly occurs in lower limbs and elderly with slight male predominancy. More than half occur in dermal/subcutaneous tissue. Its morphology includes multinodular growth and myxoid stroma with characteristic thin-walled curvilinear blood vessels. Overall mortality rate is 30-35%. Not much is there in the literature regarding the frequent expression of CD34 immunohistochemical(IHC) stain,our study will give an indepth knowledge in this regard.

**Aims:** To study the frequency of CD34+ IHC staining in MFS and its association with other clinico-pathological parameters.

**Methods:** This retrospective study was conducted at Section of Histopathology,Department of Pathology and Laboratory Medicine,AgaKhan University. H&E and CD34 IHC slides of MFS diagnosed between 2015-2021 were retrieved and reviewed. CD34 IHC staining assessment was done via Remmele scoring system.

**Results:** Patient's mean age was 56.3 (range 27-105 years), 29(67%) were male and 14(33%) females. Predominant tumor site was thigh 18(42%). 26(60%) were superficial and 17(40%) were deep. Mean tumor size where assessed was 11 cms (range 2-22 cms). In those specimens in which grading was possible 25(58%) were high grade, 13(30%) intermediate and 4(9%) low grades. CD34 IHC staining frequently showed 70% and 80% staining of cells. Strong intensity staining(3+) was seen in 29(67%), moderate(2+) in 10(23%) and weak(1+) in 4(9%) of cases.

**Conclusions**: This study shows that CD34 shows frequent strong expression in MFS. Based on these findings CD34 should be acknowledged as a prevalent IHC marker and its positivity in context with characteristic histology should prefer the diagnosis of MFS over other sarcomas, like in cases occurring at unusual sites as retroperitoneum/abdomen where distinction from commonly occurring dedifferentiated liposarcoma is difficult.

## 2.9 COMBINED FACTOR V AND FACTOR VIII DEFICIENCY: A CASE REPORT

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**Introduction**: Combined deficiency of factor V and VIII is a rare autosomal recessive disorder first defined in 1954. It has a prevalence of 1 in 1,000,000 worldwide and is more common in the Mediterranean area and where consanguineous marriages are common. This rare inherited bleeding disorder is characterized by mild to moderate bleeding tendency and presents with low levels of factor V and factor VIII ranging from 5- 20%.

**Case presentation:** 37 years old gentleman male patient with history of easy bruising and prolonged bleeding after minor wounds. He suffered prolonged bleeding from a cut after trauma at the age of 15 years for which he went to a physician. He was diagnosed as haemophilia A because of prolonged activated partial thromboplastin time (aPTT) and low factor VIII levels and was transfused fresh frozen plasma. He remained stable after that with no significant bleeding episodes. 7 years back he experienced bleeding from gums for which he visited haematology clinic and was required fresh frozen plasma transfusion. His laboratory investigations revealed partial thromboplastin (PT) of 23.7 seconds, activated partial thromboplastin time (aPTT) of 88.8 seconds, factor V was 9% and factor VIII was 7%. Based on these findings a diagnosis of combined factor V and VIII deficiency was made.
His parents had a consanguineous marriage. His younger brother also suffers from similar illness and has mild to moderate bleeding phenotype. Rest of his siblings are unaffected. He had no other significant bleeding event.

**Discussion:** Approximately 3% of all rare congenital bleeding disorders comprises of combined deficiency of factor V and VIII. Majority of patients presents with surgical, traumatic or gingival bleeding, whereas less than one third of patients suffer from haemarthrosis, typical of haemophilia A and B (4). Lectin mannose-binding protein type 1 (LMAN1, chromosome 18; 18q21) and multiple coagulation factor deficiency 2 (MCFD2, chromosome 2; 2p21) gene mutations are the primary causative coagulation pathway defects leading to the low factor V and VIII levels (5). The LMAN1/MCFD2 protein complex functions to transport the coagulation factors V and VIII from the endoplasmic reticulum to the Golgi apparatus. 70% of the cases accounts for mutations in LMAN1 gene and 30% of the cases accounts for mutations in MCFD2 gene.
Laboratory investigations revealed normal platelet count and bleeding time, prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT) along with low factor V and VIII levels. Baseline factor levels are generally greater than those with isolated factor deficiencies (6). Treatment options in case of a bleeding episode involves replacing factor V and VIII. Factor V is replaced by fresh frozen plasma and plasma-derived factor VIII concentrates, or recombinant factor VIII products can be used (7). In case of mild bleeding, administration of Desmopressin may achieve adequate haemostasis.

**Conclusion**: We presented a case of combined deficiency of factor V and factor VIII, rare inherited bleeding disorder. This 37 years old gentleman had history of prolonged bleeding from minor wounds and appearance of bruises after trauma. He was treated with fresh frozen plasma on two occasions of prolonged bleeding after trauma. His parents had consanguineous marriage and his younger brother is also affected with similar illness.

