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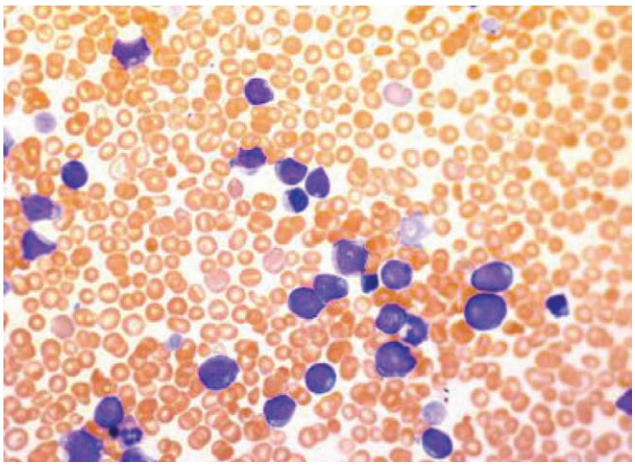
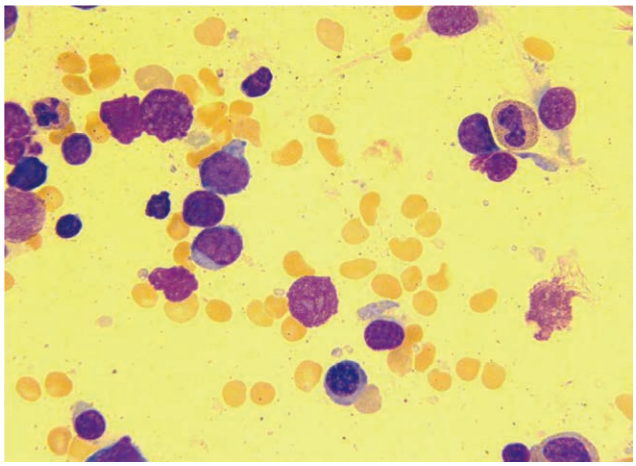
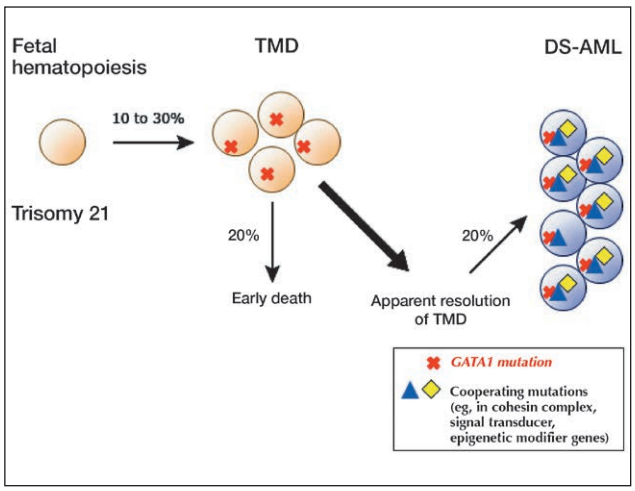
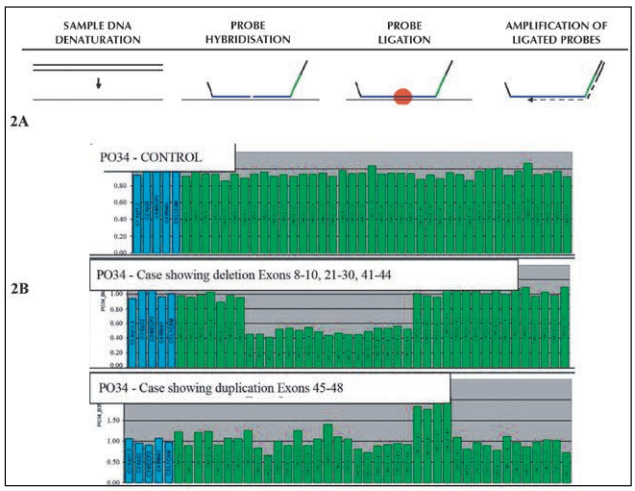
Paediatric Pathology









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The Aga Khan University Hospital, Karachi

LABRAD

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From the Editor's Desk

Pediatrics is the branch of medicine that deals with the care of infants, children and adolescents. The age limit is newborn till 21 years. Islamic writers such as Ibn Sina have contributed to Greco-Roman and Byzantine medicine. The Persian scholar and doctor Al-Razi published a thesis on diseases in children. Neonates, infants and children differ markedly from adults, physiologically. Developmental delays, genetic mutations and congenital defects are of greater concern to pediatricians as compared to physicians practicing adult medicine.

Moving a step further, pediatric pathologist is more of a generalist then a specialist. This field covers a wide spectrum of abnormalities occurring during development, extending through childhood and culminating in adolescence.

This is the first time that an issue of LABRAD has been exclusively dedicated to pathologies of the pediatric population. We have covered topics related to inherited bleeding disorders, endocrine abnormalities, malignant diseases and genetic disorders to name a few.

This year's last issue ends on a "thematic" note. Hope our readers will be enlightened as always. We will continue to publish thematic issues like these in the years to come.

Natasha Ali

Overview on Approach to Inherited Bleeding Disorders

Dr Nadia Nasir
Haematology

Inherited bleeding disorders may be due to a vascular defect, a platelet disorder or a coagulation defect. Table 1 shows the causes of inherited bleeding disorders based on this classification:

Table 1: The Causes of Inherited Bleeding Disorder

Type of Bleeding Disorder	Common Inherited Bleeding Disorders
Vascular Defect	1. Hereditary Hemorrhagic Telengiectasia 2. Ehlers Danlos Syndrome 3. Henoch-Schonlein Purpura
Platelet Defect	1. Glanzmann Thrombasthenia 2. Bournard Soulier Syndrome
Coagulation Defect	1. Haemophillia A (Factor Viii Deficiency) 2. Haemophillia (Factor Ix Deficiency 3. Afibrinogenaemia/Dysfibrinogenaemia

Approaching a child with bleeding disorder mainly consists of the following:

1. History taking
2. Clinical Examination
3. Laboratory Findings

Inherited bleeding disorders compared to acquired bleeding disorders, tend to have an early age of presentation, where bleeding is a dominant feature and usually with a positive family history. The pattern of bleeding is relatively predictable depending on the etiology. Vascular and platelet disorders tend to be associated with bleeding from the mucous membrane and into the skin, whereas in coagulation disorders the bleeding is often into joints or soft tissue as shown in Table 2.

Table 2: Adapted from Essential Haematology

	Platelet/Vessel Wall Diseases	Coagulation Diseases
Mucosal Bleeding	Common	Rare
Petechiae	Common	Rare
Deep Haematomas	Rare	Common
Bleeding From Cuts	Persistent	Minimal
Gender	Equal	Mostly Males

An appropriate and reliable laboratory approach, encompassing first-line (screening) and second-

line (specific) testing, is essential to screen, diagnose, and monitor patients who have bleeding diatheses Figure 1. The available clinical assays also can be grouped according to whether they evaluate components of primary hemostasis or coagulation factors.

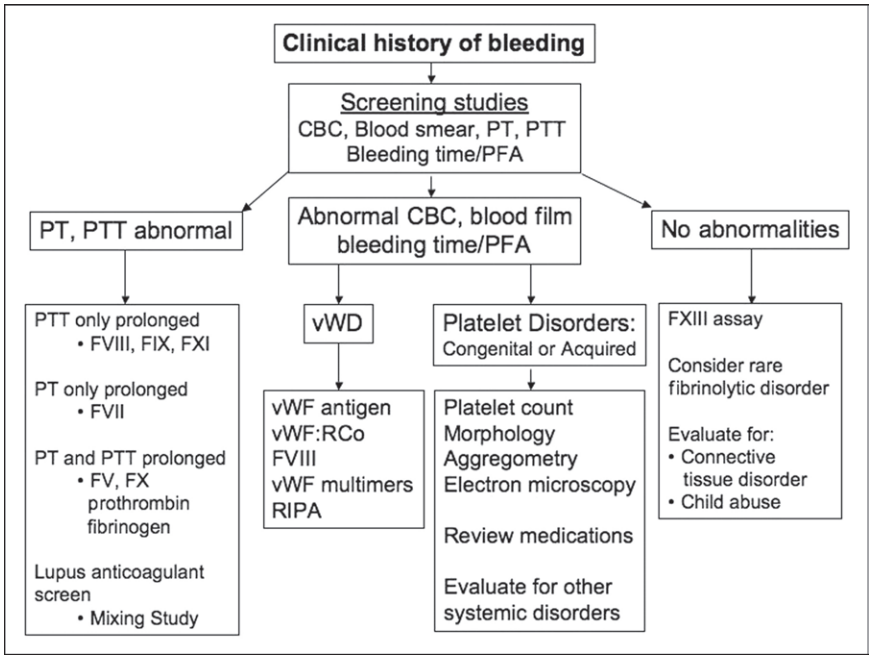


Figure 1: adapted from pedsinreview aappublications

Assays for Evaluating Primary Hemostasis

A complete blood count and evaluation of the peripheral blood smear usually constitute the required first step. The bleeding time is the historical screening test for defects of primary hemostasis, but low sensitivity and specificity in children limit its utility. Newer platelet function analyzers (PFAs) are now available in clinical laboratories but cannot be relied on to identify all patients who have von Willibrand disease or platelet function defects, and their routine use remains controversial. Therefore, when these disorders are considered, specific testing (vWF indices, platelet aggregation studies) should be performed as demonstrated in the above figure.

Assays for Evaluation of Coagulation Factor Function

The prothrombin time (PT) and activated PTT are coagulation screening tests performed on citrated plasma. The PT is reported commonly along with the International Normalized Ratio (INR) to adjust for different reagent sensitivities. The PT is a measure of the extrinsic (FVII) and common pathway (FV, FX, prothrombin, fibrinogen) clotting factors. The PTT measures the contact system (prekallikrein, FXII) as well as the intrinsic (FVIII, FIX, FXI) and common pathway clotting factors. Sensitivities of various PT and PTT reagents vary and may yield normal values in the presence of mild factor deficiencies. Therefore, specific factor assays should be performed for patients who are strongly suspected of having a bleeding disorder. Table 3 shows interpretation of laboratory results for common inherited bleeding disorder.

Table 3: Adapted from essential haematology

	Factor VII	Factor IX	VWB
Complete Blood Counts	Normal	Normal	Normal
Bleeding Time	Normal	Normal	Increased
PT	Normal	Normal	Normal
APTT	Prolonged	Prolonged	Prolonged
Mixing Studies	Pt Aptt Corrected By Normal Plasma	Pt Aptt Corrected By Normal Plasma	Pt Aptt Corrected By Normal Plasma
Factor Assays	Vii Deficiency	Ix Deficiency	Vwb Deficiency

Diagnostic Approach to Haemoglobinopathies

Dr Sidra Asad Ali and Dr Anila Rashid
Haematology

Haemoglobinopathies refer to a diverse group of inherited disorders characterized by a reduced synthesis of one or more globin chains (thalassaemias) or the synthesis of structurally abnormal haemoglobin. They encompass a heterogeneous group of disorders associated with mutations in both the alpha-globin and beta-globin genes.

Diagnosis of haemoglobinopathies, including thalassaemias, can result from either a clinical suspicion of a disorder of globin chain synthesis or from follow-up of an abnormality detected during screening. Screening may be carried out as part of a well-defined screening program or be an ad hoc or opportunistic test.

Step wise approach to Diagnosis

Complete Blood Count

Structural haemoglobinopathies may have an impact on the red cell indices, which are critical to the diagnosis of thalassaemias. The key components of the CBC include: Hb, red cell count, mean corpuscular volume (MCV), and red cell distribution width (RDW). The thalassaemias generally are classified as hypochromic and microcytic anemias. Hence the MCV is a key diagnostic indicator and reported in femtoliters, in most adult populations ranges from ~80 to 100 fL. Thalassaemic individuals have a reduced MCV, and studies suggest that an MCV of 72 fL is maximally sensitive and specific for presumptive diagnosis of thalassemia syndromes. RDW may provide information useful as an adjunct to diagnosis but is not useful as alone indicator. The RBC count is also useful as a diagnostic adjunct because the thalassemias produce a microcytic anemia with an associated increase in the RBC number. Various indices utilizing these CBC components have been developed with a view to providing a mathematical derivation to reliably differentiate iron deficiency from thalassemia minor. None are useful in all clinical settings, and probably none exceed the value of the MCV alone in selecting cases for subsequent investigations.

