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Paediatric Diagnostics

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From the Editor's Desk

Dear Readers

The past surreal year of 2020 occupies a different significance in the history of the world. So far, we all have responded positively. The technology has bridged the gap between individuals even as social distancing was practiced. During this time our LABRAD was digitalized and is now also available on AKU website: https://www.aku.edu/mcpk/pathology/labrad/Pages/from-the-editors-desk.aspx

The current issue is thematic on 'Paediatric Diagnostics'. A compilation of articles covering the various aspects of paediatric disorders with relevant investigations are being presented here. We have some interesting discussions like diagnostic tools for HIV in infants, fecal calprotectin in inflammatory bowel disease, pleuropulmonary blastoma a rare tumor of infancy, biotinidase deficiency, utility of chromosomal microarray, hemoglobin variants analysis and intussusception in paediatric patients.

Pakistan suffers from a high burden of rare diseases due to the prevalent practice of consanguineous marriages, lack of awareness, and non-availability of clinical metabolic services. Early this year, Department of Pathology and Laboratory Medicine, in collaboration with Pak-IMD-Net and Pakistan Society of Chemical Pathologists launched an e-portal 'Ek Sath' to support children/patients with inherited metabolic disorders and their families. To visit Ek Sath's website click the link: https://fal.cn/EkSath

We value your opinion and feedback regarding topic selections, educational and resource materials we can provide and ideas on how we can better network and communicate using LABRAD.

Dr Lena Jafri,
Editor LABRAD

Laboratory Diagnostic Tools for HIV in Neonates and Infants

Dr Safia Moin and Dr Mohammad Zeeshan
Microbiology

Early and optimal laboratory diagnosis has an important role in the mortality and morbidity of HIV infected children as early antiretroviral therapeutic (ARTs) interventions in less than five years of age have shown better life long outcomes. One third population in this age bracket lost their battle of lives in infancy if do not get ARTs and proper care on time. However, the limitations and challenges in laboratory diagnosis and confirmation specifically in this age group must be kept under consideration while confirming the clinical suspicion.

Proper utility of the following diagnostic tools in the first few months of life can be helpful for child's health:

1. Nucleic acid amplification test (NATs):
   - It is the recommended diagnostic platform in infants and children up till 18 months of age and must be done at 4–6 weeks of age or at the earliest opportunity thereafter for all HIV expose infants
   - HIV DNA performed on whole blood specimen or Dried Blood Sample (DBS); HIV RNA on plasma or DBS;
   - Before starting ARTs, a second specimen should be collected to confirm the initial positive virological test result

2. HIV antibody detection:
   - Due to transplacental transfer from HIV

confirmed mother, presence of antibodies in new born serum does not confirm the diagnosis and therefore are not recommended up till 18th months of child's life.

- Antibody-negative results may suggest that infants are unexposed and or uninfected, however if the infant is on breastfeeding the risk of acquisition from HIV confirm mother continues throughout the entire breastfeeding period.
- HIV-exposed infants who are well, undergo HIV serological testing at around nine months of age (or at the time of the last immunization visit). Those who have reactive serological assays at nine months should have a virological test to identify HIV infection and the need for ART.
- Infants with signs or symptoms suggestive of HIV infection undergo HIV serological testing and, if positive (reactive), virological testing.

3. Ultrasensitive p24 (Up24) antigen:
   - It is detected through ELISA-based technology and was being considered as reliable tool, however, after the availability of easy and economical NATs and difficulty in procurement of p24 commercially available kit has made this test almost redundant.

4. Rapid diagnostic test:
   - Antigen/antibody combination immunoassays that detect HIV-1/2 antibodies as well as HIV-1 p24 antigen are not recommended for 1diagnosis of HIV infection in infants.

Table 1: Summary of recommended testing approaches and required actions

Category	Test required	Purpose	Action
Well, HIV-exposed infant	Virological testing at 4–6 weeks of age	To diagnose	Start ART if HIV-infected
Infant – unknown HIV exposure	Maternal HIV serological test or infant HIV serological test	To identify or confirm HIV exposure	Need virological test if HIV-exposed
Well, HIV-exposed infant at 9 months	HIV serological test (at last immunization, usually 9 months)	To identify infants who have persisting HIV antibody or have seroreverted	1. HIV seropositive need virological test and continued follow up 2. HIV negative, assume uninfected, repeat testing required if still breastfeeding
Infant or child with signs and symptoms suggestive of HIV	HIV serological test	To confirm exposure	Perform virological test if <18months of age
Well or sick child seropositive >9 months and < 18 months	Virological testing	To diagnose HIV	Reactive– start HIV care and ART if under 24 months, or based on national start criteria if 24 months or more
Infant or child who has completely discontinued breastfeeding	Repeat testing six weeks or more after breastfeeding cessation– usually initial HIV serological testing followed by virological testing for HIV-positive child and < 18 months of age	To exclude HIV infection after exposure ceases	Infected infants and children < 24 months of age, need to start HIV care, including ART

Higher Risk: Infants born to mothers with HIV who did not receive prenatal care, did not receive antepartum or intrapartum ARV drugs, received intrapartum ARV drugs only, who initiated ART late in pregnancy (during the late second or third trimester), received a diagnosis of acute HIV infection during pregnancy, or had detectable HIV viral loads close to the time of delivery, including those who received combination ARV drugs and did not have sustained viral suppression.

Age at NAT testing	Birth	14-21 days	1-2 months	2-3 months[a]	4-6 months

[a]For higher risk infants additional virologic diagnostic testing is recommended at birth and 2 to 6 weeks after cessation of ARV prophylaxis (i.e., at 8-12 weeks of life).

Key: ART = antiretroviral therapy; ARV = antiretroviral; NAT = nucleic acid test

Recommended Virologic Testing Schedules for Infants Who Were Exposed to HIV and Who Are at Higher Risk of Perinatal HIV Transmission

Low Risk: Infants born to mothers with HIV who received standard ART during pregnancy and who had sustained viral suppression (usually defined as confirmed HIV RNA level below the lower limits of detection of an ultrasensitive assay) with no concerns related to maternal adherence.

Age at NAT testing	14-21 days	1-2 months[a]	4-6 months

[a]Test may be timed to occur at least 2 weeks after cessation of ARV prophylaxis

Key: ART = antiretroviral therapy; ARV = antiretroviral; NAT = nucleic acid test

Recommended Virologic Testing Schedules for Infants Who Were Exposed to HIV and Who Are at Low Risk of Perinatal HIV Transmission

Intussusception in Paediatric Patients: Role of Radiology in Diagnosis and Management

Dr Shaista Afzal and Dr Amna A. Saeed
Radiology and BBS Department

Introduction

Intussusception is one of the most common causes of acute bowel obstruction in paediatric patients. It typically occurs between three to 24 months of age, with a male: female ration of 2:1. It is due to invagination of intussusceptum (proximal bowel segment) into intussuscepiens (more distal bowel segment). Hypertrophy of lymphoid tissue in the wall of the small bowel plays an important role in the development of intussusception. In five to ten percent of cases, pathological lead points are identified and include polyps, Meckel's diverticulum, duplication cyst etc. The classical symptoms of intussusception include abdominal pain, vomiting, and bloody (red currant jelly) stools. However, this triad of symptoms is seen in less than 25 percent of cases, and other clinical features include a palpable abdominal lump in the right upper quadrant associated with emptiness of the right iliac fossa.

Diagnosis

Intussusception, if not diagnosed early, may lead to compromised blood supply to the bowel, which can result in bowel infarction, perforation, peritonitis and even death. Hence, prompt diagnosis is warranted to avoid these grave consequences. The diagnostic imaging approach includes x ray abdomen and ultrasound for exclusion of other causes of obstruction and for confirmation of intussusception. Although x ray abdomen has low sensitivity for detection of intussusception, i.e., around 45 percent, it helps excludes the presence of perforation and pneumoperitoneum. The imaging features seen on an abdominal radiograph are an elongated soft tissue density mostly in the right side of abdomen, with signs of bowel obstruction proximally, and paucity of gas distally (Figure 1).

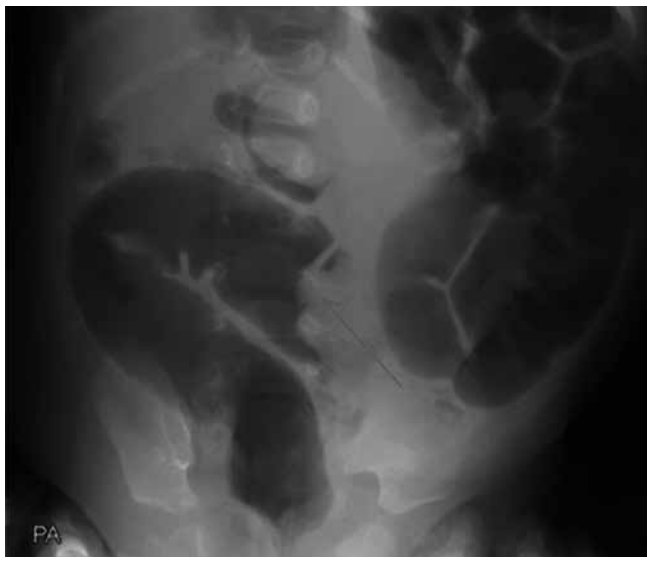


Figure 1: Soft tissue mass in lower abdomen suggesting colocolic intussusception

The reported accuracy of ultrasound in diagnosis of intussusception approaches 100 percent. The classical ultrasound features of intussusception include target/crescent in donut appearance in transverse section, and pseudo kidney/ sandwich appearance in longitudinal section (Figure 2). Ultrasound also helps in evaluation of its reducibility (Figure 3). The features associated with reduced success rates of air enema includes small bowel obstruction, more distil location, trapped inter bowel loops fluid and lack of Doppler flow in the bowel wall. However, the presence of lymph nodes within the intussusception or ascites has not been reported to affect the outcome of this procedure.

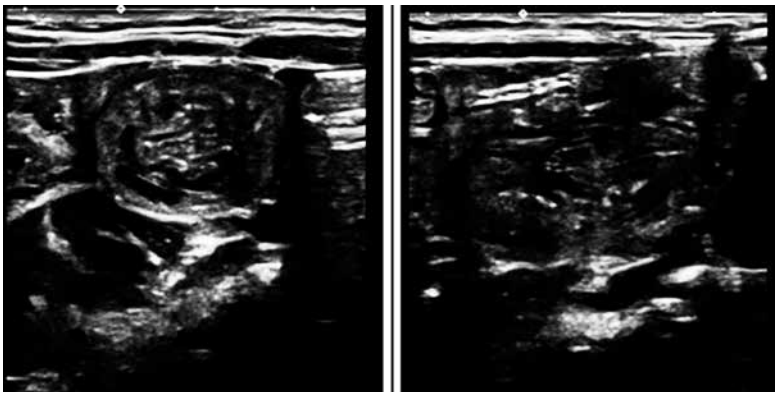


Figure 2: Ultrasound right upper abdomen shows target/ crescent in donut appearance in transverse section and pseudo kidney/ sandwich appearance in longitudinal section, consistent with intussusception

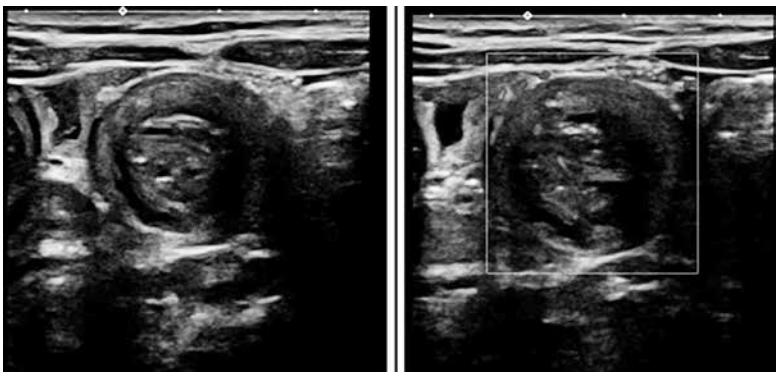


Figure 3: The ultrasound features seen with successful reduction of intussusception i.e., no trapped inter bowel loops fluid and normal Doppler flow in bowel wall.

Management

Once diagnosed on imaging, the next step towards patient's management is the use of enema as the first line treatment. The technique is to instill air, saline or contrast through rectum into the colon under real time imaging. The pressure builds up as a result thus helps to reduce the intussusception.

The success rates of air enema reduction and hydrostatic reduction under ultrasound guidance are almost similar however, the advantage under ultrasound guidance is avoidance of ionizing radiations. These two techniques are associated with low incidence of perforation. Barium enema reduction of intussusception may result in peritoneal spillage and chemical peritonitis in case of perforation and has thus become less popular. Prior to image guided reduction, surgical consultation is essential to exclude the presence of complications including peritonitis, shock, and pneumoperitoneum, as the procedure is contraindicated in such conditions.

During the procedure of hydrostatic enema, saline solution is slowly introduced by gravity into the rectum through a rectal tube that is firmly taped in place. The fluid column is maintained at a height of three feet above the patient. It is recommended to sustain pressure for not more than three minutes in case of a non-moving intussusception, and attempt the process not more than three times. The pressure thus achieved forces retrograde movement of bowel through the ileocecal valve.

The method of air reduction involves introduction of air through a rectal catheter (Figure 4). The pressure within the colon should not exceed 120 mm Hg. With the reduction of intussusception (Figure 5), the intracolic pressure falls, and hence, the presence of pressure gauge and pop off mechanism is very helpful. The reported success rate of air enema reduction of intussusception varies from 51 percent to 95 percent.