## 2.10 DIAGNOSIS OF GAUCHER DISEASE IN BONE MARROW OF AN ADULT: A CASE REPORT

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**Introduction*:*** Gaucher disease is a rare inborn error of metabolism (the most common of all lysosomal storage disorder) that affects the reprocessing of cellular glycolipids. Glucocerebroside and other related compounds that are normally degraded to glucose and lipid components gather within the lysosomes of cells. It has an autosomal recessive pattern of inheritance with an overall incidence of approximately 1:40,000 individuals, with incidence rising up to 1/800 in Ashkenazi Jews. Gaucher disease has 3 major clinical types and two other subtypes (perinatal-lethal and cardiovascular). Type 1 is the non-neuronopathic form and type 2 and 3 are the neuronopathic forms. Type 1 individuals may not have any clinical signs until late adulthood and some individuals with this genotype remain asymptomatic throughout their life

**Case presentation**: 28 years female with history of fever, generalized weakness and weight loss. Physical examination revealed pallor. Laboratory findings showed haemoglobin of 9.5 g/dl, haematocrit of 33.4 %, MCV of 85.0 fl, MCH of 24.2 pg, white blood cell count of 3.51 x10E9/l, absolute neutrophil count of 2.33 x10E9/l and platelet count of 84 x10E9/l. Bone marrow examination was done for diagnostic workup of pancytopenia.

**Interpretation**: The peripheral blood smear shows hypochromic red blood cells. The bone marrow shows gaucher cells that appear as oval shaped cells having eccentric nucleus and voluminous weakly basophilic cytoplasm with wrinkled appearance. PAS cytochemical stain on bone marrow aspirate is positive. Bone trephine section shows infiltration with the same storage cells and satin deeply with PAS immunohistochemical stain.

**Discussion:** Gaucher disease is a lipid storage disease characterized by the deposition of glucocerebroside in cells of the macrophage-monocyte system. The disorder results from the deficiency of a specific lysosomal hydrolase, glucocerebrosidase (also termed acid beta-glucosidase, glucosylceramides). The disorder is characterized by bruising, fatigue, anemia, leucopenia, low blood platelet count and enlargement of the liver and spleen. When the enzyme is defective, glucocerebroside accumulates, particularly in white blood cells and especially in macrophages (mononuclear leukocytes). Glucocerebroside can collect in the spleen, liver, kidneys, lungs, brain, and bone marrow. Gaucher disease type 1 is chronic non neuronopathic (adult) type, makes up 99% of all cases, often completely asymptomatic and is an incidental diagnosis a lot of times. It does not involve the nervous system. Most patients have splenomegaly, anemia, thrombocytopenia, and radiographic evidence of bone lesions. Gaucher disease type 2 is rare and involves severe neurological (brain stem) abnormalities. It is usually fatal within the first 2 years, and it is currently untreatable because of the severe, irreversible brain damage. Gaucher disease type 3 has a severity between types 1 and 2, causing the same symptoms as type 1 plus some neurological involvement. While patients typically have a shortened lifespan, some can live into their 50s with treatment. Learn more about Gaucher disease types 2 and 3.

## 2.11 Primary Hemophagocytic Syndrome Triggered by Dengue Infection

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**Introduction:** Hemophagocytic lymphohistiocytosis (HLH) is an aggressive and life-threatening syndrome of excessive immune activation. It most frequently affects infants from birth to 18 months of age, but the disease is also observed in children and adults of all ages. HLH can occur as a familial or sporadic disorder, and it can be triggered by variety of events that disrupt immune homeostasis. Infection is a common trigger both in those with a genetic predisposition and in sporadic cases. We are discussing a rare case of inherited HLH presented at age of 39 years that was triggered by dengue infection.

**Case Presentation:** 39-years old female presented to emergency department (ED) with a ten-day history of flu-like symptoms, fever, abdominal pain and vomiting. There was no known family history of autoimmune conditions, malignancy or consanguinity. She is a mother of three children and one of her child has been diagnosed with Charcot-Marie-Tooth disease which is an inherited disorder. . Two days after admission, there were features of severe sepsis, with the following observations: temperature $39.4^0$C, heart rate 144 bpm, blood pressure 91/45 mmHg with worsened respiratory parameters. She appeared alert but dehydrated with no palpable lymphadenopathy. However, she had tender epigastrium with palpable spleen of 4cms below left costal margin.

Complete blood count revealed Hb 8.9gm/dl, MCV 79fl, WBC 1.7 x 10E9/L, ANC 4.2 x 10E9/L and platelet count of 26 x 10E9/L. Her C-Reactive protein was elevated (42 mg/L) with normal liver and renal function tests, and a mildly elevated venous lactate (2.4 mmol/L). Chest radiograph showed bilateral pleural effusion and electrocardiogram was unremarkable.

Multiple blood cultures, urinary pneumococcal and legionella antigens, nasopharyngeal swab for COVID 19, malaria rapid immune-chromatographic tests, EBV, HSV, Hepatitis B, C, HIV serology and autoimmune profile were done, returning negative results. Acute Dengue virus infection was confirmed (through Dengue IgM serology) and subsequent bone marrow biopsy analysis demonstrated increased hemophagocytic activity (figure 1) with positive immunohistochemical stains CD68 and CD31 (figure 2).