Hb H Inclusion

Hb H refers to an insoluble Hb tetramer comprising four β-globin chains. Hb H arises in the setting of α-thalassemia where the decreased production of α-globin chains leads to β-globin excess. Oxidation of these tetramers provokes precipitation, which can be visualized microscopically. But the Hb H stain is nonspecific in that other nucleic acid or protein precipitates also stain.

In the setting of Hb H disease, a disorder in which three of four α-globin chain genes are not expressed, 30–100 per cent of red cells contain typical inclusions. In contrast, α-thalassemia minor may be associated with as few as one inclusion-containing cell in 1000–10 000 cells. The absence of Hb H inclusions therefore does not exclude thalassemia trait, but the presence of typical inclusions may be helpful in confirming a presumptive diagnosis.

Electrophoresis

Traditionally, electrophoresis has been the method of choice for identification and quantification of variant Hbs. Commercial, rapid electrophoretic methods have been developed that allow for separation at pH 8.4 (alkaline) and pH 6.2 (acid) on agarose gels.

However, it is slow, labor-intensive, and inaccurate in the quantification of low-concentration Hb variants (e.g., Hb A2) or in the detection of fast Hb variants (Hb H, Hb Barts).

Isoelectric Focusing

IEF is an electrophoretic technique with excellent resolution. Although labor-intensive and time-consuming, it has been used to identify and quantify Hbs. IEF is an equilibrium process in which Hb migrates in a pH gradient to a position of zero net charge. The narrow bands obtained on IEF allow for more precise and accurate quantification than standard electrophoresis.

Capillary Isoelectric Focusing

It is a hybrid technique combining the capillary electrophoresis sensitivity of detection with the automated sampling and data acquisition of HPLC. Many published works have described the utility of CIEF in the detection and quantification of Hb variants. Separation of the Hb in this method is related to the isoelectric point of the Hb, and this may enhance inter-laboratory reproducibility.

High Performance Liquid Chromatography

Cation-exchange HPLC is emerging as the method of choice for the initial screening of Hb variants (including neonatal screening where this is mandated) and for quantification of Hb A2 and Hb F concentrations. The Bio-Rad Variant (Bio-Rad Laboratories) is an automated cation-exchange HPLC instrument that has been used to quantify Hb A2, Hb F, Hb A, Hb S, and Hb C. College of American Pathologists studies have shown equivalence or superiority over electrophoretic methods. In comparison with haemoglobin electrophoresis, HPLC has the following advantages:

1. The analysers are automated, therefore require less staff time and permit processing of large batches.
2. Very small sample volumes (5 µL) are sufficient for analysis.
3. Quantification of normal and separated variant haemoglobins is available on every sample.
4. Provisional identification of a larger proportion of variant haemoglobins can be made.
5. δ chain variants (recognition of which is important in the diagnosis of β thalassaemia heterozygosity) are more easily detected.

DNA Analysis

After presumptive identification of hemoglobinopathies and thalassemia syndromes, and particularly for purposes of genetic counseling, defining the mutation or deletion present may be required. Typically, deletional mutations causing α-thalassemia syndromes and some rare β-thalassemias are diagnosed using Southern blot hybridization of particular restriction enzyme digests to labeled complementary gene probes. PCR techniques using allele-specific probes after globin gene amplification, allele-specific primers, or deletion-dependent amplification with flanking primers are used in definition of known globin chain mutations/deletions. For unknown mutations, several PCR-based methods, including denaturing gradient gel electrophoresis and single-strand conformation polymorphism analysis, as well as sequencing of the amplified globin gene DNA may be used.

With increasing global awareness and mass screening programs undertaken at various levels by health care system, the responsibility for laboratory personnel has greatly enhanced in detection and prevention of haemoglobinopathies. Detection of asymptomatic carriers by reliable laboratory methods is the cornerstone of prevention of this serious health problem. Awareness about the diagnostic problems as well as their solutions is also very important so that one does not miss a single case.

Biochemical work up of Nephrotic Syndrome in Paediatrics Population

Dr Khushbakht Arbab
Chemical Pathology

Childhood nephrotic syndrome can start at any age, but usually begins between the ages of two and five years. It is a rare condition that affects about 16 out of every 100,000 children at any given time, and it affects more boys than girls. It is extremely unlikely that other children in a family will also have nephrotic syndrome. While there are a few types of nephrotic syndrome which do run in families, these are very rare. Nephrotic syndrome, or nephrosis, is defined by the presence of nephrotic-range proteinuria, edema, hyperlipidemia, and hypoalbuminemia.

Along with obtaining a complete medical history, a series of biochemical tests are required in order to arrive at an accurate diagnosis that verifies the

presence of the illness. In addition, imaging of the kidneys (for structure and presence of two kidneys) is sometimes carried out, and/or a biopsy of the kidneys. Laboratory evaluation of patients suspected of nephrotic syndrome may include the following:

Urinalysis

Microscopic hematuria is present in 20 per cent of cases of nephrotic syndrome but cannot be used to distinguish between minimal change nephrotic syndrome and other forms of glomerular disease. Red blood cell casts, if present, are suggestive of acute glomerulonephritis, such as post infectious nephritis, or a nephritic presentation of chronic glomerulonephritis, such as membranoproliferative glomerulonephritis. Furthermore granular casts may be present and are non-specific to etiology.

Urine Protein Quantification

In children it is defined as protein excretion of more than >3.5 g per 1.73 m^2 per 24 hours or a first-morning urine protein/creatinine of 2-3 mg/mg creatinine or greater.

Serum Albumin

Serum albumin levels in nephrotic syndrome are generally less than 2.5 g/dL, however values as low as 0.5 g/dL are not uncommon due to renal albumin loss.

Lipid Panel

Heavy proteinuria leading to hypoalbuminemia causes compensatory increase in lipoprotein synthesis. This results in hyper lipoproteinemia. Elevated total cholesterol, low-density lipoprotein – cholesterol and triglycerides may be noted.

Serum Electrophoretic

Patterns in nephrotic syndrome show decreased albumin, and increased alpha 2-macroglobulin fraction.

Other Tests

Serum creatinine levels are usually normal unless the disorder has progressed to chronic kidney disease. Complete blood count, serum electrolytes, and serum creatinine may also be tested. To evaluate the etiology of nephrotic syndrome, antibodies to HIV, hepatitis B and C, complement studies and antinuclear antibody and anti–double-stranded DNA antibody (in selected patients) may also be done.

The prognosis for nephrotic syndrome under treatment is generally good although this depends on the underlying cause, the age of the patient and their response to treatment. It is usually good in children, because minimal change disease responds very well to steroids and does not cause chronic kidney disease.

Transient Abnormal Myelopoiesis

Dr Hira Qadir
Haematology

Introduction

Transient Abnormal Myelopoiesis (TAM) is a unique disorder of Down syndrome (DS) newborns that presents with clinical and morphologic findings indistinguishable from acute myeloid leukemia (AML). Hematologic abnormalities affecting red blood cells, white blood cells, and platelets are common in DS, particularly during childhood. Population-based studies show that risk for leukemia is 10-20 times higher in individuals with DS compared with the overall population, with a particularly striking increase of the incidence of acute myeloid leukemia in young children with DS (approximately 150-fold).

Incidence

Data regarding the incidence of TAM in DS vary depending on the screening practices and diagnostic criteria used. Reported incidence rate among infants with DS range from 10 - 30 per cent.

Disease Mechanism

TMD arises during fetal development and hematopoiesis. In the setting of trisomy 21,

megakaryocyte-erythroid progenitors (MEPs) are expanded during hematopoiesis in the fetal liver. Development of TAM appears to require the acquisition of a somatic mutation of the gene coding for the hematopoietic transcription factor GATA-1. GATA-1 mutations in TAM result in the expression of an amino-terminally truncated GATA1 protein (GATA1s). The functional consequence is impaired megakaryocytic differentiation and uncontrolled proliferation of a population of TAM blasts as shown in Figure 1.

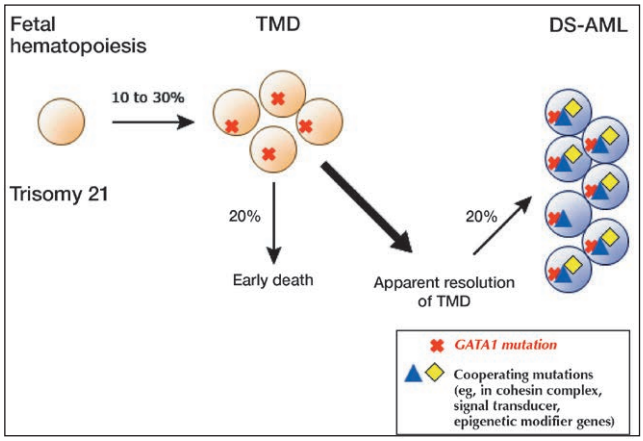


Figure 1: Disease Mechanism of TAM in Down Syndrome
Source: Xu G et al. Frequent mutations in the GATA-1 gene in the transient myeloproliferative disorder of Down syndrome. Blood 2003; 102:2960.

Clinical Presentation

Most patients are diagnosed within the first weeks of life. Some first come to clinical attention when circulating blasts are noted on a peripheral smear performed for screening purposes. Others are overtly ill with a wide range of physical and laboratory abnormalities at the time of diagnosis. Infiltration of solid organs with TAM is usually restricted to the liver, spleen, marrow, and skin. Hepatomegaly (60 per cent) and splenomegaly (42 per cent) are commonly present at diagnosis of TAM. Lymphadenopathy is infrequent in TAM (two per cent of patients). At times, skin lesions consisting of papules, vesicles, and pustules may be the first sign pointing to TAM in a newborn with Down syndrome. On biopsy, these lesions may show infiltration by immature myeloid cells.

Laboratory Features

Peripheral smear and bone marrow: TAM is characterized by the frequent presence of blasts (usually megakaryoblasts) in the peripheral blood Figure 2. Moderate leukocytosis is common (median white blood counts (28 to 40 x 109/L).

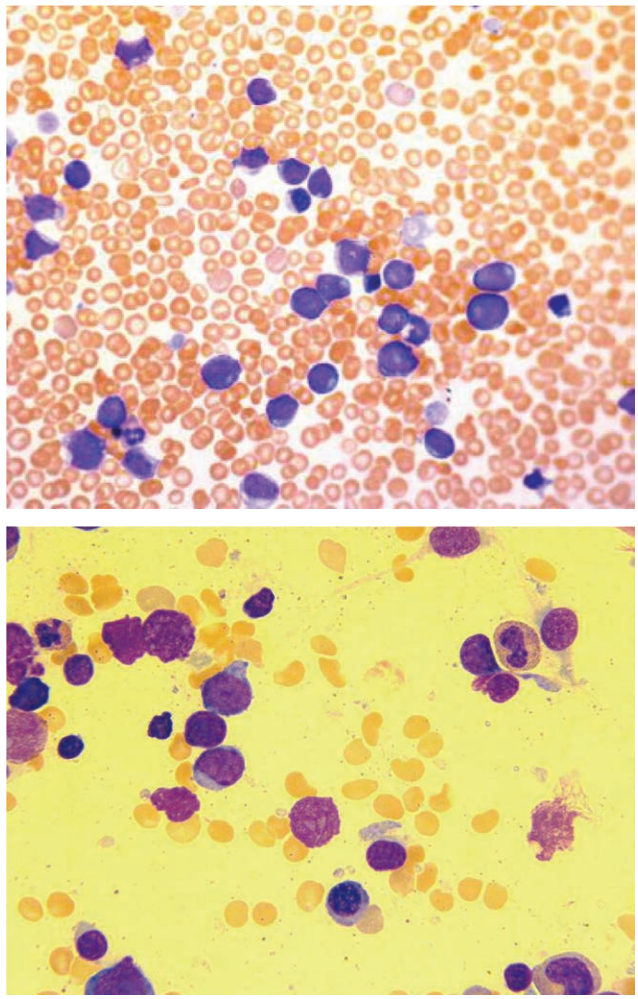


Figure 2: Blasts from the peripheral blood of 1-day-old with trisomy 21. Source: Pathology of Myeloid Proliferations Related to Down Syndrome. Author: Cherie H Dunphy, MD; Chief Editor Cherie H Dunphy

Hemoglobin (median 14.6 to 14.8 g/L) and platelet count (median 96 to 125 x 109/L) are only mildly decreased. The flow cytometry immunophenotype of the non-lymphoid, non-erythroid blasts of TAM is characterized by the expression of megakaryoblastic / megakaryocytic (CD61, CD41, CD42), stem cell (CD34, CD117), myeloid (CD13, CD33), and non-lineage (CD4, CD7, CD56) antigens. Bone marrow examination demonstrates abnormal megakaryocytic maturation in 75 per cent and dyserythropoiesis in 25 per cent.