Figure 4: Instrument for Pneumatic reduction of intussusception

In those patients with

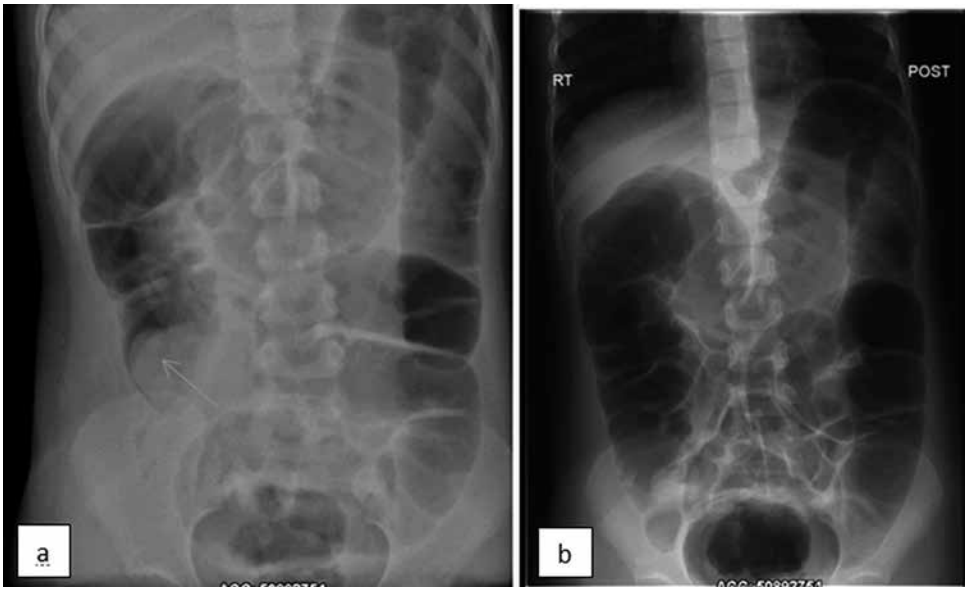


Figure 5: Soft tissue due to intussusception in the region of cecum (a), which started reducing with pneumatic reduction. Complete reduction of intussusception was achieved and free reflux of air is seen into the small bowel loops (b).

partial reduction of intussusception at first attempt, a repeat enema has been reported to be successful in around 50 percent of cases. The suggested time interval between these repeated enemas is from 30 to 60 minutes.

Recurrent intussusception after image guided reduction ranges from five percent to 20 percent and half of these occur within 48 hours. These show a higher incidence of pathological lead points. Recurrent intussusception can be managed non-operatively and does not necessarily warrant surgery, even if it occurs a number of times.

Conclusion

It is important to recognize the imaging features of intussusception for early diagnosis and to be aware of its possible radiological management options for prompt treatment and hence to reduce morbidity and mortality.

Fecal Calprotectin as an Inflammatory Marker in Inflammatory Bowel Disease

Ms Iffat Arman
Clinical Chemistry

Calprotectin (CP) is a zinc- and calcium-binding protein of the S100 family expressed mainly by the cytoplasm of neutrophils. It participates in leukocyte interactions with the endothelium, cellular adhesions leading to the recruitment of leukocytes to inflamed intestinal tissue, and with the inflammatory and thrombogenic response of endothelial cells.

Calprotectin is recommended for the diagnosis, assessment of relapses, follow up and response to treatment in inflammatory conditions, such as inflammatory bowel disease (IBD), celiac disease, necrotizing enterocolitis, acute appendicitis, intestinal cystic fibrosis etc. the fecal CP remains stable for up to one week when stored at room temperature, thus it can be used as an accurate marker for inflammatory disorders of gut, especially IBD and it is now a recommended biomarker for optimizing management of IBD patients. Its concentration is elevated in both Crohn's disease and ulcerative colitis, while treatment leads to a decrease in CP concentrations parallel to clinical improvement, reflecting mucosal healing.

In Aga Khan Hospital fecal CP test is performed and is intended for use in the quantitative determination of human CP levels in stool samples with reference range of <43.2ug/G. It is a good potential marker for diagnosis and prognosis of IBD patients. However, caution should be practiced while interpreting its results, as its levels can also be elevated in other inflammatory diseases, infectious diseases of gut and even in some neoplastic paediatric tumors.

Spectrum of Tools for the Detection of Enteric Pathogens in A Clinical Laboratory

Dr Salima Rattani and Dr Mohammad Zeeshan
Microbiology

Diarrheal diseases in children, acute or chronic, can have serious long term implications and health sequels. It contributes considerably in disease burden of low and middle income countries. The spectrum of causative agents is extensive depending upon nutritional status, socioeconomic condition, underlying comorbidities and the geographic domain.

The paradigm shift in laboratory diagnostic methods has proven the improved disease outcome. However, conventional methods are still being used as front line modalities in resource limited health care settings.

1. Stool Microscopy

a. Direct and concentration methods:
Direct microscopy is one of the oldest laboratory diagnostic procedure but still equally useful and cost effective for identification of enteric ova and parasites. It depends upon the microscopist skills; further, the conventional concentration methods are laborious and messy. Therefore, for improving the yield and reducing the cumbersome process, the utility of concentration tube with build-in Millipore filter has been introduced (Figure 1).

(a) Unassembled flat-bottomed mixing chamber and filter attached to the conical sedimentation with an inbuilt spoon
(b) The sedimentation cone scoop of fecal specimen is assembled to the mixing chamber and then vortexed with the sedimentation cone facing upward
(c) Tube is then centrifuged after inverting at 400 g for 2 min
(d) Sedimentation chamber after centrifugation
(e) Wet mount preparation from the sediment

b. Trichrome stained fixed smear:
It is a rapid and simple procedure, which produces uniformly well-stained smears of the intestinal protozoa and differentiate from human cells, yeast, and artifact material on ethanol fixed fecal smears. The main advantage is the visual clarity of the internal structure of ova, cysts and trophozoites of enteric protozoa fresh and polyvinyl alcohol fixed specimen (Figure 2).

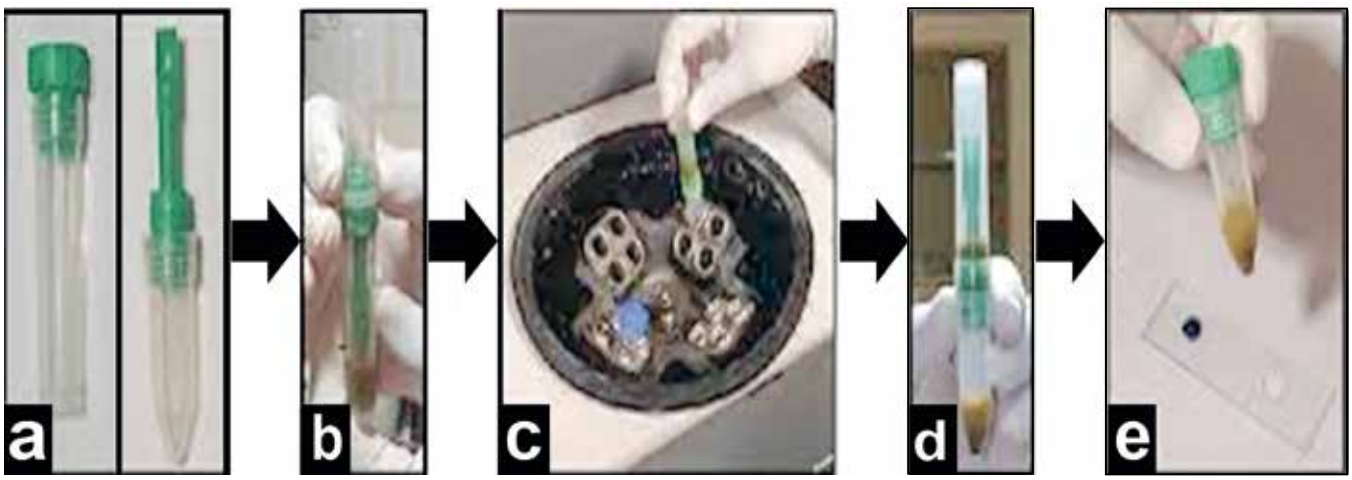


Figure 1: Fecal concentration tube and processing

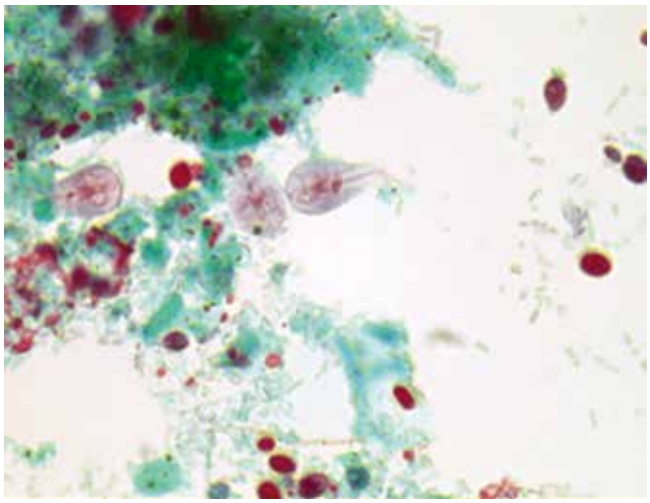


Figure 2: Trichrome stain Giardia lamblia (100 X)

c. Modified Acid-Fast fixed smear:
It is also a simple, rapid and effective staining procedure for the identification of oocysts of coccidian species (cryptosporidium and cyclospora). It can be used on fresh or formalin fixed fecal specimen and duodenal fluid. Immunocompromised population with chronic diarrhea must get evaluated by special request of modified acid-fast stain (Figure 3).

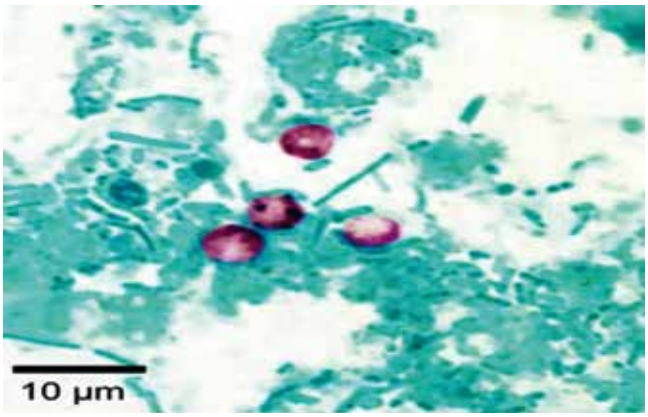


Figure 3: Cryptosporidium: Modified acid-fast stain under oil immersion (100 X)

d. Cellophane (Scotch) tape preparation:
This is used to identify the pin worm *(Enterobius vermicularis) and their ova around perianal area where female worm lays their eggs at night. Fecal specimen is not appropriate method for identify pin worm. After placing scotch tape on glass slide for microscopic evaluation is performed. For better yield, three consecutive morning sample is recommended* (Figure 4).

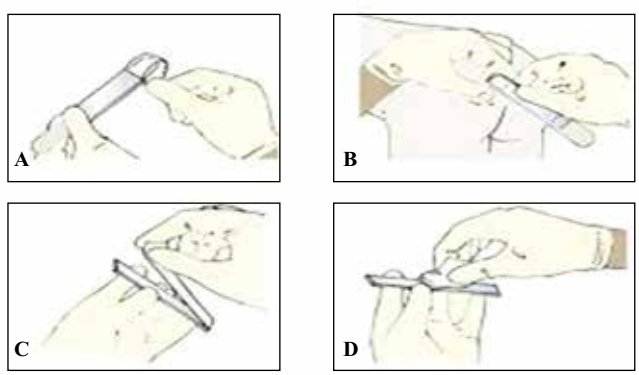


Figure 4: cellophane (scotch) tape collection and preparation method

2. Fecal Culture and Sensitivity

It is usually set for isolation and identification of epidemiologically significant enteric bacterial pathogens including ***Salmonella*** species, ***Shigella*** species, ***Campylobacter*** species, ***Aeromonas*** species and ***Vibrio*** species on fresh fecal or stored and transported fecal specimen in Clary-Blair medium. Rectal swabs can be used in those who has difficulty in specimen collection. Below is the rectal swab collection procedure:

- Pass the tip of a sterile swab approximately one in. beyond the anal sphincter.
- Carefully rotate the swab to sample the anal crypts, and withdraw the swab.
- Send the swab in Cary-Blair medium or buffered glycerol saline.

Specimen submission during the acute stage of infection (usually five to seven days) is essential because pathogens decrease in number with time. If fresh stool is submitted for culture that is not in transport medium, the specimen should be transported to laboratory and processed within two hour after collection. For best recovery in cases for delay of < 24 hours, refrigerate at 4°C.

Figure 5: Clary-Blair transport medium for fecal specimens

If the initial stool culture is negative, then additional fecal samples may be submitted for testing from different defecations on successive days (Figure 5).

3. Enzyme Immunoassays (EIA)

This method is helpful for detection of fecal antigen, and toxin. The available tests are for *Entamoeba histolytica, Clostridium difficile, Giardia lamblia, Helicobacter pylori,* Rotavirus and adenovirus.

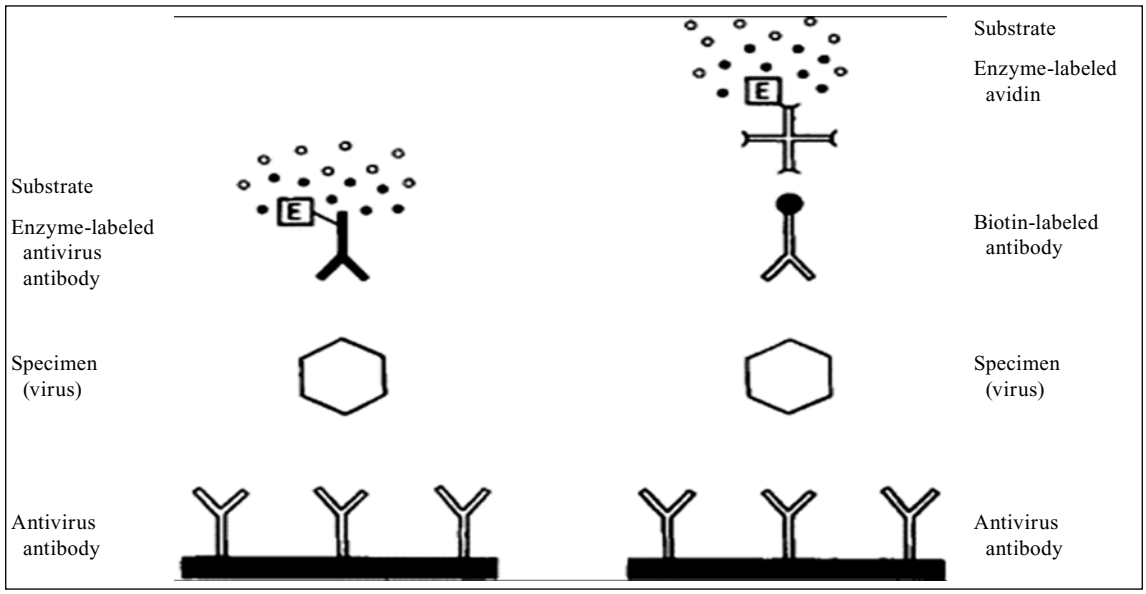


Figure 6: Enzyme-linked immunosorbent assay (ELISA) for detection of virus and/or viral antigen. Left, direct. Right, avidin–biotin.