Based on the laboratory results, her genetic work-up for primary HLH was sentit showed homozygous mutation identified at STXBP2 gene (variant c.1247-1G>C). This mutation is associated with autosomal recessive familial HLH. During her second admission, she was managed with transfusion support, broad spectrum antibiotics and HLH-94 protocol. Her condition improved in two-weeks and she was discharged from in-patient to continue the protocol in day care-based set-up at our hospital. She was counseled regarding allogeneic stem cell transplant and a search for sibling matched donor is in process.

**Discussion:** Hemophagocytic lymphohistiocytosis is a rare hyper inflammatory disorder related to macrophage activation and usually presents as prolonged fever and sepsis-like syndrome (1). Two types of HLH are seen that include primary (familial) form which is a fatal autosomal recessive disorder, whereas the secondary or reactive form is associated with viral, bacterial, fungal, or parasitic infections as well as with connective tissue disorders and malignancy. The importance of the association between HLH and infection lies in the fact that both forms of HLH may be preceded by infection. Genetic defects of HLH can present at any age and infections also can be a triggering mechanism in such patients. Five different forms of familial HLH have been described based on defects in different genetic material and genes, including mutations present on chromosome arm 9q which include (FHL1), PRF1 (FHL2), UNC13D (MUNC13-4) (FHL3), STX11 (FHL4), and STXBP2 (MUNC18-2) (FHL5). In this case of a 39-year-old female who presented with fever and splenomegaly associated with cytopenia's and raised biochemical markers, our initial working diagnosis was secondary HLH due to presence of Dengue infection and presenting age. However, genetic mutation for primary HLH was positive i.e. S*TXBP2* which has been associated with FHL5. The distinction is important as allogeneic bone marrow transplantation is the therapy of choice in patients with familial HLH as compared to sporadic HLH that has a better prognosis .

On review of largest cohort of patients with FHL5 reported so far with 37 patients from a widespread ethnic origin, deficient NK-cell degranulation seems to be a uniform finding in all patients with FHL5. Compared with other FHL types, median age of diagnosis in the FHL5 cohort seems to be like that found in patients with FHL2 months) and is slightly less than in patients with FHL3 months). In patients with FHL4 the age of onset varies widely with a median of 14 months. The most frequent symptom in 14 patients in this cohort was severe diarrhea that often affected the patients before they developed classical HLH symptoms. Most of these patients needed parenteral feeding. Diarrhea persisted during HLH treatment; this study also described an increased bleeding tendency in some of the patients with FHL5.

In our patient, STXBP2 mutation was positive categorizing her as FHL5. She had a rare presentation with respect to symptoms and presenting age triggered by infection. Early recognition and treatment with chemotherapeutic agents or bone marrow transplant may reduce mortality as initiated this case where she responded well on prompt HLH directed therapy and has been planned for allogeneic stem cell transplant.

**Conclusion:** We present the rare case of primary HLH at age of 39 years, triggered by dengue infection with underlying STXBP2 homozygous splice-site mutation. Our patient responded well to HLH-94 protocol and has been planned for allogeneic stem cell transplant.

### 2.12 The curious case of HLA-DR positive APL

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**Introduction:** Acute promyelocytic leukemia (APL) with t(15;17) (q22;q21)/PML-RARα is a subtype of acute myeloid leukemia (AML) with distinct morphologic characteristics[1]. It can become aggressive and life threatening if there is a delay in recognition of the clinical presentation and diagnosis. Patients can develop coagulopathy leading to bleeding diathesis and death[2]. However, due to improvement in diagnostic modalities and early initiation of ALL-trans-retinoic acid (ATRA) and arsenic trioxide, the outcomes of dramatically improved. The definition of disease is through the detection of t(15;17) (q22;q21)/PML-RARα mutation and this serves as the molecular basis of treatment with ATRA.
The classical morphology on peripheral smear of the abnormal promyelocytes reveals bilobed nucleus, abundant granules in the cytoplasm, presence of Auer rods and faggot cells which is pathognomonic for APL[4]. Although confirmation is through molecular studies, to aid in rapid diagnosis, flowcytometry has been widely used and extensively studied. Compared with other types of AML, the most consistent immunophenotype in APL includes absent or weak CD34, absent HLA-DR (which belongs to human leukocyte antigen class II), and positive CD117[5]. Other features in flowcytometry that are commonly seen in APL include heterogeneity of high side scatter, absent, low-level, or less frequent expression of CD10, CD11a, CD11b, CD11c, CD18, CD45RO, CD105, and CD133.
HLA-DR positive APL is a rare entity and has been scarcely reported. Mendoza et al has described 45 cases of APL of which only two were HLA-DR positive in which the clinical, morphological and molecular characteristics were similar to HLA-DR negative APL.Herein we describe the case of a 34-year-old female who presented with clinical signs and symptoms of APL and immunophenotyping by flowcytometry analysis showed HLA-DR to be positive while PML-RARα mutation was also positive.