Diagnostic Criteria

Diagnostic criteria for TAM are not universal. In most cases, the diagnosis is made based on the identification of blasts in the peripheral blood with a megakaryoblastic/megakaryocytic immunophenotype on flow cytometry. The most definitive test to identify TMD blasts is detection of a somatic mutation of GATA1, typically in exon two or three.

Prognosis and Course

TAM resolves spontaneously in the vast majority of patients. The two adverse outcomes of TAM are early death during the neonatal period and development of AML. TAM has an early mortality of up to 20 per cent and estimated five-year overall survival (OS) and event-free survival (EFS) rates of 85 and 63 per cent, respectively. Approximately 20 per cent (13 to 30 per cent) of the patients with TAM go on to develop AML within the first four years of life. Due to this substantial risk for subsequent AML, all patients with a history of TAM are followed for signs and symptoms of progression.

Introduction of Maple Syrup Urine Disease (MSUD) Profile in Biochemical Genetics Laboratory at AKUH

Dr Noreen Sherazi and Dr Sheharbano Imran
Chemical Pathology

Maple Syrup Urine Disease (MSUD) is an inherited amino acids metabolic disorder caused by a deficiency of the branched-chain alpha-keto acid dehydrogenase complex leading to a buildup of the branched chain amino acids (leucine, isoleucine, valine and alloisoleucine) and their toxic by-products (ketoacids) in the blood and urine. Clinically it can be classified as classic MSUD or other variants of MSUD.

Clinical Indications for MSUD Testing

- Maple syrup odor in urine, the first clinical sign of MSUD, detectable 12 hours after birth.
- Poor feeding by age two to three days.
- Deepening encephalopathy including lethargy.
- Intermittent apnea, opisthotonus, and stereotyped movements (such as fencing and cycling) by age four to five days.
- Coma and central respiratory failure that may occur by age seven to ten days.
- Poor growth.
- Irritability or developmental delays, later in infancy or childhood.
- Ketonuria and encephalopathy if stressed by fasting, dehydration, or infectious illness.

Biochemical Characteristics of MSUD

Elevated plasma concentrations of branched-chain amino acids (BCAAs) (leucine, isoleucine, and valine) and allo-isoleucine, accompanied by a more generalized disturbance of plasma amino acid concentration ratios, detectable by 12-24 hours of age in affected infants on a normal protein intake. Occasionally, plasma concentrations of isoleucine or valine may be low or normal, but plasma concentration of leucine is invariably elevated. Elevated plasma concentration of allo-isoleucine is specific and diagnostic for MSUD.

MSUD Profile at AKUH Clinical Laboratory

We have recently introduced MSUD profile at out biochemical genetics laboratory section of clinical chemistry. This profile includes:

- Leucine
- Isoleucine
- Valine
- Allo-isoleucine

As compared to our full plasma amino acid quantification, this shortened program allows the separation and quantification of the four branched chain amino acids. In addition to this, alloisoleucine is also identified in this profile unlike full plasma amino acid quantification. It is used as a specific and sensitive diagnostic marker. Quantitative amino acid analysis of these four branched-chain amino acids is crucial for the diagnosis and monitoring of MSUD.

Urinary Tract Infections (UTI) in Children

Dr Fizza Farooqui
Microbiology

Urinary tract infections are a common cause of unexplained fever in infants and young children. Most guidelines suggest that fever of >38°C in this age group should raise suspicion of UTI, and thus, it is of utmost importance that they have their urine tested within 24 hours.

Definitions

Guidelines proposed by National Institute of Health and Clinical Excellence (NICE) recommend that children be divided into three age groups; <3 months, three months to <3 years, and ≥3 years for the purpose of diagnosis. Children <3 months should have urgent microscopy of urine (>5 white blood cells/high power field) as well as cultures also from other sites (blood, cerebrospinal fluid if indicated) as they are at high risk of serious illness. For children three months to <3 years, urgent urine microscopy and culture is recommended, dipstick testing needs further discussion between microbiologists, pediatricians and primary care doctors. Dipstick testing is recommended for children >3 years, where a positive nitrite and leukocyte esterase denote infection. The American Academy of Pediatrics (AAP) recommends that diagnostic strategies depend on whether the clinician decides to start antimicrobial therapy or not. Diagnosis is dependent on the presence of pyuria and growth of ≥50,000 colony forming units of a single urinary pathogen from a urine specimen collected with a sterile technique.

Samples

Collection of urine samples remains highly challenging in the younger age group. Clean catch urine specimens are recommended by NICE guidelines, samples collected in urine collection bags and pads have been reported to show equal rates of contamination. If none of these are possible, in/out catheters or ultrasound guided supra-pubic aspiration are the recommended methods of choice for collection. AAP recommends in/out catheters or supra pubic aspirates as the only methods of choice for urine collection.

Antibiotic Treatment

Increase in antibiotic resistance, inconvenience, lack of compliance, and increased cost all add weight to the decision of discontinuation of antibiotic prophylaxis by the NICE guidelines. The AAP recommends that antibiotic treatment should start based on route of administration, local antibiogram, and subsequently upon susceptibility results from culture reports. The recommended duration of antibiotic therapy is 7-14 days.

Preventive Measures

NICE guidelines advise that regular bladder emptying, general hygiene measures, drinking adequate amounts of fluids, breastfeeding and circumcision may prevent recurrence of UTI. AAP guidelines suggest that ultrasonography of the kidneys and bladder should be performed to detect structural abnormalities, and a voiding cystourethrogram obtained if there is evidence of hydronephrosis, scarring, or other complex clinical circumstances are encountered.

Thus, a high index of suspicion, appropriate sample collection and testing followed by regular follow up to prevent recurrent UTI remain the mainstay for management of urinary tract infections in children.

Key points:

Age Group	Microscopy	Dipstick analysis (D/R)	Urine Culture (Clean catch/ suprapubic/ in/out catheters)
<3 months	√	-	√ (in addition to blood culture)
3 months - <3 years	√	Debatable	√
>3 years	√	√	√

Labrad Quiz

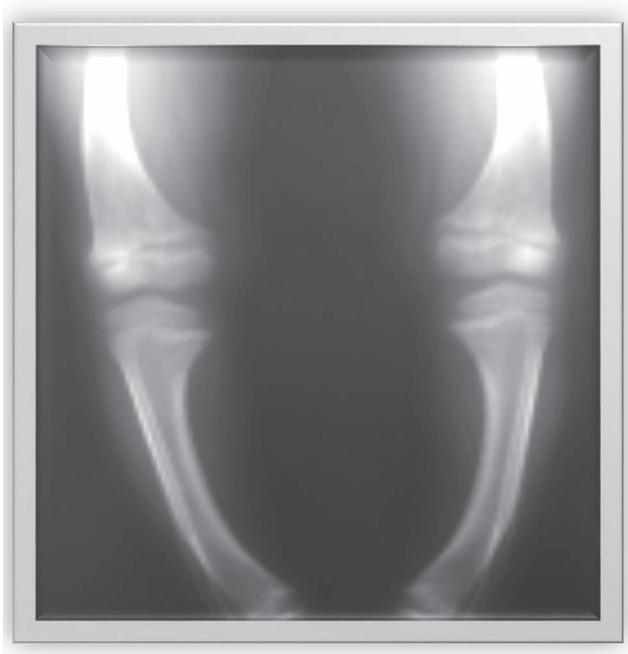
Dr Lena Jafri
Chemical Pathology

A 6 year old girl was brought to a pediatrician for not being able to walk properly since one and a half year. Mother (Para +8) explained that her marriage was consanguineous and two of their boys (12 years and 10 years old) had deformed bones and were unable to walk.. On examination, there are skeletal deformities of both upper and lower limbs, frontal bossing and pallor. Her extremities have widened wrists and ankles.

The laboratory and radiological evaluation included:

Serum Creatinine	0.5 mg/dl (0.5-1)
Serum Calcium	8.7 mg/dl (8.6-10.2)
Serum Phosphorus	1.8 mg/dl (2.5-4.5)
Alkaline phosphatase	1917 U/l (45-129)
25-hydroxy Vitamin D	32 ng/ml (>30)
Parathyroid hormone	90 pg/ml (16-87)

The pediatrician wanted to confirm his suspicion and ordered TMP-GFR in this patient.



Questions:

1. Which disease is the pediatrician suspecting?
2. Why did the pediatrician order TMP-GFR?
3. How is TMP-GFR calculated?
4. What is the pathophysiology of this disease?
5. Which other Vitamin D metabolite testing would be helpful in establishing the diagnosis in this patient?

An Overview of Congenital Adrenal Hyperplasia

Dr Sibtain Ahmad
Chemical Pathology

Introduction

Congenital adrenal hyperplasia (CAH), an autosomal recessive inherited defects of enzymes involved in adrenal steroidogenesis due to mutations in specific genes. The common functional defect in each disorder is impaired cortisol secretion, resulting in hyper secretion of corticotrophin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) and consequent hyperplasia of the adrenal glands. Those affected may present clinically as a classic (more severe) or nonclassic (less severe) type. About 75% of those with a classic deficiency will have a “salt-wasting” form that includes decreased aldosterone and leads to an excess loss of fluids, low sodium, and high potassium that, when severe, can be life-threatening. More than 90% of cases of CAH are caused by a defect in the enzyme 21-hydroxylase

(21OHD), which is caused by a mutation in the CYP21A2 gene. Four other enzyme deficiencies in the steroid biosynthesis pathway, along with one cholesterol transport protein defect, account for the remaining cases Figure 1.

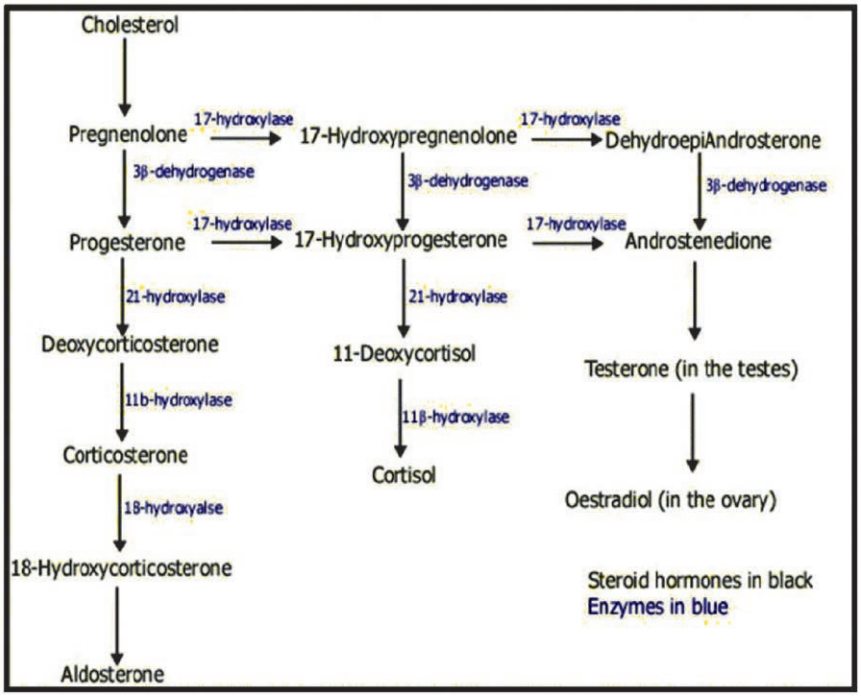


Figure 1: Pathways of Steroid Biosynthesis in the Adrenal

Signs and Symptoms of CAH

Signs and symptoms associated with CAH depend upon the type of enzyme deficiency and vary over time, less severe in non-classic diseases, getting worse in illness or stress to a life-threatening adrenal crisis in classic type.

Salt-wasting CAH signs and symptoms may include: Abnormal heart rhythm, rapid heart rate, confusion, dehydration, hyperkalemia, irritability, hypoglycemia, hypotension, hyponatraemia and vomiting. Females with classic 21OHD deficiency may have ambiguous external genitalia (external sex organs that are not clearly male or female with normal reproductive organs uterus, fallopian tubes, and ovaries) due to the diversion of pathway towards excess androgen production called as hyperandrogenism. Hyperandrogenism signs or symptoms in both males and females may include: accelerated skeletal growth (tall during childhood but short as adults), acne, deep voice, enlarged external genitalia, hirsutism in females, infertility, irregular menstruation , excess muscle growth or early development of pubic and armpit hair.

List of Tests available for Diagnosis of CAH at AKUH Laboratory

Diagnosis and differential diagnosis of CAH always requires the measurement of several steroids. Patients with CAH due to steroid 21-hydroxylase defects/ deficiency usually have very high levels of androstenedione. Additionally 17-OHP levels are usually even higher, while cortisol levels are low or undetectable Table 1. All three analytes should be tested when CAH is suspected.