4. Multipanel Nucleic acid amplification test (NAT)

This PCR base technology works on syndromic approach and utilize multiple nucleic acid sequences or gene targets of diarrhea causing agents in a single test run with in span of an hour. Therefore, the test is rapid and specific. Below is the list of causative organisms that can be detected

Bacteria	Virus	Protozoa
■ Clostridium difficile ■ Shiga-toxigenic E. coli (STEC) 0157 ■ Shiga-toxigenic E. coli non-O157 ■ Enterotoxigenic E. coli (ETEC) ■ Enteropathogenic E. coli (EPEC) ■ Enteroinvasive E. coli (EIEC)/ Shigella ■ Enteroaggregative E. coli (EAEC) ■ Salmonella ■ Plesiomonas shigelloides ■ Aeromonas ■ Vibrio ■ Vibrio cholerae ■ Campylobacter ■ Yersinia enterocolitica	■ Adenovirus F 40/41 ■ Astrovirus ■ Norovirus GI/GII ■ Rotavirus A ■ Sapovirus	■ Cryptosporidium ■ Cyclospora cayetanensis ■ Entamoeba histolytica ■ Giardia lamblia

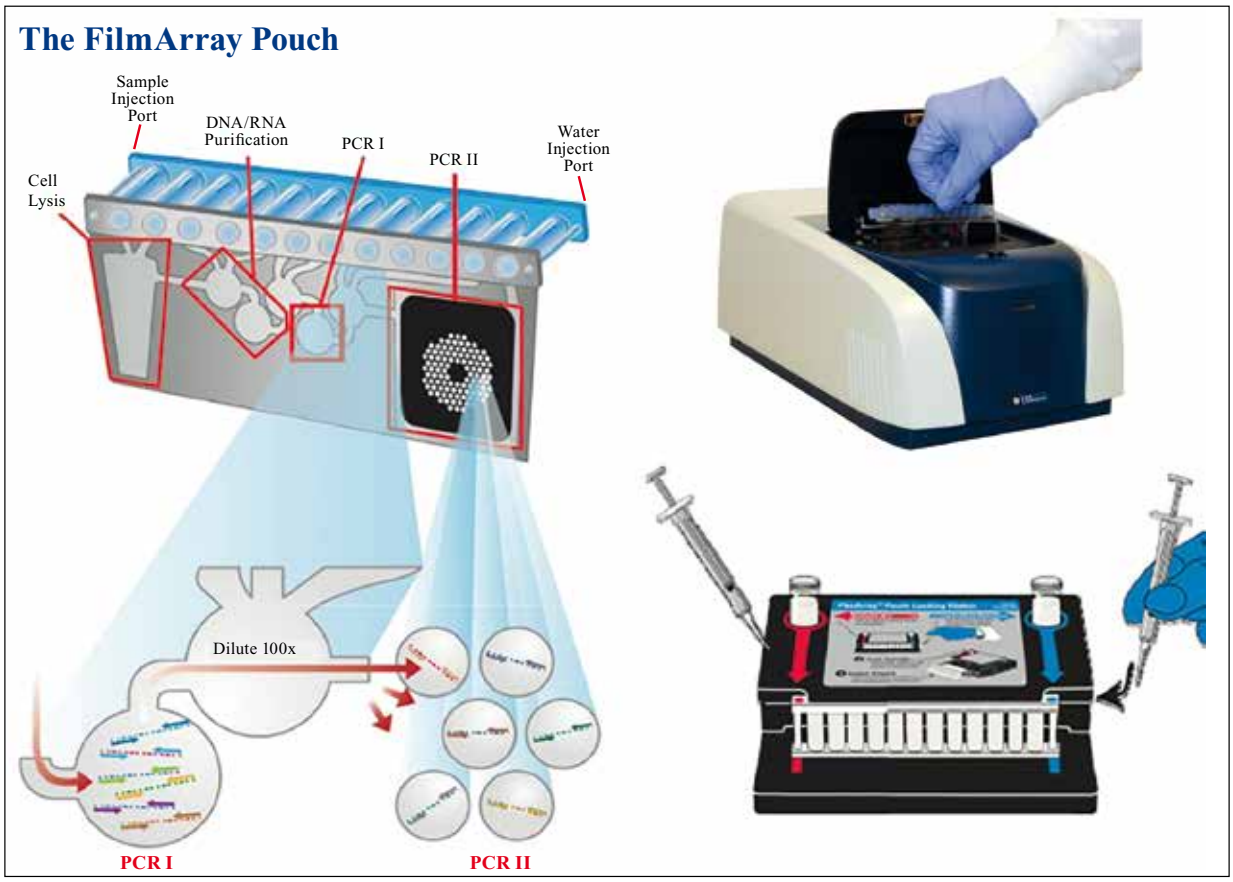


Figure 7: FilmArray multiplex nested PCR for detection and identification of several causative agents.

The conventional laboratory diagnostic tools for gastrointestinal tract infection can be effective for parasites, few bacteria and viruses, however, the detection ability is dependent strongly upon laboratory quality assurance processes and competence of technologist.

For improve patient's outcomes, it is now imperative for health care facilities to be abreast with the evolving diagnostic facilities for example syndrome based multiplex nested PCR that can have long lasting health care impact.

Pleuropulmonary Blastoma Type III with Rhabdomyosarcomatous and Chondrsarcomatous Differentiation in a Five year old Child, A Rare Case.

Dr Saman Muhammad Amin and Dr Sabeeh Uddin Siddique
Histopathology

Introduction

Pleuropulmonary Blastoma is a very rare malignant tumor of infancy and early childhood arising as a cystic and/or solid sarcomatous neoplasm in the lung or (rarely) the pleura. This embryonic or dysontogenetic neoplasm is the counterpart of neuroblastoma, hepatoblastoma, retinonblastoma

and Wilms tumor. Pleuropulmonary Blastoma tumors are subgrouped as type I (purely cystic), type II (solid and cystic) and type III (purely solid).

Case Presentation

A five years old boy presented with mediastinal mass. Patient had been initially diagnosed as "Mixed germ cell tumor" from outside AKUH and had been treated accordingly. We received the specimen coded as "Right lung upper lobe and mediastinal mass" and it consisted of a single lobe of lung with a protruding cystic mass that was already ruptured and a separately lying piece of mass. The mass had lobulated appearance. On sectioning the cut surface appeared whitish and glistening.

Microscopic examination of this mass revealed a neoplastic lesion comprising of multicystic structures that were lined by respiratory type epithelium and underlying sheets of small primitive malignant cells showing a cambium layer like zone. Blastemal cells were present along with sheets of markedly pleomorphic cells that showed abundant eosinophilic cytoplasm and relatively eccentrically placed hyperchromatic nuclei and variably conspicuous nucleoli (Rhabdomyoblastic differentiation) (Figures 1 and 2). Abundant lobules of neoplastic cartilage were also identified that showed mild to moderate nuclear atypia and increased cellularity. Fascicles of atypical spindle shaped cells are also identified. Brisk mitoses including atypical mitoses, necrosis (approximately 20 percent), hemorrhage, fibrosis and dead bone are appreciated. Immunohistochemical stains Desmin and Myogenin highlights rhabdomyoblastic cells and Cytokeratin AE1/AE3 and TTF-1 highlights native respiratory epithelium. The case was diagnosed as Pleuropulmonary Blastoma, type III (Rhabdomyosarcomatous and chondrosarcomatous differentiation).

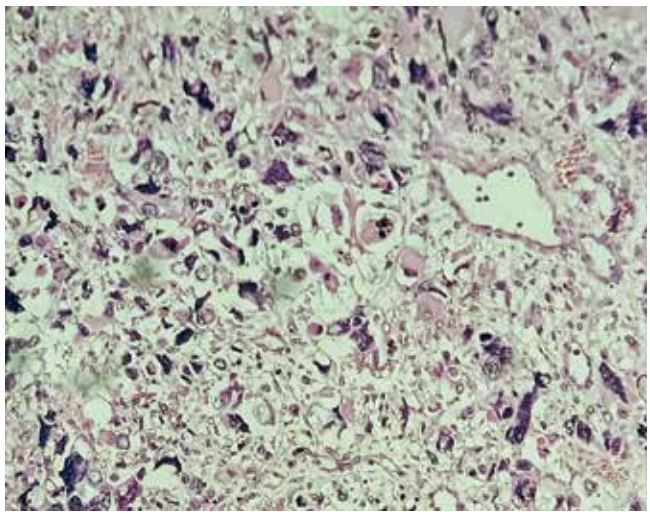


Figure 1 Rhabdomyoblastic differentiation.

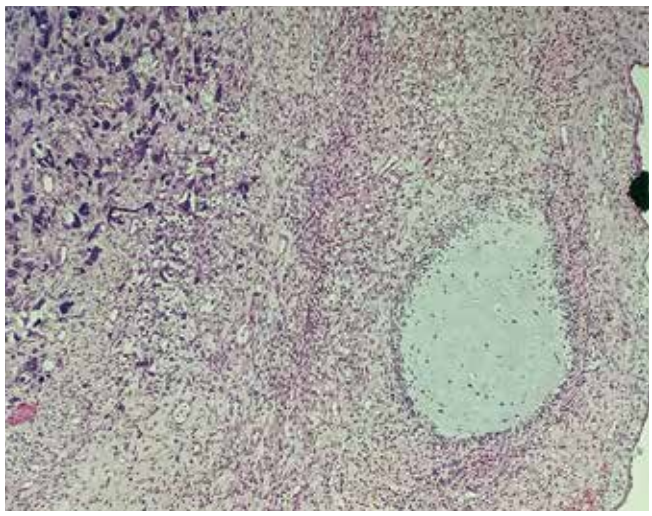


Figure 2 Pleuropulonary blastoma. Cystic structure lined by epithelium. A nodule of cartilage is present in cyst wall along with rhabdomyoblasts.

Conclusion

Pleuropulmonary Blastoma is a rare tumor and it should always be considered in the differentials when evaluating small lung biopsies taken from children that exhibit cartilaginous and mesenchymal components.

New and Emerging Paediatric Bone and Soft Tissue Tumors: An Update from the 5th Edition of WHO Blue Book

Dr. Qurratulain Chundriger and Dr. Nasir Ud Din
Histopathology

With the ongoing advances in molecular techniques new knowledge about molecular mechanisms behind etio-pathogenesis of neoplastic processes is continuously being acquired. The result is a havoc in the fields of pathology and pharmaceutical industry, because in addition to the identification of novel mutations reclassifying certain tumors as specific entities, traditionally being put under umbrella terms and waste basket diagnoses, specific therapeutic targets are being recognized and drugs are being developed by the truck load. Welcome to the era of personalized medicine!

The fifth edition of the WHO classification of Tumors of Bone and Soft tissue was recently published, seven years after the 4th edition. In addition to a different and more reader friendly pattern of the volume on the whole, a useful addition are the headings of **Essential and desirable diagnostic criteria, Cytological features,** revised **Staging** and evidence-based discussion under the heading **Prognosis and Prediction.** Furthermore, a number of new and emerging entities have been added to this edition, few of which arise mostly in the adult population, few solely in the paediatric patients and some show overlap and a wide age range from infants to the elderly. This article focuses not only on the paediatric tumors but those with a wide age range have also been discussed as they may also be encountered in patients less than 18 years of age. Some of these tumors have been established as entities while a few are still emerging with ongoing clinical, morphological, phenotypic and molecular studies.

Poorly Differentiated Chordoma (PDC)

PDC arises in children, more commonly females, with a mean age of 11 years. Axial skeleton, specifically skull base is the most commonly involved site. Clinical presentation is with headache and symptoms of cranial nerve compression.

Radiology shows origin and destruction of bone, with infiltration into the adjacent soft tissue. Heterogeneous appearance is seen on CT and MRI.

Histologically, the most striking features is absence of the physaliphorous cells typical of Chordoma. The myxoid background stroma may be only focally present. The tumor is composed of epithelioid cells, growing in cohesive nests and sheets with abundant eosinophilic “pink” cytoplasm. Cytoplasmic vacuoles may be seen, imparting signet ring like features. The nuclei are pleomorphic, round to oval with vesicular chromatin. Numerous mitoses and necrosis are generally present (Figure 1).

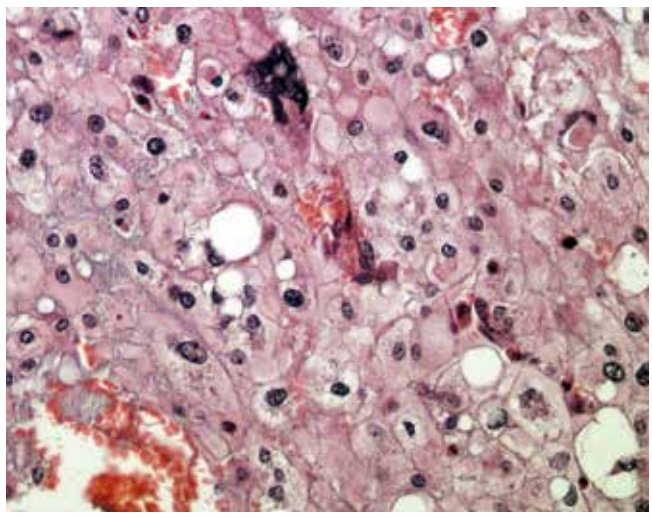


Figure 1. H&E stained sections show sheets of epithelioid cells with distinct cell borders and cytoplasmic vacuoles.

Immunohistochemically, positive expression of Cytokeratins and Brachyury, but the expression of S100 is variable. An essential diagnostic feature is the loss of nuclear INI-1, which results from deletion of the

SMARCB1 gene. Fluorescent in-situ hybridization is the method to evaluate this along with co-deletion of EWSR1 locus. This tumor shows worse prognosis as compared to Chordoma.