**Case presentation:** A-34-year-old female, presented to the emergency department of Aga Khan University Karachi, Pakistan with complaints of gingival bleeding, menorrhagia and easy bruisability for one week. On examination she had active oozing from gums and multiple, large bruises all over the body. There was no organomegaly. Baseline laboratory investigations were sent of which complete blood count showed Hb: 9.5 gm/dl, WBC: 21 x $10^9$/L, platelets: 13 x $10^9$/L. Peripheral blood film showed 75% abnormal promyelocytes with cytoplasm containing granules, Auer rods and faggot cells (figure 2). Prothrombin time (PT) was >170 seconds, INR >17, activated partial thromboplastin time (APTT) was 43 seconds, fibrinogen was 35 mg/dl and D-Dimer was >30 mg/L FEU. Liver and renal function tests were within normal limits. Based on the findings of peripheral smear, immunophenotyping by flowcytometry and molecular analysis of PML-RARα mutation was sent on peripheral blood. Bone marrow aspirate and trephine could not be performed at that time due to deranged coagulation parameters. Immunophenotype of the abnormal promyelocytes revealed positivity to CD4 (33%), CD13 (68%), CD33 (70%), cMPO (66%), HLA-DR (30%), CD117 (57%), CD34 (50%), CD36 (41%) and CD64 (66%). Due to the classical clinical and morphological findings, the patient was started on ATRA (45mg/$m^2$/day in 2 divided doses) and prophylactic dexamethasone 10mg once a day while we awaited molecular results. In 48 hours, the molecular analysis confirmed the presence of PML-RARα mutation. Her INR at that time became <1.5 and samples for bone marrow aspirate and cytogenetic were drawn. Bone marrow morphology showed diffuse infiltration with abnormal promyelocytes. These cells were large with convoluted cytoplasm containing reddish pink granules and Auer rods (figure 2). Since she was in the high risk category (WBC > 10 x $10^9$/L), induction with daunorubic and cytarabine (3+7 regimen) was also started concomitantly and she was receiving supportive care. I She responded well with induction therapy and her day 28 bone marrow along with PML-RARα mutation done post induction which showed morphological and molecular response, so she has been continued with high dose consolidation chemotherapy and she maintained molecular response, currently she is on maintenance ATRA and ATO.

**Discussion:** Acute promyelocytic leukemia is characterized by clonal proliferation of abnormal promyelocytes. The World Health Organization's (WHO) classification recognizes the importance of genetic aberrations in the diagnosis and prognostication of AML and categorizes four unique groups of AML with recurrent chromosomal translocations out of which AML with t(15;17) is specific to APL. The others include AML with t(8;21) (q22;q22), AML with inv(16)(p13q22)/ t(16;16)(p13;q22) and AML with 11q23 abnormalities. These four entities currently constitute about 25-30% of all cases of adult AML (4). The antigen HLA-DR is normally expressed on myeloblasts and loses expression at the promyelocyte stage of the maturation process. The absence of HLA-DR positivity is highly suggestive of APL and is used to distinguish AML from APL. In various studies published before, HLA-DR expression has been reported with a range of 0 – 9% 5). The percentage of expression has also been variable in the studies reported e.g., Dong et al[13] reported only one case of APL with strong HLA DR expression (20% or more) while four cases had a weak HLA-DR expression (staining 5-20% of cells). In our case, the HLA-DR expression was very strong (30%) which rarely has been reported in literature. Along with HLA DR expression, she also had positivity for CD34 (50%), which follows the same pattern of expression as HLA DR, i.e., negative expression on abnormal promyelocytes. CD34 positivity has been sparsely reported previously. Foley et al[(6)] has reported a cohort of 38 patients with APL, of which 32% of cases were CD34 positive and correlated with less differentiated APL blasts. In these patients, the incidence of early mortality is 50%. There is significant correlation between CD34 positivity and raised WBC count at presentation. Our patient also had similar parameters of HLA-DR and CD34 expression along with WBC count of 21 x $10^9$/L at presentation.

According to the National Comprehensive Cancer Network (NCCN) Guidelines, ATRA should be started before genetic confirmation in patients with clinical and pathological features of APL because early initiation of ATRA may prevent the lethal complication . . In our case, although morphology was connotative of APL, flowcytometry indicated otherwise. Based on the symptoms, clinical presentation, and deranged coagulation profile we still started ATRA and received conformation 48 hours later of APL though genetic analysis. This case consolidates the fact that with the advent of sophisticated automated diagnostic analyzers, morphological examination that is human eye dependent remains to be the backbone in leukemia diagnosis.

**Conclusion**: In rare cases, APL can present with a HLD-DR and CD34 positive immunophenotype. With a strong index of suspicion along with classical clinical and morphological findings, ATRA should be started in such patients while molecular analysis results are awaited. Although rarely reported, HLA-DR and CD34 positive cases present with high white blood cell count making them susceptible to induction mortality of 50%.

### 2.13 Proteomics for the development of novel drug intervention to restore memory deficits in Alzheimer's Disease

*Farzana Abubakar, Bushra Amin, Sara Khan, Muhammad Wasay*
*Department of Biological and Biomedical Sciences, AKU*

**Introduction*:*** Alzheimer's disease (AD) is the 6th leading cause of early death and an emergent threat to aging population all over the world including Pakistan. Immunotherapies, herbal, and alternative medicines have been suggested, yet lack effective evidence to support their use. In our previous studies we have highlighted protein deiminases (PAD) as novel therapeutic target for AD , as their expression is high in rat cerebrum at the early stages of neurodegeneration process. Moreover, abnormal activity of this enzyme and accumulation of deiminated proteins is frequently seen in the hippocampus of AD brain, further exacerbate inflammation and cause injury in brain circuitry at multiple regions.