Patients with CAH due to steroid 11 β-hydroxylase enzyme defects/ deficiency present with low levels of cortisol, aldosterone and renin. Whereas the levels of 11-deoxycorticosterone (11-DOC) are elevated because deficiency of 11 β-hydroxylase deficiency causes decreases conversion of 11-DOC to corticosterone and aldosterone.

Table 1: Biochemical Features in Different Forms of CAH

Deficiency	Cortisol	ACTH	Blood Pressure	Androgen	Estrogen
21 β-OH	↓	↑	↓	↑ Adrenal	-
11 β-OH	↓	↑	↑	↑ Adrenal	-
17 α-OH	↓	↑	↑	↓ Adrenal & Testicular	↓

The 17 α-OH enzyme deficiency is a rare variant of CAH and is characterized by hypertension in most cases. It presents with elevated levels of pregnenolone, progesterone, 11-DOC and corticosterone. However renin, aldosterone and cortisol levels are deficient.

The battery of tests performed of CAH diagnosis or monitoring are:

1. **Serum 17-hydroxyporgesterone (17OHP):**
   a. **Screening:** A 17-OHP test may be ordered as a screening test of choice for newborn screening to detect CAH due to 21-hydroxylase deficiency. The test may be

repeated if the screening result is elevated in order to confirm the initial results. Table 2 shows the normal ranges of 17-OHP.

Table 2: Normal ranges of 17Hydroxy Progesterone in Children of different age Groups

Stage	Range (ng/mL)
Cord blood	9.0 - 50.0
Premature	0.26 - 5.68
Newborn-3days	0.07 - 0.77
Pre-pubertal child	0.03 - 0.90

b. **Diagnosis:** Measurement of 17-OHP in the blood may be used to aid in the diagnosis of CAH in older children and adults who may have a milder, "late-onset" form.

c. **Monitoring:** If someone is diagnosed with CAH, a 17-OHP test may be used periodically to monitor the effectiveness of treatment.

2. ACTH stimulation test:
Administration of 250 ug of cosyntropin (a synthetic ACTH) provides a pharmacologic stimulus to the adrenal glands, maximizing hormone secretion. The different forms of CAH due to 21OHD may be determined based on baseline and stimulated values of 17-OHP, the immediate precursor to the 21-hydroxylase enzyme Table 2.

Table 2: Interpretation of Results of 17OHP; 60 minutes after ACTH Stimulation

Levels	Interpretation
<9.9 ng/mL	Likely unaffected
10- 100 ng/mL	Non-Classical congenital Adrenal Hyperplasia
>100 ng/mL	Classic Congenital Adrenal Hyperplasia

3. Dehydroepiandrosterone Sulfate (DHEA-S):
CAH due to 3 beta-hydroxysteroid deficiency is associated with excessive DHEA-S production. Lesser elevations may be observed in 21-OHD deficiency and 11 β-OH deficiency. By contrast, steroidogenic acute regulatory protein or 17 alpha-hydroxylase deficiencies are characterized by low DHEA-S levels.

4. Serum Testosterone: Approximately 10% of androstenedione is metabolized in the body to testosterone, a potent androgen. Consequently pronounced elevations are found in CAH.

5. Plasma Renin: Elevated plasma renin activity values, particularly the ratio of renin to aldosterone, are markers of impaired mineralocorticoid synthesis.

6. Serum Electrolytes: especially sodium and potassium are routinely done in monitoring of CAH patients.

7. Plasma Glucose: is tested to evaluate for hypoglycemia.

8. Additional tests: Karyotyping and pelvic and abdominal ultrasonography are further utilized diagnostic modalities. Kidney function and liver function tests may also be tested during management of these patients.

Burden of CAH in Pakistan

The true incidence of CAH in Pakistan remains unidentified due to the absence of new born screening program, lack of diagnostic testing facilities & disease awareness in the society and failure of case identification by primary physicians.

A study from our center revealed that of the 152 children registered for 17-hydroxyporegeterone (17-OHP) testing, sixty-three (41.4 per cent) were diagnosed with CAH. Among these salt wasting, simple virilization and non-classical CAH was found in 40 (63 per cent), 18(29.0 per cent) and 5 (8.0 per cent) patients respectively. Another study from Pakistan showed variable frequency of referrals from different provinces of Pakistan. Higher frequency of cases with suspected CAH was observed from Sindh (23.7 per cent) as compared to other provinces. These studies show that CAH is not an uncommon disease, thus a well-structured and appropriate screening program of CAH is the need of time.

Role of Histopathology in the Diagnosis of Paediatric Renal Tumours

Dr Nasir-Ud-Din and Dr Arsalan Ahmed
Histopathology

Introduction

Paediatric renal tumours represent a relatively common group of childhood solid neoplasms. The diagnosis, management and prognosis of paediatric renal tumours have significantly improved over the last 40 years. Pathologists have played a significant role in these developments through their work in multicentre national and international trials and studies, by recognizing new tumour entities, as well as favourable and unfavourable histological features of different tumours. This review highlights salient clinical and pathologic features of common paediatric renal tumours.

Nephroblastoma (Wilms tumor)

Nephroblastomas comprise more than 80 per cent of renal tumours of childhood. Most often, they are found in children two to five years old, and they are relatively uncommon in the first six months of life and in children older than six years. Most patients come to medical attention because of a palpable abdominal mass. Other presentations may include abdominal pain, hematu¬ria, and hypertension. Most (90 per cent) cases are sporadic; remainders are associated with one of the several syndromes, some of which are associated with abnormalities of the WT1 gene. Most are unilateral and unicentric, but bilateral tumours are present in five to 10 per cent cases, especially in familial cases. Those patients typically have a younger age at presen¬tation and are more prone to renal failure.

Nephroblastoma is derived from the nephrogenic blastemal cells and recapitulates various stages of the developing kidneys. Histologically, typically composed of variable mixtures of blastema, epithelium, and stroma (triphasic), although in some tumors only two (biphasic) or occasionally only one (monophasic) component is present. Most tumors have some degree of epithelial differentia¬tion manifested either as homologous (resembling normal nephrogenesis) or heterologous (mucinous and squamous) cell types. A variety of stromal patterns may be observed including immature myxoid and spindled mesenchymal cells, smooth muscle, skeletal muscle, and neuroglial tissue.

For therapeutic and prognostic purposes, nephroblastomas are histologically divided into two major categories; those that are highly responsive to current therapeutic modalities are designated as nephroblastomas with favorable histology, and those that do not respond well to chemotherapy are relegated to the unfavorable histology group. The latter make up about five per cent of nephroblastomas and are defined by nuclear anaplasia. Anaplasia is rare in patients younger than two years but increases in incidence thereafter. Recognition of anaplasia requires the presence of markedly enlarged nuclei that are at least three-fold of non-anaplastic nuclei and hyperchromasia and atypical multipolar polyploid mitotic figures. Anaplasia may be diffuse or focal. It should be emphasized that anaplasia per se is not an indicator of the aggressiveness of a nephroblastoma but rather predictive of its resistance to adjuvant therapy.

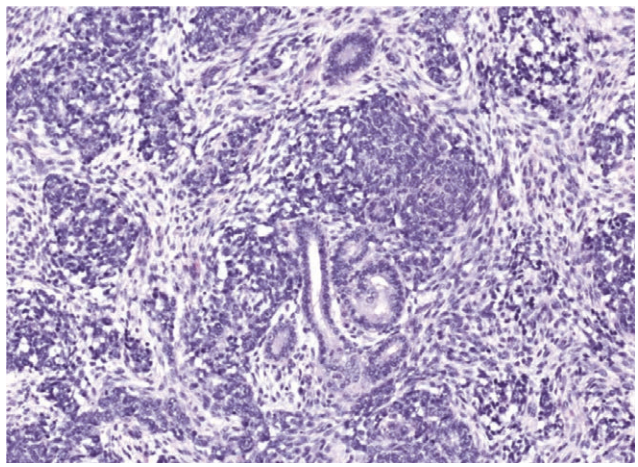


Figure: Nephroblastoma.

Triphasic nephroblastoma contains blastemal, epithelial, and stromal elements

Cystic Nephroma and Cystic Partially Differentiated Nephroblastoma

Cystic nephromas and cystic partially differentiated nephroblastomas (CPDNs) are tumours that are

currently believed to be a part of the spectrum of nephroblastoma. They present as solitary, well-circumscribed lesions, consisting of numerous cysts of different sizes and shapes with no solid areas apart from the septa. The cysts are lined by flattened, cuboidal or hobnail epithelium, whereas the septa are composed of loose or dense fibrous tissue containing only mature elements (in cystic nephroma) or blastemal or other poorly differentiated cell types (in CPDN). Cystic nephromas/CPDNs are treated only by complete surgical resection, which is why it is so critical to distinguish them from other renal tumours that may have a prominent cystic appearance, such as clear cell sarcoma of kidney, rhabdoid tumor of kidney or nephroblastoma with cysts (particularly after preoperative chemotherapy), and which are all treated with postoperative chemotherapy.

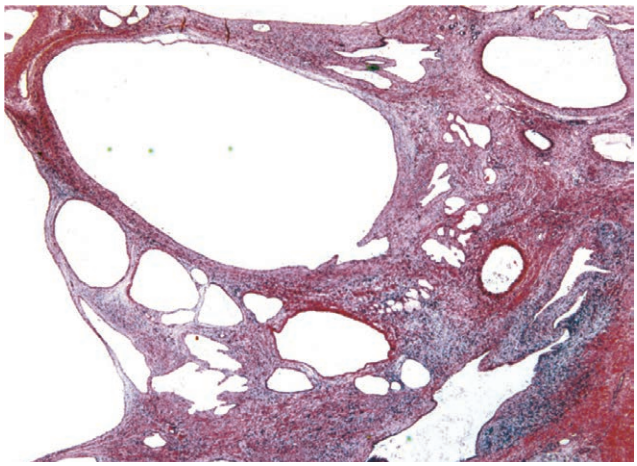


Figure: ***Cystic partially differentiated nephroblastoma.***

The multilocular cysts are lined with flattened, cuboidal, or hobnail epithelium.

Clear Cell Sarcoma of the Kidney

Clear cell sarcoma of the kidney (CCSK) is a rare malignant mesenchymal tumour representing three per cent of renal tumours of childhood. Its peak incidence is between two and three years of age, with a striking male predominance. Presentation is similar to other renal tumours, and around five per cent have metastatic disease at presentation, most commonly involving lymph nodes. Bone metastases are the most common mode of relapse, followed closely by lung metastases. Interestingly, in about 20 per cent of cases relapses occur after >3 years, unlike in nephroblastoma, where >90 per cent of relapses occur within two years after the diagnosis.

Owing to its numerous patterns, CCSK remains the most frequently misdiagnosed renal tumour of childhood, especially on needle core biopsies specimens. Diagnosis is based on morphological criteria, since there is at present no diagnostic immunohistochemical or molecular test. Immunohistochemistry is primarily useful to exclude other renal tumours,

The classical pattern demonstrates nests/cords of ovoid, epithelioid or spindled cells with bland nuclei separated by fibrovascular septa, which demonstrates a marked 'chicken wire' pattern of small blood vessels. About 90 per cent of tumours show at least some areas with the classical pattern, but other appearances are also reported, including myxoid (50 per cent), sclerosing (35 per cent), cellular (26 per cent), epithelioid (13 per cent), pallisading (11 per cent), spindle-cell (seven per cent), storiform (four per cent), and anaplastic (three per cent) patterns.

The prognosis for CCSK has improved significantly since the introduction of doxorubicin to its treatment. The only significant histological factor associated with poor prognosis is the presence of necrosis. A favourable survival rate is associated with a lower tumour stage, an age of two to four years at diagnosis and treatment with doxorubicin. The overall survival is 69 per cent but this varies from 98 per cent for stage I to 50 per cent for stage 4 disease.

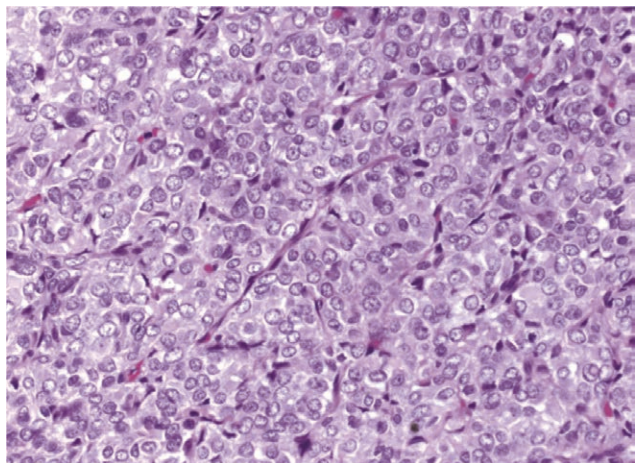


Figure: Clear Cell Sarcoma,

sheets and nests of tumor cells with pale vesicular nuclei are separated by thin vascular septa. Tumor cells are separated from each other by pale to clear extracel¬lular space that imparts a clear appearance.