BCOR Family of Tumors

This entity comprises of a group of round cell sarcomas, sharing clinical and morphological features with other round blue cell soft tissue sarcomas like Ewing Sarcoma. Before the identification of BCOR gene mutations these tumors were grouped under the heading of undifferentiated round cell sarcomas. The BCOR gene shows several types of alterations, resulting in a group of tumors which either show rearrangements and fusion with other genes – BCOR-CCNB3 most frequently – or have internal tandem duplications – BCOR-ITD. The former group belongs to patients less than 20 years of age; while, the latter along with Primitive Myxoid Mesenchymal Tumor of infancy typically arises in infants. Whatever the mechanism, the end result is oncogenic activation of the BCOR gene along with overexpression, identification of which aids in the diagnosis.

Bones of pelvis, lower extremities and paraspinal region are the usual sites of origin for BCOR-CCNB3 sarcomas. Although they may arise less frequently in soft tissue sites as well for example lung, head and neck region and kidney. BCOR-ITD and Primitive Myxoid Mesenchymal Tumor of infancy mostly occur in soft tissues of trunk, retroperitoneum and head and neck region.

Patients present with pain and swelling. Grossly the tumor may grow to large sizes of more than 10 cm. Microscopically, the distinctive feature in addition to the round cell morphology is the presence of a rich capillary network, which if growing in kidney, may mimic Clear Cell Sarcoma in an infant. The tumor cells have finely dispersed chromatin. Some tumors may exhibit spindle cell morphology mimicking a Synovial Sarcoma and a myxoid background. Strong nuclear expression of BCOR on immunohistochemistry is the most important marker for diagnosis along with expression of SATB2 and Cyclin D1, regardless of the mechanism of BCOR gene alteration.

These tumors also sometimes harbor YWHAE-NUTM2B fusions, as seen in High grade Endometrial Stromal Sarcomas and Clear cell Sarcoma of kidney. This fusion also results in oncogenic activation of the BCOR gene and nuclear BCOR expression. Cyclin B3 is additionally expressed by the BCOR-CCNB3 sarcomas.

Lung is the most common site for metastatic disease. The chemotherapy regimen and five-year survival rates for BCOR-CCNB3 sarcomas is similar to that of Ewing Sarcoma. Data on other BCOR family sarcomas is still not clear.

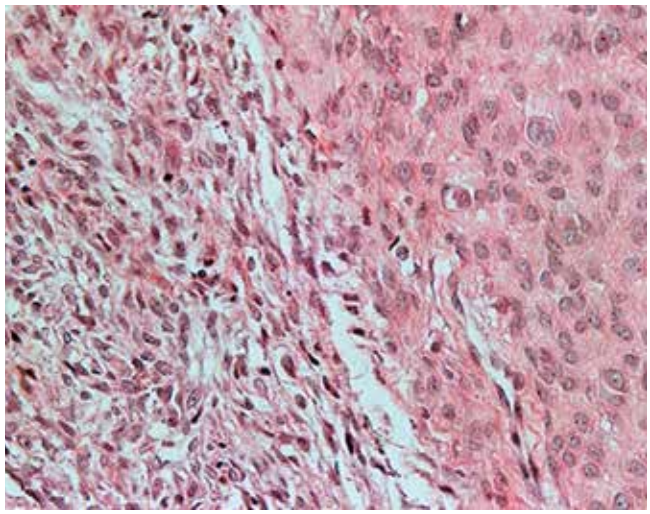


Figure 2. H&E stained section showed round and spindle cells in a BCOR rearranged sarcoma.

CIC Sarcomas

These are high grade round cell sarcomas showing rearrangements of CIC (Capicua Transcriptional repressor) gene, with several fusion partners, DUX4 being the most common. They were previously grouped under the umbrella of undifferentiated round cell sarcomas along with BCOR family of tumors and represent the most frequent subtype of ‘Ewing-like tumors.

These tumors mostly arise in adolescents with a mean age of 25-25 years although a substantial number is seen in paediatric patients as well. Deep soft tissues of extremities and trunk are the common sites of origin with head and neck region being less common, similar

to the BCOR tumor family. Presentation with mass lesion with or without pain is common, as is initial presentation with metastatic disease.

These tumors tend to be large with frequent necrosis and hemorrhage. Microscopic examination shows lobulated growth with indistinct fibrous stroma. Sheets of undifferentiated round cells with admixed minor population of epithelioid or spindle cells is seen (Figure 3). In contrast to the more common Ewing Sarcoma, CIC rearranged sarcomas exhibit nuclear pleomorphism with open chromatin and variably prominent nucleoli. Mitotic activity is brisk. These tumors express CD99 in a patchy distribution as compared to diffuse membranous staining seen in Ewing Sarcoma. NKX2-2, a marker for Ewing Sarcoma, is also negative. WT-1 and ETV4 are also expressed consistent and constitute the criteria for diagnosis along with molecular evidence of CIC mutations.

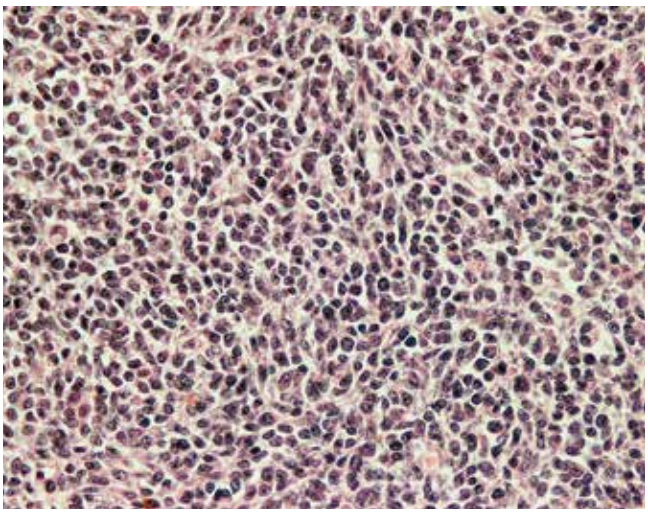


Figure 3. H&E stained sections showing variable appearance of epithelioid to round to spindle cells

CIC-DUX4 fusion is the most common alteration seen in more than 95 percent of cases, which results from either t(4;19)(q35;q13) or t(10;19)(q26;q13) translocations. Other rarer fusion partners include FOX04, LEUTX, NUTM1 and NUTM2A.

Most of these tumors behave aggressively with metastatic disease, lung being the most common site. The response to Ewing Sarcoma chemotherapy is not good and the five-year survival rates are also far worse.

NTRK-Rearranged Spindle Cell Neoplasm

The WHO defines this group as an emerging group of molecularly defined rare soft tissue tumors showing a wide spectrum of morphological features and coexpressing S100 and CD34. It is a provisional category and includes Lipofibromatosis-like neural tumors and tumors that closely resemble peripheral nerve sheath tumors. Most of these occur in patients less than twenty years of age, with the latter two seen most in children. They arise in the extremities and trunk as either superficial or deep tumors.

Microscopically they exhibit a wide range of morphology, at one end of which is Lipofibromatosis-like neural tumor and at the other end are tumors resembling Malignant Peripheral Nerve sheath tumor. The former group shows a highly infiltrative pattern of growth comprising of spindle cells with bland appearance, having indistinct cytoplasm and tapering nuclei. Mild nuclear atypia and more cellular examples may be sometimes seen. Necrosis is absent and mitosis is only scant. The latter group comprises of spindle cell tumors exhibiting solid growth, high cellularity and prominent stromal bands and perivascular keloid like collagen deposition, which is hallmark of these tumors. Although the cells are monomorphic, the growth pattern with streaming bands and patternless areas is typical. A variable mixture of these two appearances may also be seen with some less cellular areas merging with more cellular foci. Some examples may show nuclear pleomorphism and high mitotic activity with necrosis. A smaller subgroup with NTRK-1 rearrangements shows myopericytoma-like morphology.

S100 and CD34 positivity is the rule, with absent SOX10 staining and retained H3K27me3 expression, which helps in differentiation from MPNST. Majority of them show positive nuclear or cytoplasmic staining with anti-Pan-TRK monoclonal antibody, a non-specific marker for NTRK gene rearrangement. Additional testing is required for conclusive diagnosis and deciding the appropriate therapy depending on the fusion partner.

Prognosis is grade dependent. The lower grade lesions have a propensity for local recurrence due to infiltrative growth, while the higher-grade lesions exhibit an aggressive course with metastasis most commonly to lungs. Targeted therapy against receptor tyrosine kinases (TRKs) has a role in the treatment of these patients.

EBV-Associated Smooth Muscle Tumor

This entity arises in the setting of immune deficiency in one of three settings in descending order: 1- HIV/AIDS 2- after solid organ or hematopoietic stem cell transplant and 3- congenital or primary immunodeficiency. Children are the most affected population in cases of primary immunodeficiency, the other scenarios being common in adults. The latent period after the onset of immunodeficiency is long, spanning up to several years. If encountered outside the three described scenarios, the patient should be worked up for immunodeficiency.

These tumors can arise at any site in the body including solid organs. The most reported in CNS (intra and extra-axial). They can reach large sizes of more than twenty cm. Cut surface is rubbery and firm consistency. Microscopic examination shows typical morphology of smooth muscle tumors, having interconnected fascicles of spindle cells with eosinophilic cytoplasm and uniform elongated nuclei. The distinctive features of EBV associated tumors is the presence of small round primitive appearing smooth muscle cells and a variable infiltrate of T lymphocytes within the tumor, the latter being more common in the setting of HIV infection. Some tumors may exhibit hemangiopericytoma-like blood vessels. Immunohistochemistry shows positivity for Smooth muscle Actin and H-Caldesmon with patchy variable Desmin. EBER is consistently positive on In-situ hybridization and is required for diagnosis. Prognosis is largely dependent on the individuals' overall condition. Most of these tumors do not metastasize and some have been shown to regress on reduction of immunosuppression.

Myxoid Pleomorphic Liposarcoma

It is a highly aggressive and extremely rare adipocytic tumor, most commonly occurring in children and young adults. It shows features of both Myxoid Liposarcoma and Pleomorphic Liposarcoma but lacks the genetic alterations of Myxoid Liposarcoma, Dedifferentiated Liposarcoma and Atypical Lipomatous Tumor. Deep soft tissues of mediastinum is the most common site of origin.

Histologically, these tumors show a mixture of appearances with areas having delicate curvilinear vascular network, myxoid background with lipoblasts and bland primitive round cells reminiscent of conventional myxoid liposarcoma. There is gradual transition to more cellular areas with presence of more pleomorphic lipoblasts, necrosis, numerous mitoses and severe nuclear atypia. They have a non-specific immunophenotype.

Diagnosis requires absence of FUS/EWSR1-DDIT3 gene fusions of Myxoid Liposarcoma and MDM2 amplifications of well-differentiated/ Dedifferentiated Liposarcoma. Myxoid Pleomorphic Liposarcoma has aggressive clinical course with frequent metastases and poor survival.

EWSR1-SMAD3-Positive Fibroblastic Tumor

This entity is defined as a benign spindle cell tumor occurring in superficial tissues of hands and feet. A wide age range is described (one-sixty eight years). The tumor is small and painless, up to 1-2 cm in size. This tumor is usually well-circumscribed. Microscopically there is zonation, with fascicles of spindle cells growing at the periphery of the nodule, while the center is less cellular and often hyalinized. The spindle cells have a bland appearance and lack significant nuclear pleomorphism, mitotic activity or necrosis. ERG expression by immunohistochemistry is the rule, along with negative SMA and CD34. EWSR1-SMAD3 fusion is the defining genetic abnormality. Complete excision is curative with rare instances of local recurrence.

Radiology Pathology Correlation: Orthopedic Pathology

Nasir Ud Din and Dawar Khan
Pathology and Radiology

A three year old male child presented with headache. X-ray was done which showed multiple punched out lytic lesions (Figure 1)

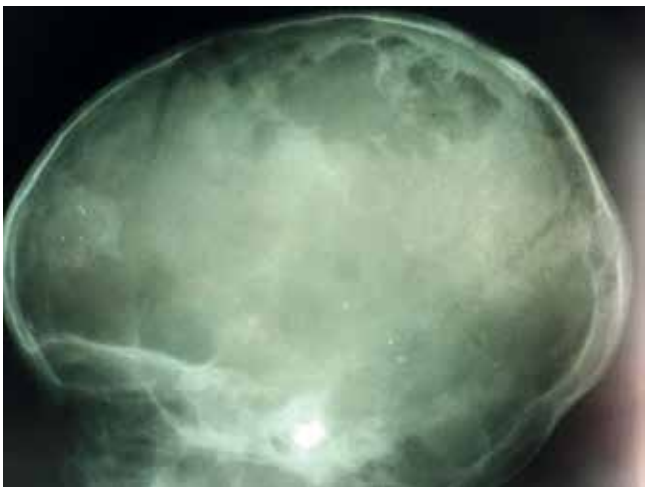


Figure 1. X-ray head lateral view showing multiple punched out lytic lesions, beveled margins and without sclerotic margins.

A biopsy of skull bone was done which histologically showed fragments of bone infiltrated by a neoplastic process composed of sheets of ovoid cells with nuclear grooves, irregular nuclear membranes and eosinophilic cytoplasm. Scattered osteoclast-like giant cells were noted. In the background, eosinophils, neutrophils and lymphocytes were seen. The neoplastic cells were positive for CD1a, S100 and CD68. A diagnosis of Langerhans cell histiocytosis was rendered after radiology-pathology correlation.