**Objective:** Our aim is to develop effective drug intervention for Alzheimer's disease to prevent the neuronal circuitry to eliminate associated memory impairments.

**Method**: In this prospective study we will generate rat model of Alzheimer's disease to mimic the neuronal loss in AD brain. The efficacy of our drug will be tested in 1, 10, and 50 mg/Kg intraperitoneal doses for the restoration of brain circuitry. Elimination/reduction of neuronal sclerosis, inflammation, and gross changes in the structure of hippocampus will be investigated with proteomics analysis. Downstream analysis will include histological examination and high-throughput proteomic analysis to detect molecular impact of drug at various brain regions and vital metabolic organs. Histological studies of vital metabolic organ i.e., liver tissue with Alzheimer's potential protein biomarker ABC Transporter Binding Cassette Protein A7 (ABCA7) demonstrate enhanced expression of protein. This is one of the potential targets for our lead drug. Association of drug effect on AD biomarker protein will be studied. Providing different dosage of the active drug intraperitoneally, a visibly sharp reduction in disease activity was observed.

Results are mean ± Standard error of mean. In current experimental course a different dosage regime will be injected.

**Conclusion:** These results will make us decide if PAD could be the novel therapeutic target for AD and halide-amines are the potential drug to treat memory deficits in AD.

### 2.14 Omics to develop reliable diagnostic biomarker(s) for early detection of Alzheimer's disease

*Farzana Abubakar, Mohammad Wasay, Sara Khan, Bushra Amin*
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**Introduction:** Alzheimer's disease (AD) is a growing threat to aging population round the globe including Pakistan. Absence of reliable biomarker delays diagnosis till advance stages of the disease where a significant and irreversible loss of critical neurons already occurred in the brain. Thus, early detection of devastating disease is a dire need and of high scientific interest. Recent genome-wide studies suggest adenosine triphosphate-binding cassette transporter A7 (ABCA7), as the strongest risk gene for AD (both for the early and late-onset). The expression of ABCA7 inversely co-relate with the accumulation of Aβ plaque in the brain.

**Objective:** To date, no study has been performed for the detection of ABCA7 as the maker for AD, both nationally and internationally. We will use high-throughput proteomics to develop ABCA7 as early diagnostic marker for AD.
**Material & Method**: In this prospective study, mild-to moderate AD patient's (N≥100) will be enrolled along with the age and gender-matched cognitively normal volunteers with comprehensive medical history and neuropsychological assessment. Saliva will be collected. Proteomic analysis will be performed for the system-wide variances in peripheral ABCA7. Immunochemical studies will further validate statistical levels of ABCA7 exclusive to AD patients.

**Result:** It is anticipated that the validated results of discovery and validation phase will possibly lead to the develop non-invasive and reliable diagnostic biomarker for the early detection of cognitive impairment in prodromal AD.

### 2.15 Frequency of TP53 mutation in patients with multiple myeloma

*Qadeer Ahmed, Salman N, Adil*
*Section of Haematology & Transfusion Medicine, Department of Pathology & Laboratory Medicine*

**Introduction:** Multiple myeloma (MM) is a malignancy characterized by clonal plasma cells secreting monoclonal immunoglobulins. Deletion of chromosome 17p, the TP53 gene locus in patients with MM is associated with poor response to treatment, low rate of complete response and rapid disease progression. The aim of the study was to determine frequency of TP53 mutation in newly diagnosed MM patients.

**Method:** This study was conducted in the section of Haematology & transfusion medicine and molecular pathology at Aga Khan university hospital, Karachi, Pakistan. All newly diagnosed patients of primary MM were included in the study. The diagnosis was made through examination of bone marrow or tissue biopsy, serum protein electrophoresis, immunofixation and immunoglobulin levels. Five ml of whole blood or one ml of bone marrow aspirate sample in EDTA tube was collected for detection of TP53 mutation by fluorescence in situ hybridization (FISH) technique. The resulting specimen DNA denatured to its single stranded form and then allowed to hybridize with the p53 (17p13.1) single color probe. Following hybridization and counter staining, detection of TP53 (17p13.1) gene conducted by microscopic examination of interphase nuclei, minimum 200 interphase nuclei were counted. Patient's characteristics and frequency of TP53 mutation were collected and expressed in mean and percentages.

**Discussion:** Multiple myeloma accounts for 1% of all cancers and approximately 10% of all hematologic malignancies.
Multiple myeloma is slightly more common in men than in women. The median age of patients at the time of diagnosis is about 65 years. Almost all patients with multiple myeloma evolve from an asymptomatic pre-malignant stage termed monoclonal gammopathy of undetermined significance (MGUS). MGUS progresses to multiple myeloma or related malignancy at a rate of 1% per year. In some patients, an intermediate asymptomatic, but more advanced pre-malignant stage referred to as smoldering multiple myeloma, (SMM) can be recognized clinically.
The diagnosis of multiple myeloma requires the presence of one or more myeloma defining events (MDE) in addition to evidence of either 10% or more clonal plasma cells on bone marrow examination or a biopsy-proven plasmacytoma. MDE consist of established CRAB i.e hypercalcemia, renal failure, anemia, or lytic bone lesions) features as well as three specific biomarkers: clonal bone marrow plasma cells ≥60%, serum free light chain (FLC) ratio ≥100 (provided involved FLC level is ≥100 mg/L), and more than one focal lesion on MRI.