Rhabdoid Tumour of Kidney

Rhabdoid tumour of kidney (RTK) is a distinctive, highly malignant paediatric renal tumour, usually metastasizes widely and causes the death of the

patient within 12 months of diagnosis. The tumour has a predilection for infants, with a median age of 11 months; over 80 per cent of patients are under two years of age, and it virtually never occurs over five years of age.

Hema¬turia is the most common presentation, and hypercalcemia is a frequent finding. Reportedly, 15 per cent of infants with rhabdoid tumor of the kidney have an associated PNET of the posterior fossa midline. Until recently, the definitive diagnosis could sometimes be difficult, but with the recent improved understanding of the genetic basis of the disease and the availability of specific immunohistochemical markers, the diagnosis can now be made with certainty, even on a needle biopsy specimen. Characteristic histological features are the presence of large, non-cohesive tumour cells with eccentric large nuclei and very prominent eosinophilic central nucleoli, and focally hyaline intracytoplasmatic inclusions. Immunohistochemically, vimetin demonstrates characteristic strong dot-like perinuclear positivity in a significant proportion of cells and INI1 immunopositivity is characteristically absent in the nucleus of cells of rhabdoid tumour.

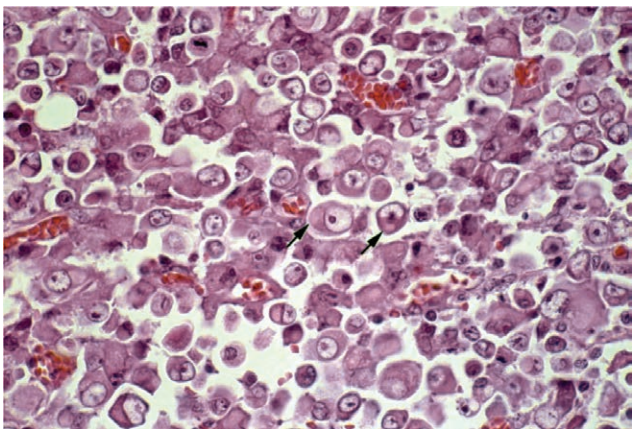


Figure: Rhabdoid Tumor of Kidney.

Classic features include large nucleoli, abundant pink to amphophilic cytoplasm, and intracytoplasmic globules (arrows)

Congenital Mesoblastic Nephroma

Congenital mesoblastic nephroma (CMN) is a low grade fibroblastic sarcoma of kidney comprises two per cent of all paediatric renal tumors. It is the most common congenital renal neoplasm, with more than 90 per cent of affected patients are less than one year of age, about two thirds are diagnosed in the first three months of life and virtually never occurring after the age of three years. Most patients present with a palpable abdominal mass, although an increasing number of tumours are detected sonographi¬cally in utero.

Histologically, CMN is categorized into two major histologic variants: the classic variant (22 per cent), which is composed of fascicles of bland fibroblastic cells with infrequent mitosis and resembles infantile fibromatosis; and the more common cellular variant (40-60 per cent), composed of solid sheets of ovoid cells with frequent mitosis and focal necrosis which is essentially identical to infan¬tile fibrosarcoma. A mixed pattern is recognized when features of both are present in a single tumor. Necrosis and hemorrhage are naturally more frequent in cellular variants. Tumour cells are usually positive with vimentin and focally for smooth muscle actin, although the diagnosis is based on morphological criteria alone. The cellular variant shares the same genetic abnormality as infantile fibrosarcoma: a t(12;15)(p13;q25) translocation with resultant ETV6-NTRK3 fusion. The main factors of importance as risk factors for local recurrence are cellular subtype and incomplete local excision at primary surgery. They relapse within 12 months after the diagnosis, so during this period a close follow-up is recommended.

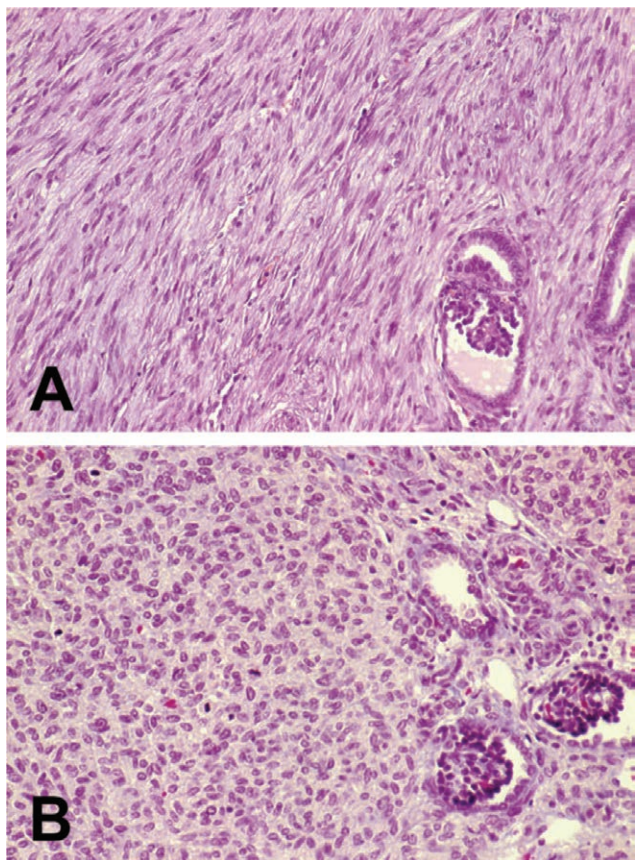


Figure: A. Classic form of ***Congenital Mesoblastic Nephroma.***

Fibromatosis-like growth of well-differentiated spindle cells with entrapped glomerulus and tubules. B, Cellular congenital mesoblastic nephroma. High cell density, mitotically active spindle cell proliferation, also with entrapped glomeruli.

Renal Cell Carcinomas

Renal cell carcinomas (RCCs) represent around five per cent of all paediatric renal tumours, hence they are more common than CMN, CCSK or RTK. They show distinguishing histological and genetic features from RCCs seen in adults. These tumours are defined by several different translocations. A group of Xp11.2-translocation carcinomas resulting from fusion of the TFE3 gene is now recognized as a separate entity. Histologically, they show a typical nested appearance, but may also be papillary, composed of cells with granular eosinophilic cytoplasm or clear cells. Immunohistochemically, tumour cells show strong nuclear positivity for TFE3, which is diagnostic. A t(6;11)-translocation RCC is another translocation-associated paediatric RCC which has been rarely reported, and is seems to be less aggressive than Xp11.2-translocation RCC. Histologically, they feature nests and small acini of polygonal epithelioid cells with well-defined cell borders, separated by a thin-capillary vasculature, but also another population of smaller epithelioid cells clustered around nodules of hyaline basement membrane material. A number of these tumours occur as post-chemotherapy tumours, including after neuroblastoma and nephroblastoma. Papillary carcinoma can also occur in older children. Carcinomas of the kidney need to be considered in children with von Hippel-Lindau disease and tuberous sclerosis. RCCs with lymph node metastases generally appear to have a better prognosis in children.

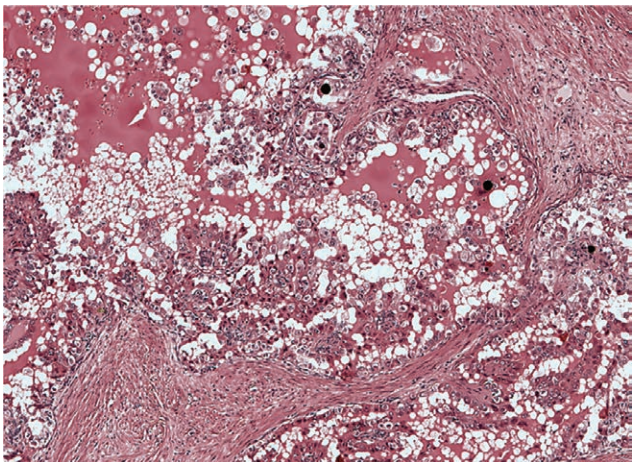


Figure: Xp11 Translocation Carcinoma.

Large cells with eosinophilic cytoplasm and psammoma bodies with papillary and compact architecture.

Renal medullary carcinoma is a rare, highly aggressive tumour occurring in children and young adults with sickle cell trait or disease. Microscopically, it is very infiltrative, and is composed of large cells with large, vesicular nuclei and prominent nucleoli (rhabdoid features), usually accompanied by a marked acute and chronic inflammatory infiltrate and stromal desmoplasia. Interestingly, tumour cells may be negative for INI1 marker. Patients usually present with widespread metastases and show no response to chemo- or radio-therapy, resulting in a poor survival (mean 4 months).

Conclusion

Renal tumours of childhood are a fascinating group of tumours where huge progress in classification, treatment and understanding of molecular biology has been made. Since these tumours are rare, they still represent a diagnostic problem and central pathology review in multicentre trials is essential for assigning the appropriate treatment. Molecular biology markers are likely to play an even more important role in future trials.

Role of Histopathology in the Diagnosis of Paediatric Bone and Soft Tissue Small Round Cell Tumours

Dr Nasir-Ud-Din and Dr Arsalan Ahmed
Histopathology

Introduction

Paediatric bone and soft tissue small round cell tumours (SRCTs) include Ewing sarcoma, rhabdomyosarcoma, poorly differentiated synovial sarcoma, lymphoblastic lymphoma, desmoplastic small round cell tumour, small cell osteosarcoma, mesenchymal chondrosarcoma, melanotic neuroectodermal tumour of infancy, and neuroblastoma. Accurate diagnosis is crucial as treatment modalities are different for each tumour. In this paper we will discuss salient features of each of these tumours and discuss role of histopathology in their diagnosis.

Ewing Sarcoma

Ewing sarcoma (ES) is a high grade sarcoma most often occur in children and young adolescents, with a male sex predilection. It commonly involves the diaphysis of long bones especially femur followed by sacrum and spinal column. Extraosseous ES is rare but increasingly recognized due to greater awareness of this tumor and better techniques of diagnosis. A typical ES is histologically characterized by sheets of uniform round cells with scant clear to eosinophilic cytoplasm, regular nuclear contours, finely dispersed chromatin and inconspicuous nucleoli. Mitotic activity is scarce and areas of geographic necrosis are frequently seen.

A rare histologic variant of ES called atypical ES is characterized by sheets of large cells with irregular nuclei and prominent nucleoli, thus mimicking a large cell lymphoma. Despite their worrisome features, atypical ESs are similar to typical lesions in clinical behavior.

A high content of glycogen is seen in cells on periodic acid-Shiff (PAS) stain. Up to 99 per cent of ES show diffuse strong membranous expression of CD99. FLI-1 one is another sensitive marker positive in approximately 75 per cent cases of ES. In the appropriate clinical setting, morphology and diffuse strong positivity of CD99 (and negative stains of other SRCTs) is diagnostic of ES. In difficult cases, cytogenetic analysis of EWSR1 by FISH or RT-PCR is needed for diagnosis.

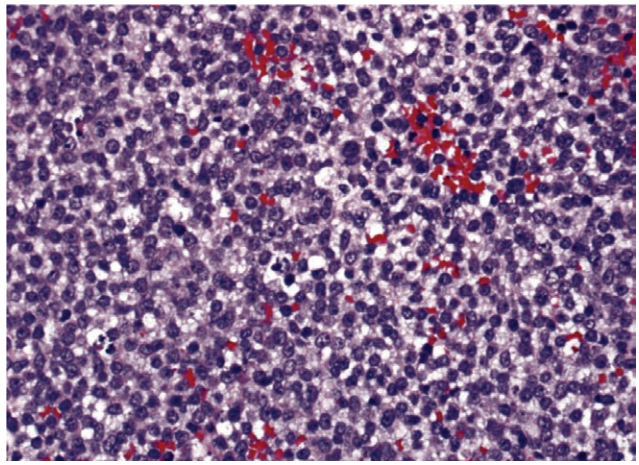


Figure 1: Conventional Ewing Sarcoma

showing uniform cells with round, regular nuclei containing finely dispersed chromatin and scant clear to slightly eosinophilic cytoplasm. (original magnification 400x).