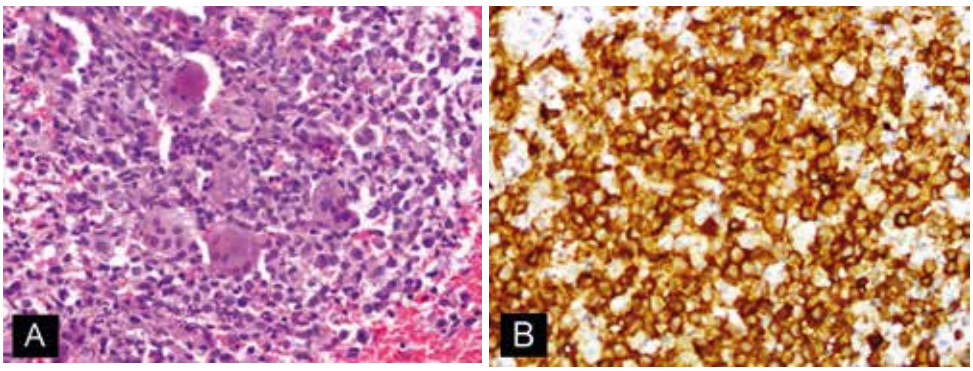


Figure 2. A) H&E showed sheets of Langerhans cells with scattered osteoclast-like giant cells. B) CD1a positivity in Langerhans cells

Discussion

Langerhans cell histiocytosis (LCH) also known as eosinophilic granuloma, is a clonal proliferation of Langerhans cells. Langerhans cells regulate the immune system and normally found throughout the body, especially in the skin, lymph nodes, spleen, lungs, liver, and bone marrow. The disease usually diagnosed during the first three decades of life. The disease can be unifocal or multifocal. It commonly involves skeleton (skull>femur>pelvis>ribs). Monostotic form is more common than polyostotic. Skin, lymph node and lung are other common sites on involvement. In bone, it comprises <1 percent of all bone tumors. Clinical symptoms depend on the site involved. Radiographically, in long bones, present usually as well-defined lytic lesions, but may have ill-defined margins. In skull (50 percent involvement) it appears as discretely punched out lesions involving tables of skull unevenly resulting in beveled edges. Radiological differential diagnosis include osteomyelitis, metastasis, primary bone tumor, lymphoma and leukemia.

In the affected organs, there infiltration of Langerhans cells, accompanied by a variable admixture of eosinophils, giant cells, neutrophils, and foamy cells. The Langerhans cell express CD1a, CD207 (langerin), S100 and CD68 immunohistochemical stains. Histologic differential diagnoses include osteomyelitis, granulomatous inflammation, chondroblastoma, Hodgkin lymphoma and Rosai-Dorfman's disease. LCH is differentiated from osteomyelitis by ovoid cells with grooved nuclei and CD1a positivity. Chondroblastoma contain polygonal cells with nuclear grooves and S100 positive, but negative for CD1a and preferentially involves epiphysis of long bones.

Monostotic skeletal involvement has excellent prognosis. Multifocal disease has guarded prognosis.

High Performance Liquid Chromatography for Hemoglobin Variants Analysis in Paediatric Population

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

Hemoglobin

Hemoglobin (Hb) is an iron binding protein that not only transports oxygen but also body's respiratory carbon dioxide. The red colour of cells (hence the name) is due to this protein.
There are four subunits of Hb each having one polypeptide chain and one heme group. The heme group has iron protoporphyrin IX associated with a polypeptide chain of 141(α) or 146 (β) amino acid residues. The adult Hb has two types of polypeptide chains known as alpha and beta chains. Both embryonic and adult Hb have similar alpha chains. The other (non-alpha chains) include the beta chain of normal adult Hb (α2β2), the gamma chain of fetal Hb (α2β2), and the delta chain of HbA2.

Normal hemoglobin types include:

- Hemoglobin A: 95-98 percent of Hb found in adults comprises of HbA; it's a combination of two alpha and two beta protein chains.
- Hemoglobin A2: Adults have only 2-3 percent of HbA2 comprising two alpha and two delta protein chains.
- Hemoglobin F (fetal hemoglobin): It is the major Hb found in fetal stage whereas makes up to 1-2 percent of Hb found in adults. It is composed of two alpha and two gamma protein chains. HbF is replaced by HbA by one-two years of age.

Mutations in Hb

Hemoglobinopathy is defined as genetic defect that results in abnormal structure of one of the globin chains of the Hb molecule. Hemoglobinopathies are generally single gene defects inherited as a dominant trait in majority of cases.

As a result of mutations in the globin genes there could be either a quantitative reduction in production of Hb or a qualitative defect in the protein produced. Quantitative defects lead to development of thalassemia, whereas qualitative defects, also called Hb variants, result in a wide range of diseases including sickle cell disease, unstable Hb, decreased oxygen affinity, increased oxygen affinity and methemoglobinemia. Pakistan being a resource constrained country and where consanguineous marriages are commonly practiced, is a victim to a huge number of patients suffering from hemoglobinopathies. This pose a massive health problem. In one recent study, the estimated frequency reported was 34.2 percent. Another estimate states that a total of 5000–9000 children with β-thalassemia are born per year, although no documented registry is available in Pakistan. An approximate carrier rate is five–seven percent, with projected 9.8 million carriers in our total population.

Indications for Testing

1. Complete blood count shows RBC indices suggestive of thalassemia trait and/or blood smear shows features of an abnormal hemoglobin.
2. Presenting signs and symptoms in a child suggestive of severe anemia and/or failure to thrive such marked shortness of breath, weakness, lethargy, pale skin, abnormal bossing of facial bones and splenomegaly.
3. Episodes of pain in chest, hands and feet, ulcers formation on bony surfaces (suggesting a specific form of abnormal Hb i.e. sickle cell disease).
4. Family history of hemoglobin disorders; as part of family screening.
5. Prenatal screening in a pregnant female: when either of the parent is high risk or there is a diagnosed/suspected thalassemic child

previously born to the couple.
6. As a part of state legislation for neonatal screening programs, where indicated.

Sample

Venous sample stored in EDTA tube is required for testing. In neonates/infants, a finger stick/heel stick (samples taken by pricking the finger or heel of the neonate) sample may be collected due to difficult phlebotomy and low blood volume in neonates. Pretesting special preparation is not required, however results of blood transfusion interfere with proper interpretation of test. A pre-transfusion sample should be preferably sent in cases requiring transfusion.

Principal of High Performance Liquid Chromatography (HPLC)

HPLC forms one of important tools for testing for hemoglobinopathies. Different types of hemoglobins are separated based upon the ionic charges they carry. An analytical cation exchange cartridge uses a preprogrammed buffer gradient. The hemosylate passes through the cartridge and separates hemoglobin fractions upon interaction with resin. There's a flow cell where absorbance is measured at 415 nm and again at 690 nm to reduce background noise. Separated fractions pass through this flow cell. Changes produced in absorbance due to passage of fractions of hemoglobins are measured and recorded over time producing a chromatogram (absorbance vs. time).

Each type of hemoglobin has a specific retention time which is characteristic to it, hence leading to its identification. This retention time is measured from the time of sample is injected into the HPLC to the highest point achieved by each peak. This peak determines the maximum elution for that type. While assessment, there can be other peaks in the chromatogram which are labeled as Unknown. These peaks are due to hemoglobin fractions that elute at a retention time which is not pre-programmed according to the dictionary of the instrument.

HPLC leads to separation as well as quantification of HbF and HbA2. It also detects variant hemoglobins (sickle cell Hb, Hb C, HbD, HbE) along with thalassemia.
HPLC is equally capable for identifying hemoglobinopathies from both newborns/paediatric and adult populations and takes the benefit of the algorithm/software/instrument specification.

Interpretation of Results

The report provides the presence of normal as well as vairant hemoglobins in order of their quantification. Along with pathologist review, the results are interpreted in close relation with patient's sign and symptoms and family history.

The following table provides some examples of test interpretation: (This table suggests the role of genetic mutation contributing towards the pathogenesis: final conclusion requires genetic studies).
Heterozygous: One gene copy for variant Hemoglobin
Homozygous: Two gene copies for variant Hemoglobin

Results seen	Condition	Possible Genetic Pathology
Hb A: 50-65 percent Hb S: Up to 45 percent	Sickle cell trait	Heterozygous for HbS
Hb S: More than 80 percent Hb F: 2-20 percent Hb A: Absent	Sickle cell disease	Homozygous for HbS
Hb C: More than 80 percent Hb A: Absent	Hemoglobin C disease	Homozygous for HbC
Hb F: More than 80 percent Hb A: Present in small amount or completely absent	Beta thalassemia major	Both beta genes mutated
Hb A: More than 80 percent Hb A2: Increased (4-8 percent) Hb F: Usually increased slightly as compared to reference values	Beta thalassemia minor/ Beta thalassemia trait	Mutations in one beta gene leading to decrease production from one beta globin chain

Conclusion

HPLC forms a reliable method for both diagnosis as well as screening for suspected hemoglobinopathy in paediatric population. It is a simple technique which is highly reproducible with automation, high resolution and rapid results with a short turnaround time thus facilitating patient and physicians. The definitive diagnosis may require genetic testing in small fraction of patients only.

Updates in Reporting Wilms Tumor (Nephrectomy) Specimen

Dr Qurratulain Chundriger, Dr. Zoonish Ashfaq and Dr Nasir-Ud-Din
Histopathology

Paediatric medicine has changed a lot since the advent of more advanced molecular diagnostic techniques and neo-adjuvant therapy. This is particularly true for solid paediatric tumors. Talking about renal tumors, in contrast to getting a resection specimen for primary diagnosis, more and more patients are being first treated with neo-adjuvant chemotherapy followed by surgical resection. This has resulted in refined and revised guidelines for reporting and treatment. Wilm's tumor is one of the most commonly occurring malignant paediatric renal tumors in children up to six years of age. The most common presentation is a mass felt in the abdomen, mostly by the mother. Some cases of presenting with tumor rupture have also been reported. Radiologically it presents as a solid mass in the kidney with sometimes associated foci of calcifications. In certain parts of the world including south Asia, pre-operative chemotherapy has become standard in Wilms tumor patients.

In this article, we will review the parameters for reporting of post chemotherapy nephrectomy specimen and their implications in further treatment of patients.

Wilms Tumor

Nephroblastoma or Wilms tumor is the most commonly encountered surgical pathology specimen in the histopathology lab with status-post chemotherapy resection. The guidelines for reporting the changes secondary to chemotherapy have evolved over the years. The WHO blue book for renal tumors discusses two groups of thoughts in this regard. The two main stakeholders are the Children's' oncology group (COG) and Society of Paediatric Oncology (SIOP). The COG advocates initial resection of tumor, followed by therapy, based on histological findings of grade and stage, while the SIOP applies to resection after the administration of neo-adjuvant Chemotherapy. Under COG, only special circumstances allow for the administration of pre-operative therapy which include Congenital solitary kidney, inoperable tumor, bilateral tumors, vena caval tumor thrombus above the level of hepatic vein, direct extension into adjacent structures like pancreas, spleen colon and presence of extensive metastases in the lung.

Post chemotherapy resection provides the advantage of tumor shrinkage, less instances of per-operative rupture and hence stratification into lower stage group. The SIOP guidelines for these specimen help in deciding the next step in management. While the COG takes into account the tumor biology and chooses the therapy regimen and protocol based on the primary appearance and histology of the tumor. Both groups have shown more or less similar outcomes in terms of disease stratification according to stage and overall survival.

The following table highlights differences in COG and SIOP staging systems, as described by the WHO blue book (bold text):

Stage	COG	SIOP
I	Tumor limited to kidney (**may show minimal infiltration of renal sinus soft tissue but without vascular invasion**) and completely resected	Tumor limited to kidney **without any infiltration of renal sinus soft tissue. Presence of necrotic tumor outside these parameters does not upstage it.**
II	Tumor extends beyond kidney (renal capsule invasion, lymphovascular invasion, renal vein invasion with negative renal vein margin and renal sinus fat invasion) but is completely resected with negative surgical margins	Tumor extends beyond kidney (renal capsule invasion, lymphovascular invasion, renal vein invasion with negative renal vein margin and renal sinus fat invasion) but is completely resected with negative surgical margins

III	Residual tumor (positive margins, rupture, surgical spill, positive nodes, piecemeal excision and **pre-operative biopsy**)	Residual tumor (positive margins, rupture, surgical spill, positive nodes, piecemeal excision and **necrotic tumor or chemotherapy-induced changes at resection margins or in lymph nodes**)
IV	Metastasis (hematogenous or lymph node beyond the renal drainage system, i.e. outside abdomino-pelvic region)	Metastasis (hematogenous or lymph node beyond the renal drainage system, i.e. outside abdomino-pelvic region)
V	Bilateral renal involvement on initial diagnosis (both kidneys to be staged separately)	Bilateral renal involvement on initial diagnosis (both kidneys to be staged separately)

Post-operative Chemotherapy

These changes in the staging system are significant for choosing the next step in patient treatment. Post-surgical therapy is a routine according to COG except in patients who are less than two years at diagnosis, favorable tumor histology tumor of less than 550 gms, stage I as defined above with negative lymph nodes. Similarly, SIOP also recommends post resection chemotherapy in all patients except those with Stage I disease and low risk tumors.

Post-operative Radiation

COG: recommends radiating the tumor bed in stage III disease.
SIOP: recommends radiating the entire abdomen in patients with high risk histology, tumor rupture or peritoneal deposits. The importance of favorable or unfavorable histology also comes into account when decision for including the lungs in radiation field needs to be taken.

Reporting of surgical pathology specimen:

Gross Observations of Intact Specimen

The following points are mandatory for synoptic reporting of gross (and microscopic) findings:

- Intactness of Gerota's fascia is an important parameter in staging the tumor
- Status of renal capsule
- Weight of the specimen
- Tumor focality
- Size of the tumor
- Presence of renal sinus involvement grossly and microscopically
- Involvement of Renal vein with mention of resection margins
- Involvement of adjacent organs if present in the resection specimen
- Status of resection margins

Following parameters can be assessed exclusively on histological examination:

- Histologic type – define histology (favorable, focal anaplasia, diffuse anaplasia)
- Post-therapy histologic classification

Anaplasia in Wilms Tumor

The WHO defines Anaplasia as "Cells with huge hyperchromatic nuclei associated with multipolar mitotic figures". To classify as anaplastic, the largest dimension of these nuclei should be more than three times the largest dimension of tumor cell nuclei in other areas. Atypical mitotic figures are larger than normal mitoses and exhibit more than two poles. Another term worthy of mentioning is **"Severe nuclear unrest"** which is defined as severe nuclear atypia approaching the criteria of anaplasia but not meeting the criteria.

Focal anaplasia is presence of 1 or few sharply outlined tiny foci of anaplasia within the intrarenal tumor. The remaining tumor must not exhibit severe nuclear unrest.

Diffuse anaplasia is the presence of any amount of anaplasia in any of the extrarenal sites i.e. renal sinus, lymphatic/vascular channels, extra capsular tumor deposits, lymph nodes or other metastatic sites. Presence of any anaplasia, albeit a single cell or a single atypical mitotic figure in a random biopsy qualifies for diffuse anaplasia.