Bone marrow studies at the time of initial diagnosis should include fluorescent in situ hybridization (FISH) probes designed to detect t(11;14), t(4;14), t(14;16), t(6;14), t(14;20), trisomies, and del(17p) for prognostication.
t(4;14), ), t(14;16), t(14;20), del(17p), gain(1q) is considered high risk.

**Result:** A total of 113 newly diagnosed MM patients were included in the study. Of 113, 77 (68.1%) were male while 36 (31.9%) were female and mean age was 59.4 (±12.98) years. TP53 mutation was found in 04 (3.5%) patients.

**Conclusion:** In this study presence of TP53 mutation was found to be infrequent in MM patients. However more studies with larger data are required to validate findings of this study. It is also appropriate to perform FISH studies for the high-risk cytogenetics at baseline.

### 2.16 Development of novel tags for enhanced sample multiplexing in OMICS

**Objective:** The specific goals of our study are as follows:

- Development of labeling tags
- Higher sample multiplexing capabilities of cPILOT
- Improve identification and quantification of macromolecules in complex biological mixtures

**Background**: Our laboratory has developed a novel quantitative proteomics approach that combines precursor isotopic labeling and isobaric tagging (cPILOT) [1, 2.] This doubles the number of samples to be simultaneously analyzed in a single shot. To improve the overall sample-throughput of cPILOT this work focuses on enhancing the number of sample channels (up to 100) available for multiplexing. In this work we have developed a small library of novel N-terminal specific reagents that can be coupled to proteolytic peptides from biological samples. Additionally, incorporating protein blocking or tagging of lysine amines helps to facilitate N-terminal specificity. Current progress and limitations of this work is presented.

**Conclusion:** Nic-NHS and DMB-NHS have pH-dependent site-specificity, while peptide labeling is pH-independent

- Labeling with endogenous peptides is highly reproducible and have tested with various samples
- Molar excess does affect the Binding Efficiency of our novel labels though a minimum of 10-50-fold excess is needed for endogenous source labeling
- NHS ester can be incorporated into cPILOT workflow
- Effect of Reaction Temperature and Time on Binding Efficiency of NHS esters is have also monitored**.**

### 2.17 Application of enhanced sample multiplexing approaches to elucidate aging and allied processes

**Background:** Our laboratory has developed an enhanced sample multiplexing approach called “combined precursor isotopic labeling and isobaric tagging (cPILOT)” to simultaneously analyze up to 24 different samples. Using large-scale quantitative proteomics, we are able to provide a near-global view of cellular proteins sensitive to aging process. In a single shot analysis, we have identified 2997 proteins and quantified 1814 protein groups in vital metabolic peripheral organ. we detected pronounced variations in crucial metabolic pathways involved in mitochondrial dysfunction, fat, and protein degradations.

**Objective:** The specific goals of study are as follows:

- Applications of enhanced sample multiplexing approach to elucidate complex biological processes
- Study aging-sensitive proteins in vital metabolic organ
- To discover aging-associated pathways and crucial proteins involve in the process.

**Method:** High-throughput quantitative proteomics approach was used using cPILOT labeling to simultaneously detect 14-different biological samples of various ages. Low pH di-methylation and high pH Tandem mass-labeling was performed.
LC-MS/MS analysis was carried out to identify thousands of hepatic proteins while, MS3 analysis has facilitated their quantification and pathway detection**.**

**Conclusion:** Aging globally effects cellular and organismal metabolism across a range of mammalian species, including humans.

- Rabbits are an attractive model system of aging due to their genetic similarity with humans and their short lifespans.
- Pronounced variations in fat metabolism, mitochondrial dysfunction, and protein degradation was detected. Such changes are consistent in the liver across several mammalian species.
- We have identified 2,586 liver proteins, among which 45 proteins had significant ($p < 0.05$) changes with aging. Seven proteins were differentially expressed at all ages.
- Fatty acid binding protein, aldehyde dehydrogenase, enoyl-CoA hydratase, 3-hydroxyacyl CoA dehydrogenase, apolipoprotein C3, peroxisomal sarcosine oxidase, adhesion G-protein coupled receptor, and glutamate ionotropic receptor kinate are aging-sensitive proteins in liver.
- Insights of aging-sensitive alterations in metabolism that affect protein expression in liver have been gained.

### 2.18 Inflammatory myofibroblastic tumor occurring in head and neck: unusual sites of an unusual entity

*Anam Ghauri, Nasir Uddin, Zubair Ahmad*
**Section of Histopathology, Department of Pathology & Laboratory Medicine*

**Objective:** The study aims to describe the clinicopathological features and occurrence of Inflammatory myofibroblastic tumor (IMT) in different locations in the region of head and neck. Although the lung is the best known and most common site, IMT occurs in diverse extrapulmonary locations and in any age group.