Rhabdomyosarcoma

Rhabdomyosarcomas (RMSs) are the most common malignant soft tissue tumours in children, and embryonal RMS is most common type, which commonly arises in the head and neck region with a minority of cases in the musculature of extremities. Histologically, embryonal RMS typically displays alternating areas of hyper and hypocellularity. The cells vary considerably in differentiation ranging from primitive and stellate to others having moderate eosinophilic cytoplasm and still others showing terminal differentiation with abundant eccentric eosinophilic cytoplasm and cross-striations. The hypocellular areas contain myxoid stroma.

Clinical behavior and treatment varies for subtypes of RMS, so subclassification becomes just as critical

as diagnosis. Presence of anaplasia in embryonal RMS should be mentioned in histopathology report as it is associated with aggressive behavior. The diagnosis of RMS is made on morphology coupled with muscle markers desmin, myogenin and Myo D1.

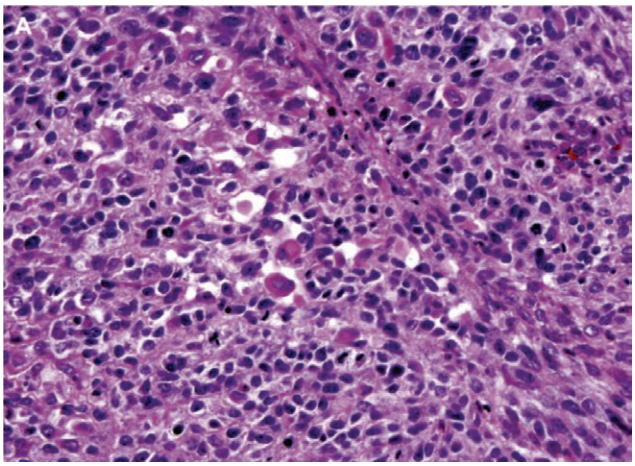


Figure 2: An embryonal rhabdomyosarcoma

contains rhabdomyoblasts with central or eccentric nuclei and prominent, brightly eosinophilic cytoplasm (hematoxylin and eosin, original magnification x40).

Poorly Differentiated Synovial Sarcoma

Synovial sarcoma (SS) is a relatively common soft tissue sarcoma that most frequently occurs in extremities of adolescents and young adults with a peak incidence between 10 and 35 years. The three histologic types of SS are monophasic, biphasic and poorly differentiated. Poorly differentiated synovial sarcoma (PDSS) tends to occur more proximally than classical SS, and is composed of sheets of rounded or ovoid cells with increased mitotic activity, focal necrosis, and high proliferation index (Ki67), and resembles small round cell tumors, such as ES or lymphoma. PDSS shares a similar immunophenotype to classic SS such as EMA, Cytokeratin AE1/AE3, as well as CK7 and CK19. Almost all cases show nuclear expression of TLE1, a very sensitive but not specific marker for SS. Variable number of tumours express CD99, thus causing confusion to discriminate from ES. In those cases, molecular analysis of specific chromosomal translocation t(X;18)(p11,q11) by FISH or RT-PCR helps in arriving a correct diagnosis as treatment is entirely different for both tumours.

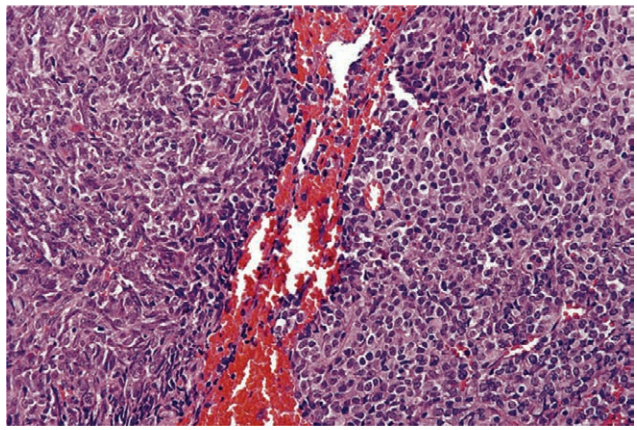


Figure 3: Poorly differentiated synovial sarcoma.

Note the predominantly round cell cytomorphology and transition from spindle cells.

Mesenchymal Chondrosarcoma

Mesenchymal chondrosarcoma (MC) is a high grade sarcoma which mainly affects young adults and teenagers. Two thirds of cases involve bones especially ribs and jaw bones and one third of cases occur in soft tissues adjacent to the craniospinal axis including paraspinal musculature. Histologically, MC is characterized by primitive small round cells arranged in sheets, vague nests or both, often in a hemangiopericytoma-like vasculature. These cells usually show an abrupt transition to single or lobules of well-differentiated hyaline cartilage.

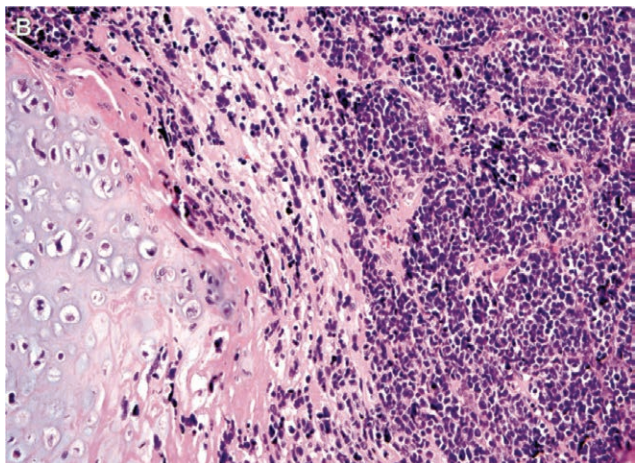


Figure 4: The small blue cell portion of mesenchymal chondrosarcoma resembling other types of SRCTs, in particular atypical ES. A diagnosis can be made by finding areas containing the low-grade chondrosarcoma component.

The differential diagnosis includes other SRCTs such as atypical ES, and lymphoma, particularly when matrix component is not evident because of sampling error. Hematopoietic immunostains will rule out

lymphoma. Potential overlap with ES is exacerbated by the fact that >90 per cent of MCs show strong diffuse membranous positivity for CD99. Alternatively, FLI-1 has been shown to be positive in 75 per cent of ES and MC is negative. SOX9 is a useful marker for MC. The stromal component of small cell osteosarcoma may resemble MC. Moreover, small cell osteosarcoma also can show positivity with CD99. Careful attention paid to the type of matrix production-osteoid in osteosarcoma and cartilage in MC- is the best way to make the distinction. Sometimes this requires additional tissue.

Desmoplastic Small Round Cell Tumour

Desmoplastic small round cell tumour (DSRCT) is an aggressive small round cell neoplasm that predominantly affects children, adolescents, and young adults, with a male preponderance. The tumor characteristically occurs in the abdomen or pelvis, often with widespread serosal involvement. DSRCTs almost always consist of small nests of uniform round cells with hyperchromatic nuclei with inconspicuous nucleoli and scant cytoplasm set in a highly vascular, desmoplastic stroma.
DSRCT is of uncertain histogenesis, but it shows polyphenotypic differentiation, displaying immune reactivity for epithelial (cytokeratin, EMA and BerEP4), muscle (desmin), and neural (NSE, synaptophysin). Desmin staining is usually dot-like, but muscle specific markers myogenin and MyoD1 are negative. DSRCTs are highly aggressive, with more than 90% of patients dying from tumour progression within just a few years.

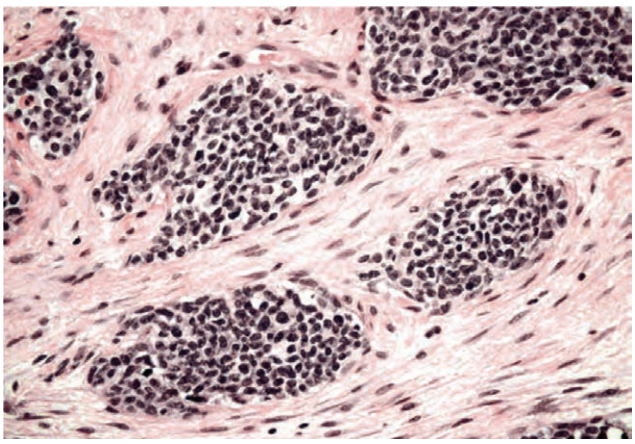


Figure 5: Desmoplastic Small Round Cell Tumour.

Classic appearance, with sharply demarcated nests of small round cells within a desmoplastic stroma containing spindle-shaped myofibroblasts embedded in a matrix of loose or myxoid extracellular material and collagen.

Small Cell Osteosarcoma

Small cell osteosarcoma is a rare histologic subtype of osteosarcoma composed of small cells simulating the appearance of ES or lymphoma. However, tumour cells produce osteoid or bony matrix, at least focally. The distinction between ES, especially atypical ES, and a small cell osteosarcoma can be difficult because of overlapping histologic and radiologic features.

Some Ewing sarcomas show fibrinous deposits among tumor cells, whereas the osteoid in small cell osteosarcoma is mineralized. Ancillary studies play an important role in cases in which osteoid production is questionable. Hematopoietic immunostains will distinguish lymphoma from small cell osteosarcoma. Small cell osteosarcoma can be immunoreactive with CD99, so it is not a useful stain in ruling out Ewing sarcoma. FLI-1is a better marker because most Ewing sarcomas are positive but small cell osteosarcomas are negative. Small cell osteosarcoma is an extremely uncommon tumor with a poor prognosis.

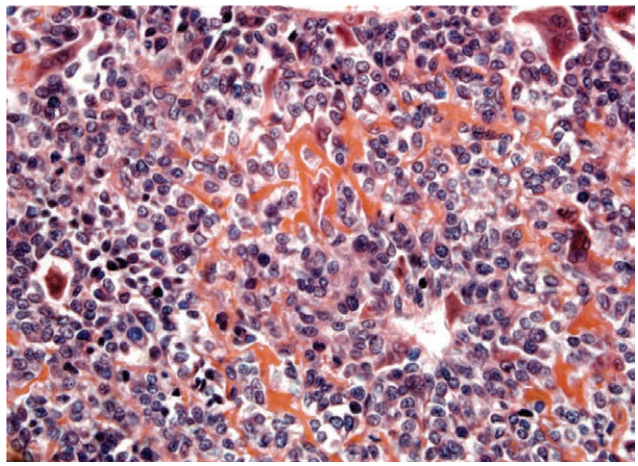


Figure 6: The neoplastic cells in small cell osteosarcoma are small, round, and only occasionally spindle-shaped. The lacelike pattern of osteoid production differs from reactive bone sometimes seen in other types of SRCTs of bone.

Neuroblastoma

Neuroblastoma are malignant childhood tumours derived from primordial neural crest cells. Neuroblastoma is the third most common childhood malignant tumor, after hematolymphoid and central nervous system neoplasms, but the most common solid tumor of infancy. Patients with metastatic neuroblastoma to bone are almost always younger than age five years. A chest and abdominal CT scan usually detects the primary site in cases where the

initial diagnosis is made or suggested via a bone biopsy.

Melanotic Neuroectodermal Tumour of Infancy (Retinal Anlage Tumour)

Melanotic neuroectodermal tumour of infancy is a rare tumour usually present in the first year of life with a moderate predominance in boys. More than 90 per cent of cases arise in the head and neck region, with an overwhelming predilection for the maxilla. The tumour has characteristic two principal cellular components: small basophilic neuroblast-like cells, often set in a fibrillary matrix, and larger epithelioid, eosinophlic cells with vesicular nuclei and containing variable amounts of melanin pigment. The latter cells are arranged in alveolar or pseudoalveolar structures set in a dense fibroblastic stroma. Immunohistochemically, both cell types stain positively for NSE and synaptophysin; in addition the larger cells show consistent immunopositivity for both cytokeratin and HMB-45, in parallel with the findings in pigmented retinal epithelium.

B-Lymphoblastic Leukemia/Lymphoblastic Lymphoma (B-ALL/LBL)

B-Acute Lymphoblastic leukemia/lymphomas occur primarily in children. Bone marrow is nearly always involved and peripheral blood is also usually involved. The most frequent extramedullary sites to be involved by B-LBL include skin, soft tissue, lymph node and bone. The clinical presentation is related to pancytopenia secondary to involvement of bone marrow. Lymphadenopathy and hepatosplenomegaly are also common. The involved site is usually composed of sheets of small to intermediate sized monotonous lymphoblasts which have indented or convoluted nuclei and inconspicous to prominent nucleoli. On immunohistochemistry, the lymphoblasts of B-ALL/LBL show positive expression for B-cell markers (PAX-5, cC79a, CD19, CD 24 and cCD22). Of all these markers, PAX-5 is considered as the most sensitive and specific marker to demonstrate B-cell lineage. The lymphoblasts are also positive for CD10 and TdT and negative for surface immunoglobulin (Ig). Expression of CD20, CD45 and CD34 is variable. There can be aberrant expression of myeloid-associated antigens CD13 and CD33 but Myeloperoxidase (MPO) will be negative. B-ALL with t(9;22)(q34;q11.2), t(v;11q23) and hypodiploidy are associated with poor prognosis. B-ALL with (12;21)(p13;q22) and hyperdiploidy are associated with favorable prognosis. Ages of less than one year and more than 10 years, high white blood cell count, presence of minimal residual disease after therapy and CNS disease at the time of diagnosis are associated with adverse outcome. B-ALL/LBL has a better prognosis in children than in adults.