Post-Therapy Histologic Classification

Chemotherapy-induced changes include necrosis, xanthomatous histiocytic foci, hemosiderin deposits and fibrosis. Chemotherapy-induced maturation may also be seen in some instances. The most commonly seen pattern of differentiation is skeletal muscle. Post-chemotherapy histologic findings are used to classify patients into groups with low, intermediate or high

risks of poor survival and aggressive behavior of the disease.

The categories outlined by the COG and SIOP apply only to Wilms tumor with favorable histology. These categories are based on quantifying the proportion of viable, blastemal tumor. The staging for post therapy nephrectomy specimens differs only in the interpretation of areas of necrosis outside the kidney. The presence of necrotic tumor or chemotherapy-induced changes in a lymph node or at the resection margins is regarded as proof of previous tumor with potential microscopic residual disease, and therefore the tumor is assigned stage III. The overall idea is tumors with most of the tumor being viable and composed of Blastema after chemotherapy is going to behave more aggressively. The following table explains the risk groups based on the amount of viable tumor with the proportion of Blastema. Also refer to Figures 1 and 2 for histologic appearance of tumor.

Risk Stratification	Parameters
Low risk	No viable tumor. Residual nephrogenic rests may be seen
Intermediate risk	Viable tumor, <33 percent of the mass regardless of histology
	Viable tumor, >33 percent of the mass, with <66 percent Blastemal histology in viable areas
High risk	Viable tumor, >33 percent of the mass, with >66 percent Blastemal histology in viable areas

Reference: https://documents.cap.org/protocols/cp-paediatric-wilms-biopsy-19-4000.pdf

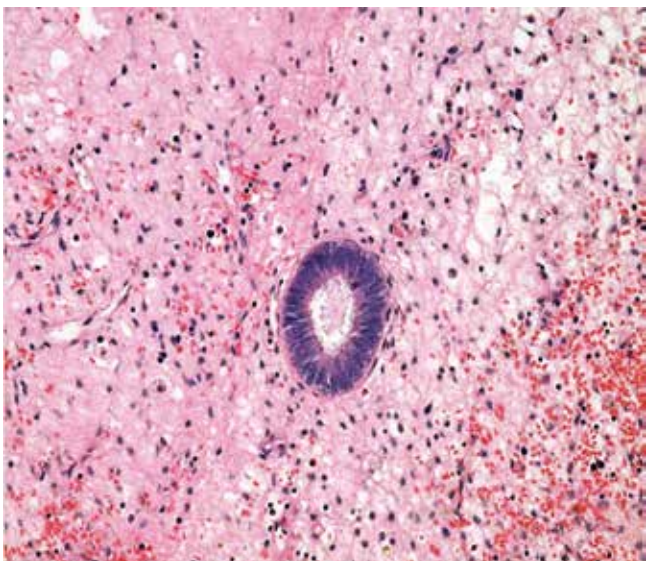


Figure 1 This image shows a few residual tubules surrounded by a zone of fibrosis and hemorrhage with scattered inflammatory cells (taken from webpathology.com).

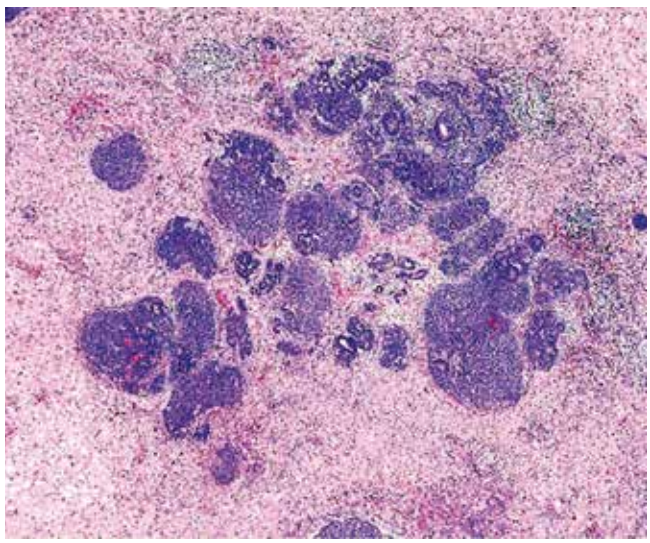


Figure 2 Few residual nodules of blastema and scattered tubular elements. Post-chemotherapy specimens in which blastemal component constitutes more than 2/3 of the viable tumor are considered high risk tumors (taken from webpathology.com).

Conclusion

Paediatric renal neoplasms are being studied in the context of molecular testing with the aim of developing targeted and more sophisticated therapeutic options. Our understanding of the biological mechanisms underlying pathogenesis and potential for aggressive behavior are evolving. This area of cell biology and cancer genomics is definitely going to see a huge growth in the upcoming years, with the advent of newer drugs like immunotherapeutic agents recently developed for a number of solid tumors. Our aim as physicians and health care providers is to provide the best therapeutic options catered to the individual patients' needs and reporting of all the parameters which will help and guide in this regard.

Biotinidase Deficiency- a Rare but Treatable Disorder

Dr Sibtain Ahmed
Chemical Pathology

Biotin, widely known as hair and skin vitamin, is a water-soluble vitamin present in small amount in various foods. It is recycled in the biotin cycle by the action of the enzyme Biotinidase a glycoprotein detectable in dried blood spots, serum, leukocytes, fibroblasts, and hepatocytes. Biotinidase Deficiency (BD), is an inherited autosomal recessive disorder caused by absent or markedly deficient activity of the enzyme biotinidase. As biotin serves as the cofactor of the four human carboxylases, BD results in multiple carboxylase deficiency, a potentially life-threatening disorder with impairment of gluconeogenesis and organic acids metabolism.

The incidence of BD is 1:40,000 to 1:60,000 births in the world; however, in countries with higher consanguinity rate, such as the United Arab Emirates and Turkey, the frequency is significantly higher, 1:8300 and 1:14,800 respectively. Additionally, the carrier frequency in the general population is shown to be approximately one in 120.

From a clinical perspective, cases with BD present with broad spectrum of neurological, dermatological and systematic manifestations, ranging from seizures, hypotonia, feeding problems, delayed motor and cognitive development, ophthalmologic issues, hearing difficulties, hair loss, eczematous skin rash and recurrent infections.

BD is included in the Recommended Uniform Screening Panel for Newborn screening (NBS) by the Department of Health and Human Services, United States of America, which traces back to 1985 when a swift colorimetric assay for evaluating biotinidase activity on dried blood spots was developed. Collection of blood onto an absorbent paper card, often referred to as a 'Guthrie card', is the commonest type of NBS sample as shown in figure 01. A few drops of blood from a heel prick are collected onto a filter paper and sent to the laboratory for analysis.

In countries, like Pakistan that lack NBS, high risk screening of suspected cases of BD is usually built upon clinical findings, hyperammonemia, metabolic ketolactic acidosis and the presence of 3-hydroxyisovaleric acid, lactic acid, 3-hydroxypropionic acid, 3-mehtylcrotonyl glycine and methyl citrate in the urine organic acid analysis by gas chromatography mass spectrometry. For the confirmation of diagnosis quantification of biotinidase enzyme activity in serum/plasma is usually recommended using tandem mass spectroscopy. Based on the results, a diagnosis of profound (less than 10 percent of the mean normal biotinidase activity) or partial (10-30 percent of mean normal biotinidase activity) BD is established. Molecular analysis based on identification of biallelic pathogenic variants in biotinidase gene is considered when the results of enzymatic testing are equivocal in differentiating profound from partial BD and further evaluation is necessary.

Cases with BD identified by NBS continue to be symptom free and show normal development if biotin therapy is introduced promptly and uninterruptedly. The simple, relatively inexpensive and widely available treatment is usually based on 5-10 mg of oral biotin per day. Furthermore, cases with profound and partial BD, even diagnosed at later stages due to lack of NBS have shown good outcomes and better prognosis on treatment with oral biotin. In context of cost effective screening tests, availability of confirmatory assays, in expensive and widely available treatment, the prognosis of BD is better compared to other enzyme defects and organic acidemias, provided timely initiation and compliance with therapy. The monitoring is done clinically and periodic follow-up visits with the metabolic physician are needed to ensure that the features of the condition have resolved with therapy.

Figure 1 showing Heel Prick on dried blood spot filter paper for DBS-Biotinidase analysis

Renal Tubular Disorders and Biochemical Diagnostics

Dr Siraj Muneer and Ms Iffat Arman
Clinical Chemistry

Renal tubules play an important role in fluid, electrolyte and acid-base homeostasis. Normal reabsorption of electrolytes, glucose, calcium, magnesium, phosphates and amino acids and secretion of protons occur in various specialized parts of the renal tubule. Tubular dysfunction should be observed in all children with hypokalemia and metabolic acidosis, failure to thrive, polyuria, refractory rickets. Common disorders of renal tubular dysfunction are shown in Table 1.

Table 1: Common Disorders of Tubular Functions

Segment	Function	Disorder
Proximal tubule	Amino acid transport	Aminoaciduria
	Bicarbonate transport	Proximal RTA
	Phosphate transport	Hypophosphatemic rickets
	Glucose transport	Renal glucosuria
Ascending limb of Henle	Chloride, potassium, sodium transport	Bartter syndrome
Distal tubule	Sodium, chloride transport	Gitelman syndrome
	Proton (H+) secretion	Distal RTA
Collecting duct	Sodium, potassium transport	Liddle syndrome
		Pseudohypoaldosteronism
	Water transport	Nephrogenic DI

Assessment of Renal Tubular Acidosis (RTA)

The clinical features suggestive of renal tubular disorders are refractory rickets, unexplained hypertension (Liddle's syndrome), growth retardation, failure to thrive, polyuria, polydipsia; preference for savory foods, renal calculi, nephrocalcinosis etc. On laboratory investigation these patients present with hypercalciuria with normal serum calcium, hyponatremia with hyperkalaemia, metabolic alkalosis with or without hypokalemia and hyperchloremic metabolic acidosis. The following laboratory findings are helpful in evaluating and classification of RTA.

Plasma anion gap: Anion gap shows the difference of unmeasured anions and cations in the plasma, and is measured as follows: *Anion gap = $Na^+ – (Cl^- + HCO3^-)$.* Normal plasma anion gap is 10-12 mEq/L. Normal anion gap in the presence of acidosis (hyperchloremic metabolic acidosis) suggests elevated urinary (proximal RTA) or gastrointestinal loss (diarrhea) of bicarbonate or impaired excretion of H+ ions (distal RTA).

Urine anion gap: Urine anion gap (net charge) (urine $Na^+ + K^+ – Cl^–$) gives an estimate of urinary ammonium ($NH4^+$) excretion and is beneficial in the evaluation of hyperchloremic acidosis. Under normal circumstances, urine anion gap is positive due to the presence of dissolved anions ***e.g.,*** sulfates, phosphates. Metabolic acidosis is link with a compensatory rise in $NH4^+$ production, resulting in a negative urine anion gap. Patients with RTA typically represents impaired renal $NH4^+$ excretion and a positive urine anion gap.

Urine pH: Urine pH is an estimate of the number of free H^+ ions in the urine which are secreted in response to metabolic acidosis. The presence of alkaline urine during metabolic acidosis represents defective renal acidification, as in distal RTA. However, alkaline urine may also be found in patients with metabolic acidosis due to extra-renal disorders, as in diarrhea.

Fractional excretion of bicarbonate: The proximal tubule normally reabsorbs almost all filtered bicarbonate (fractional excretion below five percent). A value more than 10 percent indicates proximal RTA while levels are in the normal range in distal RTA. Proximal tubular handling of bicarbonate can be evaluated by fractional excretion of bicarbonate.

$$\text{Fractional excretion of bicarbonate} = \frac{\text{urine bicarbonate x plasma creatinine}}{\text{plasma bicarbonate x urine creatinine}} \times 100$$

Tests for Potassium Handling

Renal tubular disorders may be associated with both hypokalemia and hyperkalemia.

The fractional excretion of potassium (FEK) may also be used as an indicator of tubular function; FEK values <2 indicates extra-renal causes while >2 indicates renal causes.

$$\textit{Transtubular gradient of potassium} = \frac{\textit{urinary potassium x plasma osmolality}}{\textit{plasma potassium x urinary osmolality}}$$

We can differentiate patients into three types of tubular disorders based on these routine biochemical tests, shown in table 2. Identification of correct type of disorders is critical to patient management. Hence it is imperative that all patients suspected of any renal tubular disorder are investigated thoroughly and managed accordingly.

Table 2: Differential Diagnosis in various types of Renal Tubular Acidosis

Primary Defect	Type I (Distal)			Type II (Proximal)	Type IV
	H+ Secretion in the distal tubules			HCO-3 reabsorption in the Proximal CT	Disruption of the renin-angiotensin-aldosterone axis
	Classic	With HCO-3 wasting (Type III)	Hyperkalemic		
Metabolic Acidosis (Hyperchloremic)	Yes	Yes	Yes	Yes	Yes
Serum K	Normal/Low	Normal/Low	High	Normal/Low	High
Urinary Anion Gap	Positive	Positive	Positive	Negative	Positive
Urine pH	>5.5	>5.5	>5.5	<5.5	<5.5
Fractional HCO^-_3 Excretion	<5 percent	>5-15 percent	<5 percent	>10-15 percent	>5-10 percent
Fractional K^+ Excretion	High	High	Low	Normal/High	Low
$NH4^+$ Excretion	Decreased	Decreased	Decreased	Normal	Decreased
Calcium Excretion	Increased	Increased	Increased	Normal	Normal/Decreased
Citrate Excretion	Decreased	Decreased	Decreased	Normal	Normal
Nephrocalcinosis/Lithiasis	Often Present	Often Present	Often Present	Absent	Absent
Bone Involvement	Rarely Present	Rarely Present	Rarely Present	Often Present	Absent
Other Tubular Defects	Absent	Absent	Absent	Often Present	Absent

Utility and application of Chromosomal Microarray (CMA) in Clinical Diagnostic

Zeeshan Ansar, Asghar Nasir, Nazneen Islam
Molecular Pathology

Introduction

The chromosomal microarray, also termed cytogenetic microarray, molecular karyotyping, or genomic copy number array, is a deoxyribonucleic acid (DNA)-based testing method used to identify copy-number variants (CNVs), which are either gains (i.e. duplications) or losses (i.e. deletions) of genomic material. This assay is a high resolution, whole-genome screening technique that can identify most of the chromosomal imbalances detected by conventional cytogenetic analysis, as well as smaller submicroscopic deletions and duplications that are referred to as copy-number variants (CNVs).Various human disorders may be caused by CNVs, including neurodevelopmental disorders and congenital anomalies such as cardiac defects. CMA is recommended as the first-tier test in the postnatal evaluation of congenital abnormalities and neurodevelopmental disorders as well as other constitutional or congenital diseases.