**Methods***:* 44 cases of IMT were retrieved from the archives of Histopathology at The Aga Khan University Hospital between years 2006-2021. Out of these, 13 cases involved various locations in the head and neck region. H&E slides were evaluated for characteristic histopathologic findings. Relevant IHC stains performed were CKAE1/AE3, ASMA, ALK protein, Desmin, CD34, S100 and CD117. FISH analysis for ALK gene rearrangement had been done on two cases. Treatment and follow up details were obtained by calling the patients.

**Results:** The described unusual tumor sites were eye(n=1), orbit (n=2), vocal cords (n=2), intracranial but extra-axial (n=2), cheek (n=2), and parotid gland, oropharynx, epiglottis, and supraclavicular area (each with n=1). 07 patients were females and 06 were males. Ages ranged from 3.5-53 years with a mean age of 27 years. Specimen were received as trucut biopsies, excised masses or in multiple pieces. The IHC results were CKAE1/AE3(+ve in n=00), ASMA(+ve in n=11), ALK protein(+ve in n=05) Desmin (+ve in n=00). Other immunohistochemical markers performed included CD34, S100, CD117, Caldesmon, MyoD1, Myogenin, EMA, p40, SOX10 etc. Follow up of 07 patients was attainable. 01 found to have expired while on chemotherapy. Of the remaining 6 alive patients, 03 were alive with recurrence. Of the 03 alive patients without recurrence, 01 had received radiotherapy while 02 of them were treated by resection alone.

**Conclusion:** IMT is an uncommon neoplasm with intermediate biological behavior. Its occurrence should always be suspected whenever a spindle cell lesion with marked inflammation arises at unusual sites in body specially in children and young adults. Long-term follow up with serial imaging techniques may be recommended to clinch possible local aggressive behavior and recurrence.

### 2.19 MELANOTIC NEUROECTODERMAL TUMOR OF INFANCY: REVIEW OF 7 CASES OF PEDIATRIC POPULATION

*Alka Rani, Nasir Ud Din*
**Section of Histopathology, Department of Pathology & Laboratory Medicine, AKU*

**Introduction:** Melanotic neuroectodermal tumors of infancy (MNTIs) commonly affect the head and neck region of infants, arising usually in the maxilla (68%–80%), mandible (5.8%), brain (4.3%) or skull (0.8%). It has been reported at other sites as well such as femur, epididymis, ovaries, uterus and mediastinum) with uncertain clinical behavior. MNTIs are the neural crest cell origin and are regarded as benign tumors, fast-growing, locally aggressive with tendency to invade surrounding bone leading to higher incidence of recurrence that varies between 10% and 60% and 6.6 % risk of malignant change. Histological examination shows non-encapsulated lesion exhibiting a biphasic pattern composed of abundant large epithelioid cells arranged in clusters, tubules and alveolar pattern that contain melanin pigment arranged around small neuroplastic cells. Epithelioid cells are positive for immunohistochemical stain CKA1/AE3, HMB45 while small cells are positive for synaptophysin and NSE. The aim of this study is to review clinicopathological features of a series of MNTIs with follow-up.

**Method**: H&E slides of diagnosed cases of MNTI at Aga khan hospital reported between 2015-2021 were retrieved and reviewed. Demographic data was obtained from patients' medical records surgical reports and follow up was taken.

**Result:** 7 cases of MNTI were reported with the age range 1 to 6 months. Male to female ratio was 4:3 with mean tumor size 4 cm. Tumors occurred in maxilla (57%), mandible and post auricular (14 % each). Primary surgery was performed on all patients, with no known status of margins. Follow-up of 6 patients was available. There was no local reoccurrence with survival rate of 66% at time of follow-up. 1 case had metastasis to other site with primary site of post auricular. 4 patients were alive in a follow up duration of 2-3 years. Two patients died one within 2 months of surgery and other patient developed metastasis to chin and parietooccipital region within a period of 6 months, underwent surgery and chemotherapy and died within a year.

**Conclusion:** Even though MNTI is usually a benign tumor, owing to its fast growth and locally destructive behavior early diagnosis and treatment is important to bound local expansion and a favorable outcome for the patient. As MNTIs tumors rarely occur, delay in diagnosis, results less desired outcome in patient.

## 2.20 Tuberculosis infection with concomitant pleomorphic adenoma in submandibular gland: a rare case report

*Alka Rani, Nasir Ud Din*
**Section of Histopathology, Department of Pathology & Laboratory Medicine, AKU*

**Introduction***:* Pleomorphic adenomas are the most common benign salivary gland tumors accounts 45-75% of all salivary gland tumors and most commonly occur in the superficial lobe of the parotid gland. They can involve the submandibular and minor salivary glands. Tuberculosis rarely involves the salivary gland, despite being the commonest infectious disease in the developing countries and tuberculosis represents 15-20% of infection in extrapulmonary sites. Pleomorphic adenomas with coexistence of tuberculosis is extremely rare. Only a few cases are reported in the literature with coexistence of tuberculosis and benign tumors including mixed tumor and Warthin's tumor and parotid was the involved gland. We present a case of 45-year-old female with pleomorphic adenoma of submandibular gland and concomitant tuberculosis.