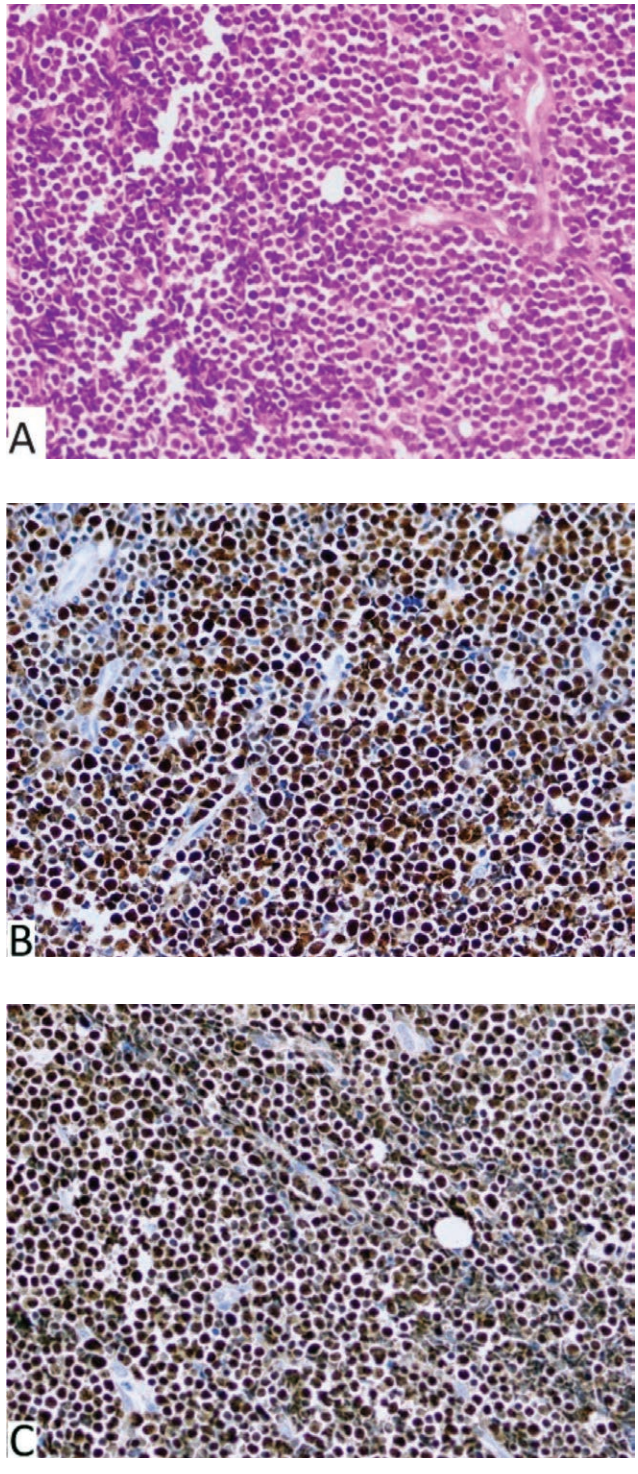


Figure 7: B-ALL/LBL A. The neoplastic cells are dyscohesive, are fairly uniform, have hyperchromatic nuclei, inconspicuous nucleoli and scant cytoplasm. The B-lymphoblasts show characteristic positive expression for TdT (B) and PAX 5 (C) immunostains. (original magnification 40x).

Laboratory Workup of Neonatal Anaemia

Dr Anila Rashid
Hematology

The most common cause of anaemia in young infants is "physiologic anaemia", which occurs at approximately six to nine weeks of age. Erythropoiesis decreases dramatically after birth as a result of increased tissue oxygenation and a reduced production of erythropoietin. In healthy term infants, hemoglobin (Hb) levels are high (>14 g/dL) at birth and then rapidly decline, reaching a nadir of approximately 11 g/dL at six to nine weeks of age, which is called "physiologic anaemia of infancy". Pathologic anaemia in newborns and young infants is distinguished from physiologic anaemia by any of the following.

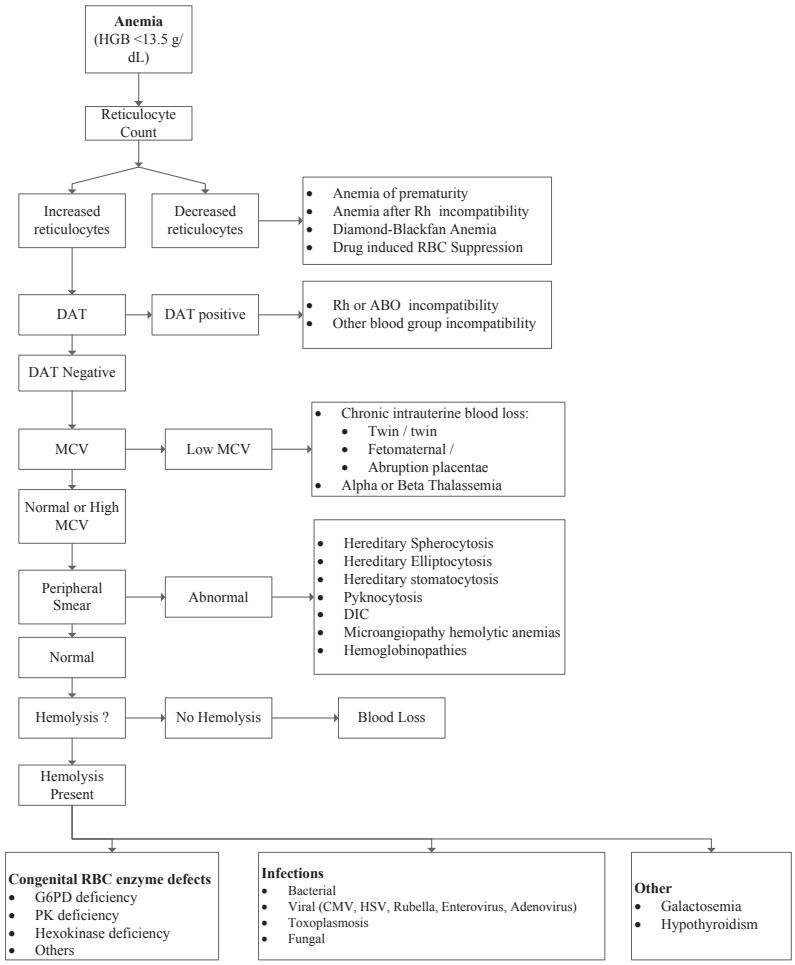
- Anaemia (Hb <13.5 g/dL) within the first month of life
- Anaemia with lower Hb level than is typically seen with physiologic anaemia (ie, <9 g/dL)
- Signs of hemolysis (eg, jaundice, scleral icterus, or dark urine) or symptoms of anaemia (eg, irritability or poor feeding)

Common causes of pathologic anaemia in newborns include blood loss, immune hemolytic disease (ie, Rh or ABO incompatibility), congenital infection, twin-twin transfusion, and congenital hemolytic anaemia (eg, hereditary spherocytosis, glucose-6-phosphate dehydrogenase [G6PD] deficiency). Hyperbilirubinemia in the newborn period suggests a hemolytic etiology; microcytosis at birth suggests chronic intrauterine blood loss or thalassemia.

Thorough history taking and physical examination are the primary steps in identifying the condition and establishing an etiology, but further investigations in the form of laboratory testing are often required to differentiate the many possible causes.

Laboratory investigations can aid in determining the underlying cause of neonatal anaemia; however, it is often difficult to decide which tests to obtain and how to interpret the results. It is therefore beneficial to proceed with investigation in a stepwise manner to avoid unnecessary testing that will delay and confuse proper diagnosis. The following algorithm can be easily followed to help in establishing a diagnosis of anaemia and its underlying etiology.

Diagnostic approach to anaemia in the newborn



HGB: hemoglobin; RBC: red blood cell; DAT: direct antiglobulin test; MCV: mean corpuscular volume; DIC: disseminated intravascular coagulation; PK: pyruvate kinase; G6PD: glucose-6-phosphate dehydrogenase; CMV: cytomegalovirus; HSV: herpes simplex virus.
Source: Gallagher PG. The neonatal erythrocyte and its disorders. In: Nathan and Oski's Hematology and Oncology of Infancy and Childhood, 8th Ed, Orkin SH, Fisher DE, Look AT, et al (Eds), WB Saunders, Philadelphia 2015. p.52.

Evaluation of Inborn Errors of Metabolism (IEM) In a Nutshell

Dr Noreen Sherazi
Chemical Pathology

Inborn Errors of Metabolism (IEM) are single gene defects, responsible for the abnormalities in the synthesis or catabolism of proteins, carbohydrates or fats by way of defective enzymes or transport proteins, resulting in a block of metabolic pathway. They are mostly inherited in autosomal recessive pattern and parent consanguinity increases chance of inheritance in offspring. IEM was first described by Garrod at the beginning of the 20th century; several hundred new disorders have now been defined, as new robust biochemical and molecular diagnostic tools became available.

IEM can affect any organ system and usually affect multiple organ systems. Manifestations vary from those of acute life threatening disease to sub-acute progressive degenerative disorders. For evaluating an IEM it is essential to consider following five important aspects i.e.:

- **Detailed Clinical History:** Proper history from parents has a role in suspecting IEM (Table 1) as detailed history is necessary for further diagnosis.
- **Physical examination:** To look for dermatitis, alopecia, facial dysmorphia, cataract etc.
- **Metabolic screening tests:** These include complete blood count, Urine D/R, plasma glucose, plasma ammonia, plasma lactic acid, arterial blood gases and electrolytes
- **Follow up/Selective screening:** The test is performed on the basis of clinical context which includes quantitation of amino acids, organic acids, carbohydrate and other metabolites, Long chain fatty acids, MPS separation and speciation. Clinical laboratory AKUH started first local diagnostic biochemical genetics laboratory in the country and offering testing facility for screening and diagnosis of IEMs Table 2.
- **Definitive diagnostic tests:** To confirm the disorder detected, specific enzyme assays in leucocytes, plasma/serum or red cells, immunoassays and DNA/ mutation based analysis will be tested.

Table 1: Risk Factors in Suspected IEM Disorders

Prenatal Factors	Antenatal Factors	Postnatal Factors
Consanguinity	CNS anomalies	Healthy neonate at birth became sick
		after few hours or days of life
Previous sibling death	Hydrops fetalis	Refusal to feed
	Echogenic kidneys	Sepsis, lethargy, hypotonia
	Stippling epiphyses	Ketosis, hypoglycemia
		Dysmorphic features

Table 2: Initial and Follow Up Testing Facility at Clinical Laboratory AKUH for IEM Disorders

Metabolic Screening Panel	Selective or Follow up Testing Services
✓ CBC,	✓ Plasma/ Urine/ CSF quantification of Amino Acids
✓ Urine D/R	✓ Qualitative Urine Organic acids
✓ Blood Glucose	✓ Urine Succinylacetone
✓ Plasma Ammonia	✓ MSUD Profile
✓ Plasma Lactic Acid	
✓ Arterial Blood Gases	
✓ Electrolytes	

The role of follow-up testing or selective screening in narrowing the differential diagnosis in suspected IEM individuals is crucial as per National Academy of Clinical Biochemistry. (NACB) practice guidelines Table 3.