CMA in particular is recommended when genetic analysis is performed in cases with clinical structural anomalies and/or karyotypical anomalies identified.

CMA should be considered as further evaluation when an apparently balanced de novo rearrangement is detected by karyotyping to exclude an imbalance at one or both of the translocation breakpoints.
CMAs study is also proved to be helpful tool in identifying the chromosomal origin and gene content of marker or ring chromosomes identified with conventional karyotype

Indications of CMA

Chromosomal microarrays are recommended as a screening test by the American College of Medical Genetics when there is a family history of chromosomal abnormalities and there is a normal karyotype but suspected genetic conditions. Investigations could include; 1) identification of congenital genetic defects, 2) characterization of acquired genetic changes 3) determination of genomic variations and polymorphisms 4) individuals with multiple congenital anomalies, developmental delay/intellectual disability, and/or autism spectrum disorders, with clinically significant findings reported in approximately 15 percent of cases with normal conventional karyotypes

Therefore, CMA should be considered for clinical presentation of:

- Isolated autism spectrum disorder (ASD) or ASD plus other findings
- Isolated global developmental delay or intellectual disability
- Multiple congenital anomalies in the absence of a syndrome diagnosis
- Unusual physical features (dysmorphisms)
- Recurrent miscarriages

Limitations of CMAs

Limitations of the use of CMAs include:

1. The inability to detect genetic events that do not affect the relative copy number of DNA sequences, for example., molecularly balanced chromosomal rearrangements. However, CMAs may reveal copy number changes in apparently "balanced" chromosomal rearrangements, i.e., gains or losses, at or near the chromosomal breakpoint sites.

2. Low-level mosaicism for unbalanced chromosomal rearrangements and aneuploidy may not be detected by CMAs. The sensitivity of the microarray for detection of mosaicism will be influenced by the platform, sample type, copy number state, DNA quality, data quality, and size of imbalance.

3. The chromosomal mechanism of a genetic imbalance may not be clarified.

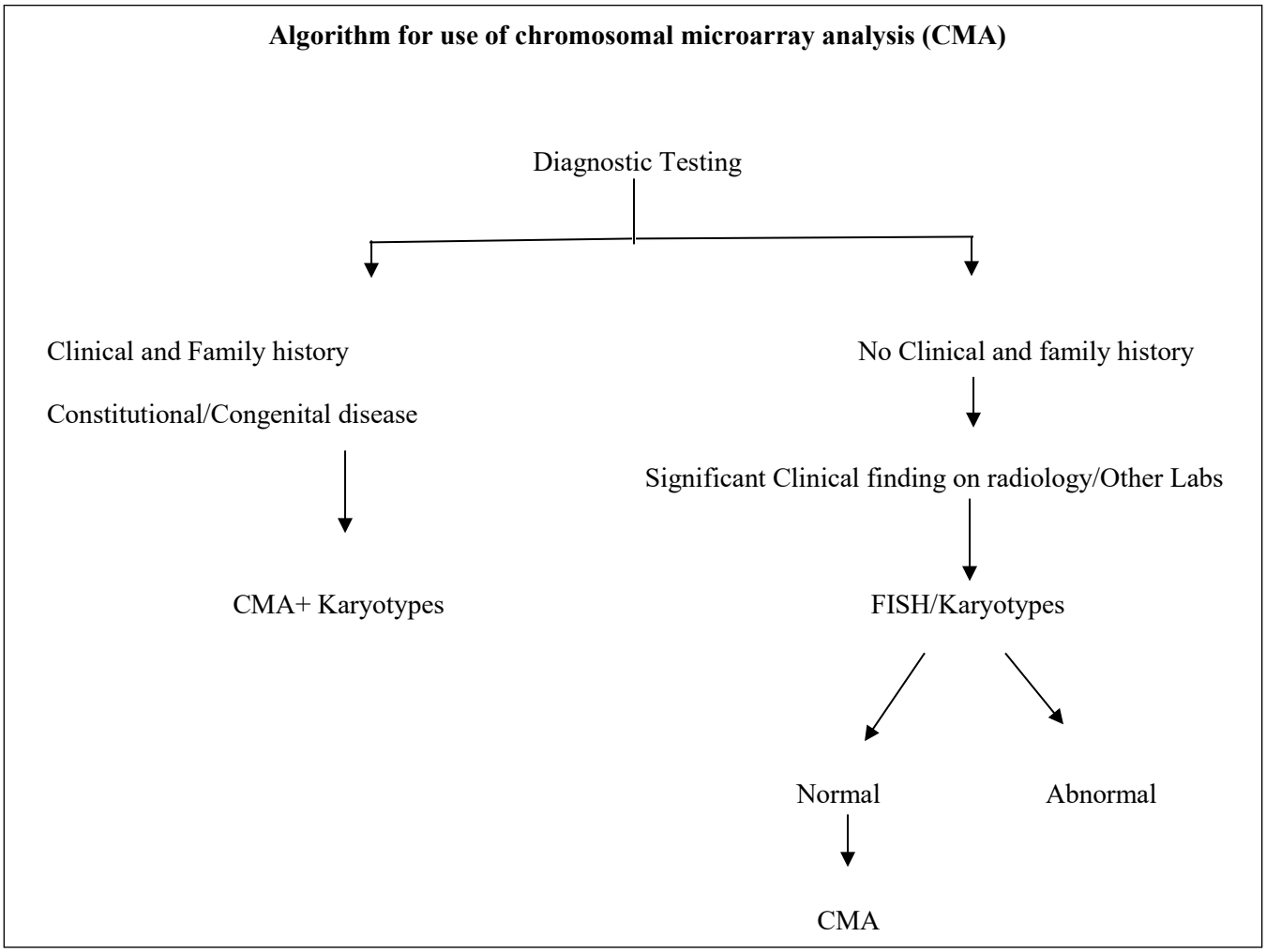
4. Tetraploidy or other ploidy levels may not be detected or may be difficult to detect.

5. Genomic regions copy number variations (CNVs) is not represented on the platform.

6. Current CMA technologies are not designed to detect duplications and deletions below the level of detection according to probe coverage and performance, point mutations, gene expression, and methylation anomalies that may contribute to the patient's phenotype.

7. No microarray platform will detect all mutations associated with a given syndrome. Therefore, failure to detect a copy number alteration at any locus does not exclude the diagnosis of a disorder associated with that locus.

Developmental delay (DD) and intellectual disability (ID) are estimated to affect ~ 1 percent of the children across the world. Genetic factors play a major part in DD/ ID (up to ~ 47.5 percent). Identification of the genetic cause is crucial for accurate etiological diagnosis and refined clinical management. Chromosomal microarray analysis (CMA) has been recommended as a first-tier genetic test for unexplained DD/ID and congenital malformations. The reported diagnostic yields of clinical CMA vary between 12 and 20 percent, depending on the population and methods used.



TSH Receptor Antibodies (TRAb) in Neonatal Hyperthyroidism: What We Need to Know?

Dr Zaib-un-Nisa
Clinical Chemistry

Neonatal hyperthyroidism, also known as neonatal Graves' disease (NGD), is a rare disease occurs due to the Trans-placental passage of maternal stimulating TSH receptor antibodies (TRAb). It is mostly transient and permanent non-autoimmune neonatal hyperthyroidism is rare and is due to activating mutations of TSH receptor. Exposure to topical iodine has also been reported as a rare cause of hyperthyroidism in newborns. If it is not diagnosed early and treated correctly, can leads permanent intellectual disability in the baby.

Graves's hyperthyroidism occurs in approximately 0.2 percent of women, and approximately one to five percent of infants born to these mothers developed NGD. TRAb are Immunoglobulin of G class and freely cross the placenta. Most NGD occurs in the setting of active or treated Graves's hyperthyroidism in the mother. Thus, NGD would be expected to occur in approximately 1: 25,000 neonates and affects males and females equally.

Any infant with unexplained tachycardia, goiter or stare, unexplained petechiae, hyperbilirubinemia, or hepatosplenomegaly, history of persistently high TRAb titer in mother during pregnancy, persistently high requirement for any thyroid medication in mother during pregnancy, thyroid ablation for hyperthyroidism in mother or previously affected sibling should be further investigated for NGD. The clinical manifestation of NGS are shown in Table 1.

Table 1: Clinical Spectrum of the NGD presenting in prenatal period and neonates.

Age Group	Clinical Features
Prenatal Period (Fetus)	• Unexplained tachycardia, • Failure to thrive • Intrauterine growth retardation • Goiter • Advanced bone age • Prematurity • Craniosynostosis, microcephaly • Fetal death
Neonate	• Irritability, hyper excitability, sleep disorders • Tachycardia, hypertension, cardiac failure • Flushing, sweating, Goiter, stare • Respiratory distress, pulmonary hypertension • Increased appetite but no/poor weight gain • Diarrhea • Unexplained petechiae, hyperbilirubinemia • Craniosynostosis, microcephaly, • Death

For evaluation of any neonate suspected with the NGD, serum TRAb, TSH and FT4 testing is recommended. A low TSH, high FT4 along with TRAb levels higher than three-fold the upper limit of normal within 7 days of birth is predictive of the NGD. And if the TRAb levels are not detectable, the baby is very unlikely to become hyperthyroid. At Aga Khan Clinical Laboratories we are performing serum TRAb levels, using Chemiluminescence immunoassay..

Webinar 'Newborn Screening Program: An Impetus for change' in a webinar series 'Rare Links'

Dr Hafsa Majid
Clinical Chemistry

An online educational webinar series 'Rare Links' was initiated by Section of Chemical Pathology since December 2019, from the platform of Pakistan Inherited Metabolic Disease Network (Pak-IMD-Net), a working group of Pakistan society of Chemical Pathology. Aim of Rare links webinar series is to provide a platform where national and international experts can share their knowledge and experience of developed and developing newborn screening programs in and approaches to developing this in Pakistan. The webinar will feature presentations on the elements of a newborn screening program; talks on experiences and challenges of implementing newborn screening programs. Third webinar of this series was conducted via Zoom video conferencing on 'Newborn Screening Program: An Impetus for change' on 22nd September 2020.

The focus of workshop was on understanding the basic concepts, components of a complete newborn screening program, and relate criteria for disorder selection to their individual circumstances. Webinar started with introduction of all participants followed by a talk by chair Pak-IMD-Net, Dr Lena Jafri. She briefed the participants about the aims and objectives of this working group and how Pak-IMD-Net is raising awareness about NBS. This was followed by lecture about 'Laboratory Aspects of Newborn screening: What you need to know for establishing a NBS laboratory' by Prof. Dr. Aamir Ijaz, co-chair, Pak-IMD-

Net. This was followed by introduction to "rare links' webinar series by Dr. Hafsa Majid. She shared the experience of establishing a successful NBS program at the Aga Khan University. Next was a talk given by Dr Aysha Habib Khan, Advisory council member for Society for Studies on inherited metabolic disorders, to further our understanding of the selecting the disorder to be include in NBS program established for Pakistani setup. She presented findings of the consensus report from the conference on 'NBS in Pakistan' conducted in 2019.

The program concluded with panel discussion by national and international expert on 'Developing Policy Recommendations for starting Nationwide NBS program in Pakistan'. The panelist included Dr Dianne Rosemary Webster, Director NBS program of New Zealand, Prof. Dr. Layachi Chabraoui, President Moroccan Society for Study of Inborn Errors of Metabolism, Dr. Khadija Humayun, Associate Professor and Consultant Paediatric Endocrinologist, Paediatric and Child Health, Aga Khan University, along with the presenters.

It was a remarkably successful event, with more than 70 participants from 3 different countries, 17 different cities, and 34 different institutes of Pakistan. The participants were from different areas of medicine including Paediatrics, Laboratory Medicine, Gynecology and Obstetrics, nursing, Family Medicine, Internal Medicine, and Public Health sectors. This was the third webinar of this series, and it was very well appreciated by all the participants. The participants encouraged organizers to conduct more such activities under the banner of 'Rare Links'.

The Best of the Past

Radiologist # Paediatricimaging # Followtheirlead

Interviewee: Associate Prof Naila Nadeem

Interview recorded by Dr. Shayan Anwar

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

Prof Naila Nadeem: Every moment of my clinical experience in AKU has been worth narrating, however the most AHAA moments were when I worked with the multi-disciplinary team in management of "Conjoint Twins", starting from imaging, then surgery and management was challenging. It is one of the best example of team work. I just could not stop my tears when I met the twins, separately held by parents, both healthy and happy.

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

Prof Naila Nadeem: Long journey, I have worked in four different government hospitals immediately after graduation, did my minor Diploma (MCPS) in radiology. The quest for better training made me apply in the residency program of AKU. I was fortunate to get selected as a resident in AKU in 1997. I have no regrets to leave my consultant position in government job for a better training. My young and energetic batch mates kept me on my toes. Colleagues were supportive, respectful but challenged me every day. University is very supportive, I was encouraged to excel not only as a clinician but also as an educationist, I did MHPE which has further tested my endurance but is rewarding. I enjoy Paediatric radiology and medical education.

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

Prof Naila Nadeem: Discipline, excellent work-place, lifelong learning desire all have contributed, but what has really affected me most is "altruism". This is the splendor of AKU, it induces into us the "selfless concern for the well-being of our patients and for our colleagues".

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

Prof Naila Nadeem: When I joined Paediatric imaging group, Dr. Yousuf was the only name, I am grateful to him for leading and building the team with open mind. Now Paediatric imaging is an established separate field in Pakistan. It is established in Karachi, Lahore, Multan and Islamabad. We now have our representation in radiology Society of Pakistan. At College of Physician Surgeons Pakistan (CPSP) level we are trying to establish fellowship programme. In next ten years I see further progress of paediatric radiology, as more and more dedicated centers for paediatric care are established. Still long way to go but we are very hopeful.

5. Any advice for Junior Radiologists?

Prof Naila Nadeem: Be happy and gratified physicians, live up to your title "The Doctor". Work with dedication for your patients. Role models like Dr. Gaffar Billoo teach us there is no age limit for learning and work hard. The smile on your patients' face is the biggest reward, try to earn that.