**Objective:** Benign salivary gland tumor (pleomorphic adenoma) with coexistence of chronic granulomatous inflammation (tuberculosis) is a very rare entity with case report of Parotid gland involvement. We present a case of 45-year-old female with pleomorphic adenoma of submandibular gland and concomitant tuberculosis.

**Case Presentation:** We had received specimen of 45-year-old female with a history of swelling in the right submandibular area for 3 months associated with cough. She had no history of fever, weight loss or contact with tuberculosis. Smear was negative for mycobacteria tuberculosis. Grossly specimen was received in multiple pieces. Microscopic examination showed ruptured fibrous capsulated lesion composed of three components. Epithelial ductal component lined by polygonal cells surrounded by spindle-shaped myoepithelial cells in a myxoid stroma, hence the diagnosis of pleomorphic adenoma was made. In the adjacent submandibular gland and tumor fragments multiple granulomas composed of epithelioid cells, lymphocytes and Langhan's giant cells were seen with extensive areas of caseous necrosis. Special stain. AFB and Gene expert is negative for acid-fast bacteria.

**Conclusion:** Pleomorphic adenoma with concomitant tuberculosis of a submandibular salivary gland is very rare and only a few cases have been reported in the literature. In developing country where the incidence of tuberculosis is very high, coexistence of tuberculosis infection in a benign lesion must be kept in mind.

# RESEARCH REPORT

## 2019 – 2021(*till September*)

# DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE

## Publications in the year 2019 – 2021(*till September*)

| Sections | 2019 | 2020 | 2021 *(Till September 30, 2021)* |
|---|---|---|---|
| **Histopathology** | 44 | 61 | 34 |
| **Microbiology** | 46 | 41 | 27 |
| **Chemical Pathology** | 21 | 14 | 20 |
| **Molecular Pathology** | 7 | 4 | 4 |
| **Haematology and Transfusion Medicine** | 16 | 13 | 10 |
| **Total** | 134 | 133 | 95 |

## Publications in International and National Journals 2019 – 2021 (*till September*)

| | 2019 | | 2020 | | 2021 | |
|---|---|---|---|---|---|---|
| | **National** | **International** | **National** | **International** | **National** | **International** |
| **Histopathology** | 10 | 34 | 6 | 55 | 4 | 30 |
| **Microbiology** | 4 | 42 | 3 | 38 | 1 | 26 |
| **ChemicalPathology** | 5 | 16 | 2 | 12 | 2 | 18 |
| **MolecularPathology** | 3 | 4 | 0 | 4 | 0 | 4 |
| **Hematology& Transfusion Medicine** | 0 | 16 | 2 | 11 | 1 | 9 |

## Types of Publication 2019 – 2021 (Till September)

| 2019 | | | | | | |
|---|---|---|---|---|---|---|
| | **Original Article** | **Review Article** | **Case report** | **Letter to Editor** | **Editorial** | **Book Chapter** |
| **Chemical Pathology** | 19 | - | 2 | - | - | - |
| **Haematology & TransfusionMedicine** | 12 | 2 | 2 | - | - | - |
| **Histopathology** | 27 | - | 16 | - | 1 | - |
| **Microbiology** | 39 | 1 | 5 | 1 | - | - |
| **Molecular Pathology** | 5 | 2 | - | - | - | - |

| 2020 | | | | | | |
|---|---|---|---|---|---|---|
| | **Original Article** | **Review Article** | **Case report** | **Letter to Editor** | **Editorial** | **Book Chapter** |
| **Chemical pathology** | 11 | - | 1 | 2 | - | - |
| **Haematology & Transfusion Medicine** | 6 | 1 | 4 | 2 | - | - |
| **Histopathology** | 18 | 8 | 20 | - | - | 15 |
| **Microbiology** | 32 | 4 | 4 | - | 1 | - |
| **Molecular Pathology** | 3 | 1 | - | - | - | - |

| 2021 | | | | | | |
|---|---|---|---|---|---|---|
| | **Original Article** | **Review Article** | **Case report** | **Letter to Editor** | **Editorial** | **Book Chapter** | **Guidelines** |
| **Chemical Pathology** | 15 | 3 | 1 | - | 1 | - | - |
| **Haematology & Transfusion Medicine** | 5 | - | 3 | 2 | - | - | - |
| **Histopathology** | 9 | 10 | 7 | - | - | 8 | - |
| **Microbiology** | 24 | 1 | 2 | - | - | - | - |
| **Molecular Pathology** | 3 | 1 | 0 | - | - | - | - |

# AWARDED GRANTS 2019 – 2021 (till September)

| | 2019 | 2020 | 2021 |
|---|---|---|---|
| **Extramural grants** | 4 | 11 | 8 |
| **URC** | 2 | 2 | 2 |
| **Seed Money** | 0 | 0 | 1 |
| **SoTL** | 0 | 0 | 0 |
| **RRG** | 4 | 4 | 5 |
| **Institutional** | - | - | 5 |

# AUTHOR INDEX