The Best of the Past

Interview of the Professor of Pathology and Laboratory Medicine,

Interviewee: Professor Aysha Habib Khan

Interview Recorded by Dr Syed Bilal Hashmi

1. Please share a brief journey/experience of establishing a Biochemical Genetics Laboratory?

Professor Aysha Habib Khan: I take pride in the 10 years I have invested in realization of BGL and have a great sense of gratitude when I look back. In 1995 after moving from paediatric residency to chemical pathology, I felt a major gap in the diagnostics of inborn errors of metabolism, this then became my passion to contribute towards this area of metabolic medicine. There was a genuine need of Biochemical Genetic Laboratory (BGL) for the community and healthcare. Establishing BGL was not an easy task and consumed 10 extensive years after my post-graduation.

Starting from advocacy down to planning and implementation and now, representing AKU and Pakistan in international forums are accomplishments that are difficult to describe and express. All I know is a strong foundation has been laid and now the entire BGL team including my technologists and faculty have got to give their best to reap fruits. Complete diagnostic setup will take its time but continuing in the right direction and developing & training younger lot to take it forward with updated knowledge and latest technique is the need for future.

2. What challenges did you face in establishing and leading the Biochemical genetics laboratory?

Furthermore, what were the success factors that helped you respond to these challenges?

Professor Aysha Habib Khan: As I said earlier, advocacy was the major challenge, which consumed significant time. Establishing a new specialty requires substantial resources, and with limited funds for entire hospital, it was not easy convincing decision makers to invest on something very new. New in a sense that biochemical genetic diagnostics specialty did not existed in Pakistan, with no data to prove that inherited metabolic disorders exist in sufficient numbers to be of public importance. The good part was that related clinical fraternity was cognizant of the missing facility and therefore, supported this endeavor voice fully.

My perseverance and focused approach to achieve genetic lab objective was the main factor in overcoming the challenge. Despite of non-availability of funds, always enhancing my knowledge and remaining abreast of the happening and advancements taking place in the field further strengthening my zeal to equip the lab with genetic diagnostic facilities.

3. Can you share any success story or "AHA Moment" where you felt very excited while making a diagnosis?

Professor Aysha Habib Khan: The AHA moment to me was also the first milestone for us, in diagnosing the first case with inherited metabolic disease in "real time'. It so happened that before our services were operationalized; we were approached by Dr Bushra Afroze for a patient admitted in NICU for a clinical suspicion of a metabolic disorder and requested our support to determine the diagnosis. It was a weekend and on analysis it was identified to be a case of isovaleryl CO A dehydrogenase deficiency. Why I called it a milestone was with the reason that the lab team had before operationalizing got its first result tested, verified and communicated to the treating physician. Actual benefit was saving a precious neonate's life, which otherwise would not have happened, unless the specimen was sent abroad for testing for verification. A heartening thank you note from Dr Bushra Afroze was not only a source of excitement for the team, but also a sense of accomplishment and a valuable contribution in the treatment of children, who were otherwise delayed for multiple reasons.

4. What contribution is the Biochemical genetics laboratory paying towards patient benefit?

Professor Aysha Habib Khan: Now that the lab is formally accredited by college of American Pathologist; the most important and valuable contribution is access and prompt diagnosis from within AKU, thus saving a lot of time and providing financial relief to the family. The second important factor is close interaction and trust between treating physician and pathologist.

5. What are the plans or vision for the next ten years?

Professor Aysha Habib Khan: We started small with just two equipments, but with a test menu that had maximum diagnostic yield. Now we are moving forward on optimizing and validating assays on HPLC and LCMSMS. With this, we will be able to expand our test menu and capture disorders that were not picked earlier. This would also provide opportunity to study the spectrum of diseases within Pakistan.

We have also laid down infrastructure and standard operating procedures for newborn screening program at AKUH. We started with screening for congenital hypothyroidism and now moving to screening for congenital adrenal hyperplasia. We hope to expand it for expanded newborn screening once the assays are validated.

We have focused on human resource capacity building to oversee the specialty. These include eight faculty members including myself and 12 technologists. Intention is to train more faculty and technologist to ensure that gap does not not arise at any stage. Our faculty is now leading from front and we are collaborating with various international organizations including Mayo Laboratory in US and Society of Study of Inborn Errors of Metabolism (SSIEM).

At the same time, we have worked on developing the national capacity of physicians, pathologists and technologists across Pakistan through numerous conferences, continuing medical education, seminars, hands on workshops and other training ventures. Our intentions is to contribute nationally, developing human resource capacity to carryout diagnostics and interpretation of biochemical genetics data and facilitate families in all cities, regions and provinces

of Pakistan. This is how we would be able to serve community at large in line with Chancellor's vision. We hope to develop patient's registries and support groups for raising awareness and education on genetic diseases for masses in future.

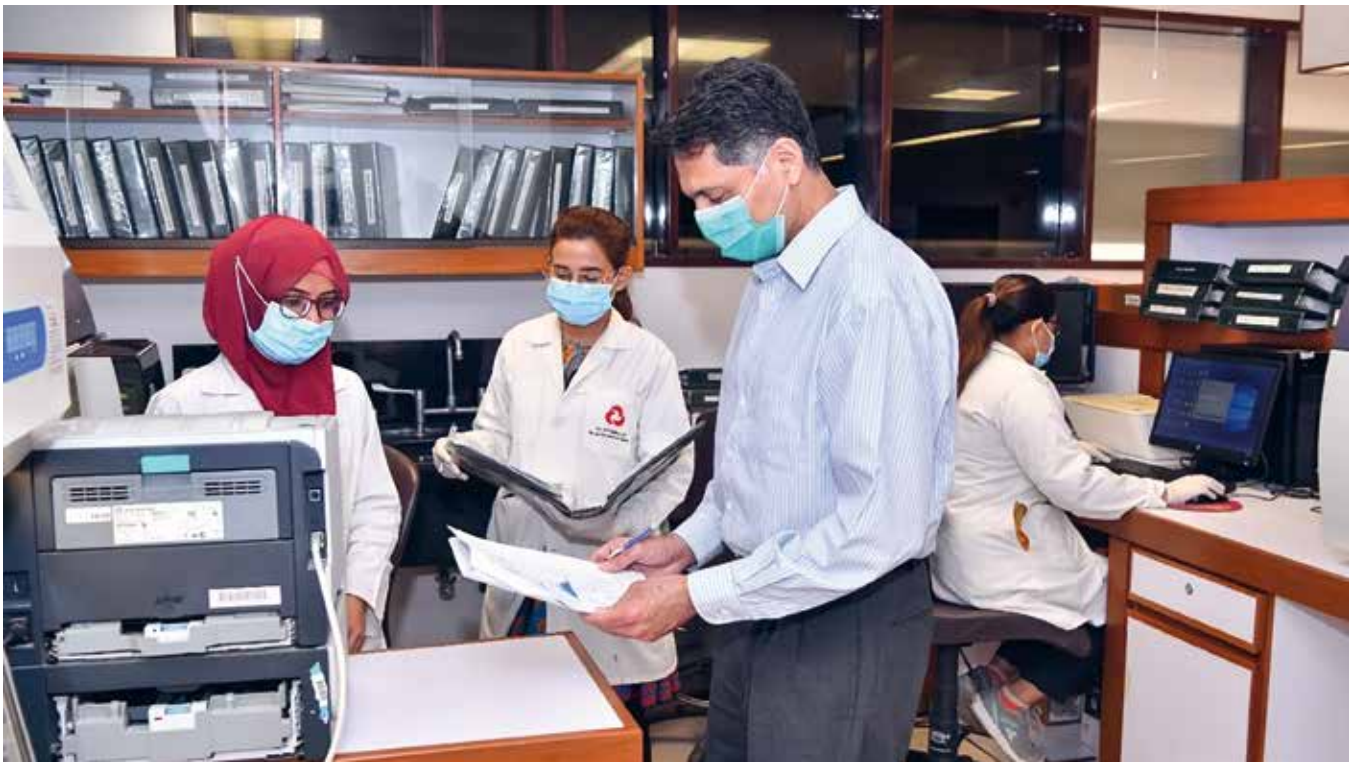
6. What advice will you give to the junior pathologist persuading career in the field of Biochemical genetics?

Professor Aysha Habib Khan: I would encourage participation from the seniors to nurture the juniors and for the juniors and newcomers to take on this field with zeal and enthusiasm. Biochemical genetics will now grow at a faster pace in Pakistan and youngsters wishing to pursue a career in this specialty will have a very bright future. To get into this field, one must have analytical approach and research aptitude. A strong foundation has been laid by AKUH. Developing & training younger lot to take it forward with updated knowledge and latest technique is the need for future.

Polaroid

Histopathologists after conducting Paediatric Tumor Boards of multiple subspecialties

Polaroid



Reporting of Flow Cytometry cases of Paediatric Hematological Malignancies

Laboratory technical staff processing Paediatric Blood Culture bottle that was flagged positive in continuous blood culture monitoring system

Polaroid

Positive Latex Particle Agglutination (LPA) test for Neisseria Meningitidis in Paediatric patient with suspected community acquired Meningitis

A Lab Scientist performing Sweat Chloride testing to Screen for Cystic Fibrosis in a patient

Polaroid

Faculty and Technologists of Chemical Pathology discussing the Chromatograms of Plasma Amino Acids and Urine Organic Acids in Departmental Consultation Conference

Paediatric Imaging Team Aga Khan University Hospital at Radiological Society of Pakistan: (left to right) - Dr Kiran Hilal, Dr Naila Nadeem, Dr Yousuf Husen, Dr Waseem Mirza and Dr Basit Salam

Polaroid

Faculty, Laboratory technologist (Genetics bench) and Laboratory Manager at the section of Molecular Pathology, Clinical Laboratory, AKUH that are involved in paediatric molecular diagnostics

Computer analysis is used to compare a patient's genetic material to that of a reference sample. A difference between a patient's DNA and the reference sample is called a genetic variant.

Polaroid

Haematology resident and blood bank staff performing and interpreting neonatal blood group by gel card method

Chief Guests at the Launch of Ek-Sath- an eportal for the support of patients and families with rare inherited metabolic defects (IMDs) in Pakistan

THE AGA KHAN UNIVERSITY HOSPITAL CLINICAL LABORATORIES

UPDATE
Chromosomal Microarray for Constitutional genetics

VOL.XXIV No. 8, 2019

INTRODUCTION

Chromosomal microarray (CMA) is a high resolution genetic test to investigate chromosomal aberrations such as gains and losses (copy number variants, CNV) that could contribute to genetic defects. CMA can identify structural abnormalities in chromosomes at a much greater resolution than conventional karyotyping (G-banded karyotype) and allows identification of clinically significant copy number aberrations amongst patients with multiple constitutional disorders. CMA can identify genomic microduplications or microdeletions that are a few hundred basepair (bp) in size. High resolution CMA platforms that incorporate single-nucleotide polymorphism (SNP) genotyping enable even greater diagnostic yields. However, CMA cannot be used to determine balanced translocations.

Genomic microarrays are the first line screening test recommended by the American College of Medical Genetics for a number of conditions; 1) identification of congenital genetic defects, 2) characterization of acquired genetic changes 3) determination of genomic variations and polymorphisms. Therefore, CMA should be considered for clinical presentation of:

- Isolated autism spectrum disorder (ASD) or ASD plus other findings
- Isolated global developmental delay or intellectual disability
- Multiple congenital anomalies in the absence of a syndrome diagnosis
- Unusual physical features (dysmorphisms)

PRINCIPLE

The Applied Biosystems CytoScan 750K enables the detection of high resolution copy number across the genome, providing allelic imbalance information from SNPs. This high-density array contains greater than 750,000 markers for copy number and approximately 200,000 genotype-able SNPs, which provide high-resolution copy number, and accurate breakpoint estimation. The SNPs on this array are from the public SNP database (dbSNP) and maximize genomic coverage, genotyping accuracy and optimize detection of homozygosity. There are 550,000 unique non-polymorphic probes to cover constitutional and cancer genes which are covered by each assay.

REPORTING

CMA will be used to identify deletions greater than 400 bp and or duplications greater than 500 bp in regions which contain at least one known gene.

SPECIMEN COLLECTION:

5 ml whole blood in EDTA tube is required

SCHEDULE:

The test is performed 1st Monday of every month and report will be issued after 15 days.

THE AGA KHAN UNIVERSITY HOSPITAL CLINICAL LABORATORIES

UPDATE
Neonatal Dried Blood Spot (DBS) Biotinidase

VOL. XXVI No. 8, 2020

INTRODUCTION:
Biotinidase deficiency is the major cause of late-onset multiple carboxylase deficiency. It is an autosomal recessive inherited disorder, caused by absent or deficient activity of biotinidase, an enzyme releasing biotin (also known as vitamin B7 or H) from dietary proteins. Mammals cannot synthesize biotin and, therefore they must obtain the vitamin from their diet and by recycling endogenous biotin.

The disorder can lead to a decrease in biotin availability and if untreated, it is associated with the neurologic and cutaneous consequences. Symptoms usually appear between 2 and 5 months of age but may not be evident until several years of age. The clinical presentation can vary, but abnormalities involving the central nervous system are often the first features to occur. The symptoms can include development delays, seizures, alopecia, ataxia, hypotonia, respiratory problems, conjunctivitis, visual problems, hearing loss, skin rash, or metabolic compromises and may be life threatening.

Newborn screening of Biotinidase activity from dried blood spots can identify affected patients shortly after the birth. Because the screening is done with a direct enzyme assay, it is not influenced by dietary protein or biotin intake. It is also possible to start biotin supplementation for the infant and repeat the test after a couple of months.

Principle: Fluorometric Enzyme Immunoassay.

Specimen Collection:
- Blood sample is collected on dried blood spot filter paper (DBS) by trained phlebotomist / nurses.
- For new born screening blood samples is collected from the pricked heel of the baby 3-5 days after birth.
- The blood spot should be air-dried for at least 3 hours. Once dry, sample should be mail to the laboratory within 24 hours in a separate paper envelope, along with humidity indicator card, desiccant pouch to protect against moisture.

Cut Off:
Normal Biotinidase Activity > 60 nmol/min/dL

SCHEDULE:
Performed on: Second Wednesday of the month
Reported on: Following Friday

hospitals.aku.edu/Karachi/clinical-laboratories