Table 3: Common Disease-Specific Follow-Up Testing Recommendations by NACB

	Follow-up Analyses	Follow-up Markers	Additional Testing
Amino Acids Disorders			
Phenylketonuria	Plasma Amino acids	Phenylalanine	Urine Pterin*, Dihydropteridine reductase activity*
Tyrosinemia Type 1	Urine organic acids	Succinylacetone	No additional testing
Tyrosinemia Type 2	Plasma amino acids	Tyrosine >1000 μM on presentation	
Maple Syrup Urine Disease	Plasma amino acids	Isoleucine + valine + leucine + alloisoleucine	No additional testing
Homocytsinuria	Immunoassay	Homocysteine	Folate/Vitamin B12 status should be investigated
	Plasma/Urine amino acids	Methionine, Homocystine	Disorders of cobalamin metabolism should also be considered
	Urine Organic Acids	Methylmalonic acid	Methylmalonic CoA mutase activity*
Argininosuccinic acidemia	Plasma/Urine amino acids	Argininosuccinate, Citrulline	No additional testing
Organic Acid Disorders			
Methylmalonic acidemia	Urine organic acids	Methylmalonic, 3-OH propionic, tiglylglycine, methylcitrate	Complementation analysis, B12 studies*
Isovaleric Acidemia	Urine organic acids	3-OH isovaleric acid, isovaleryl glycine	No additional testing
Propionic acidemia	Urine organic acids	3-OH propionic, tiglylglycine, methylcitrate, propionylglycine	B12 studies*
Multiple Carboxylase Deficiency	Urine organic Acids + Plasma Acylcarnitine*	3-OH propionic, 3 OH isovaleric, tiglylglycine methylcitrate, 3-MCC (glycine), lactate	Biotinidase activity, Isolated carboxylase activities + biotin*
Glutaric Aciduria Type 1	Urine Organic acids	Glutaric, 3-OH Glutaric	
Glutaric Aciduria Type 2	Urine Organic acids	Glutaric, 2-OH Glutaric, adipic, suberic, sebacic ethylmalonic, 3-OH isovaleric, isobutyric	No additional testing

**Not available at Clinical Laboratories AKUH*

Clinical Utility of Immature Platelet Fraction – An advanced Parameter in Laboratory Hematology

Dr Sidra Asad Ali and Dr Muhammad Shariq Shaikh
Haematology

Platelets, first described by a German anatomist as 'spherules' nearly a century ago, are multifunctional anucleated cells that play a vital role in hemostasis. They are cytoplasmic fragments of megakaryocytes with dimensions of approximately 2.0–4.0 by 0.5 µm and a mean volume of 7–11 fl. Platelets act as an initial hemostatic wave through their adhesive and cohesive properties leading to the formation of platelet plug with subsequent activation of coagulation pathways in order to consolidate the primary hemostatic plug. Average platelet count ranges between 150 and 410 × 109/L of blood. However, in patients with thrombocytopenia, count alone does not provide precise assessment of bleeding risk and platelet production from bone marrow. Analogous to red cell reticulocytes, immature platelets or reticulated platelets are young platelets that circulate in the peripheral blood and provide functional status of platelet production by bone marrow.

Owing to their high ribonucleic acid (RNA) content, immature platelets can be differentiated from their mature counterparts. This was first demonstrated by Kienast and Schmitz in 1990 utilizing flow cytometry. Since then, several modifications have been developed utilizing multi-color flow cytometric analysis and different flouro-chromes providing simple, rapid and precise assessment of reticulated platelets. In modern automated hematology analyzers equipped with this sophisticated method, fluorescent dyes penetrate the cell membrane through a breach created by surfactant and label the RNA. It is reported as percentage of the total platelet count (%-IPF). The normal reference range for IPF ranges from 1.6-7.1 per cent in adults and 1.0-6.8 per cent in pediatric population.

Since IPF provides status of bone marrow thrombopoiesis, it can be utilized for diagnosis and management of various disorders. Raised IPF levels are seen in conditions with high platelet turn over like disseminated intravascular coagulation (DIC), thrombotic thrombocytopenic purpura (TTP) / hemolytic uremic syndrome (HUS), immune thrombocytopenic purpura (ITP), blood loss and others. Whereas, low levels are observed in individuals with bone marrow suppression such as aplastic anemia and other bone marrow failure syndromes, nutritional deficiencies and drug induced myelosuppression.

Besides its role in aiding the diagnosis of above disorders, IPF can be utilized to monitor the diseases process and to assess need of platelet transfusion. In disorders with high platelet turn over like ITP, initially high IPF values tend to decline as the disease responds to treatment. Similarly, low IPF values in drug induced myelosuppression and viral hemorrhagic fevers will return to normal range with improvement in disease process. Since IPF levels decline around 24-48 hours before improvement in platelet counts, unnecessary platelet transfusion can be prevented in such conditions.

In conclusion, IPF is a unique, rapid, non-invasive and in-expensive tool to assess bone marrow thrombopoietic activity and is available 24/7. All these properties are highly desirable specifically in paediatric settings where bone marrow examination may not be always practical. At AKUH clinical laboratories, state of the art fully automated hematology analyzers of Sysmex XE class, incorporate this sophisticated method of IPF measurement. This test will soon be started andenable us to provide highly reliable and important information about thrombopoietic activity on a single blood sample.

Multiplex Ligation-Dependent Probe Amplification (MLPA) to Screen for Mutations Leading to Duchenne Muscular Dystrophy (DMD)

Dr. Zeeshan A Ahmed, Dr Asghar Nasir and Sheeba Parveen
Molecular Pathology

Duchenne Muscular Dystrophy (DMD) is an inherited X-linked recessive genetic disorder. Individuals who inherit it have a defective gene related to a muscular protein called dystrophin. This protein keeps muscle cells intact, and its absence causes rapid muscular deterioration. DMD is a fatal condition and most sufferers pass away during their twenties. In the later stages of the disease, sufferers are completely disabled and require full-time care. The condition is degenerative. DMD symptoms may begin to appear between age two and six and include: difficulty walking or loss of ability to walk, enlarged calves, learning disabilities, lack of motor skills development, fatigue and rapidly worsening weakness in the legs, pelvis, arms, and neck.

Dystrophin, the largest known human gene (2.4 Mb) is located on the short (p) arm of the X chromosome at position 21.2. The DMD gene is located from base pair 31,119,219 to base pair 33,339,609 on the X chromosome. Mutations in the dystrophin gene alter the structure or function of dystrophin or prevent any functional dystrophin from being produced. This protein is located primarily in muscles used for movement (skeletal muscles) and in heart (cardiac) muscle. In skeletal and cardiac muscles, dystrophin is part of a group of proteins that work to strengthen muscle fibers and protect them from injury as muscles contract and relax. Muscle cells without enough of this protein become damaged and the damaged fibers weaken and die over time, leading to the muscle weakness.

Molecular Diagnostic approach to Screen for Mutations DMD

In suspected DMD patients, an initial investigation involves studying the levels of creatine kinase (CK). Normal serum CK levels at presentation excludes DMD; however an elevated serum CK level obviates the need for a muscle biopsy with an assay for dystrophin protein. Molecular testing is useful to confirm a clinical diagnosis in affected patients.

For DMD it is recommend to initially screening for deletions and duplications, followed by a screen for point mutations if the clinical diagnosis is certain but a deletion/duplication has not been found, See Figure 1. Since whole exon deletions are the predominant type of mutation in the DMD gene (65 per cent), an initial screen which detects the majority of deletions should be the minimum level of diagnostic test offered. Currently the most widely used methods for screening are Multiplex PCR and Multiplex Ligation-Dependent Probe Amplification (MLPA).

Screening of DMD by MLPA (Multiplex Ligation-Dependent Probe Amplification)

The principle of Multiplex Ligation-dependent Probe Amplification (MLPA) is based on the amplification (by use of a single PCR primer pair) of multiple probes, each of which detecting a specific DNA sequence of approximately 60 nucleotide in length. MLPA reagents together with a wide range of MLPA probe mixes can be used to detect deletions and duplications in a DNA sample.

Figure 2A shows a typical MLPA reaction. After denaturation of the sample DNA, a mixture of MLPA probes is added to the sample. Each MLPA probe consists of two oligonucleotides that must hybridise to immediately adjacent target sequences in order to be ligated into a single probe. Each probe in an MLPA probemix has a unique amplicon length, typically ranging between 130-500 nucleotides. During the subsequent PCR reaction, all ligated probes are amplified simultaneously using the same PCR primer pair. One PCR primer is fluorescently labeled, enabling the amplification products to be visualized during fragment separation. This is done on a capillary electrophoresis instrument, yielding a specific electropherogram. The relative height of each individual probe peak, as compared to the relative probe peak height in various reference DNA samples, reflects the relative copy number of the corresponding target sequence in the sample.

A deletion of one or more target sequence thus becomes apparent as a relative decrease in peak height while an increase in relative peak height reflects amplification. Figure 2B shows MLPA results with probe P034 in normal control sample (top panel) compared with a case of deletion (middle panel) and a case of duplication (bottom panel).

DMD can be caused by deletions, duplications or point mutations in the DMD gene that encodes the protein dystrophin, however to what extent DMD manifests depends on whether the translational reading frame is lost or maintained. Partial gene deletions or duplications in the DMD gene account for as much as ~65% of cases of these dystrophies. This extremely high percentage may be due to the nature of the protein and the gene's extreme length (> 2.2 Mb). The MLPA based DMD probemix contains probes for each of the exons of the DMD gene (79 exons) on Xp21.2 chromosome. Performing MLPA reactions is thus sufficient to investigate the copy number of all exons.

In conclusion, MLPA offers a more extensive screening than many other methods used in DMD analysis as it makes possible to find deletions/ duplications that were previously overlooked.

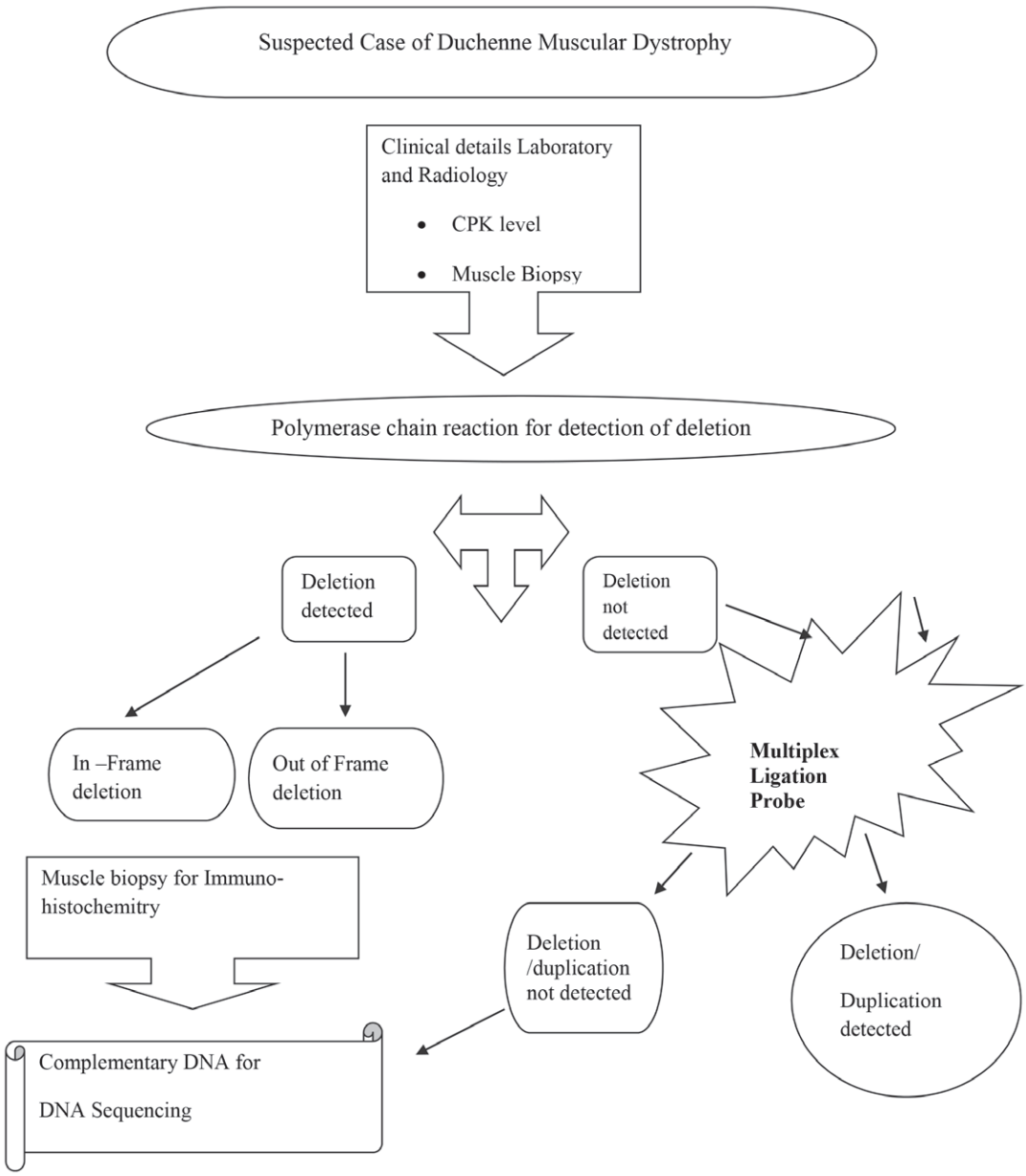


Figure 1: Molecular Diagnostic approach to Screen for DMD Gene Mutation

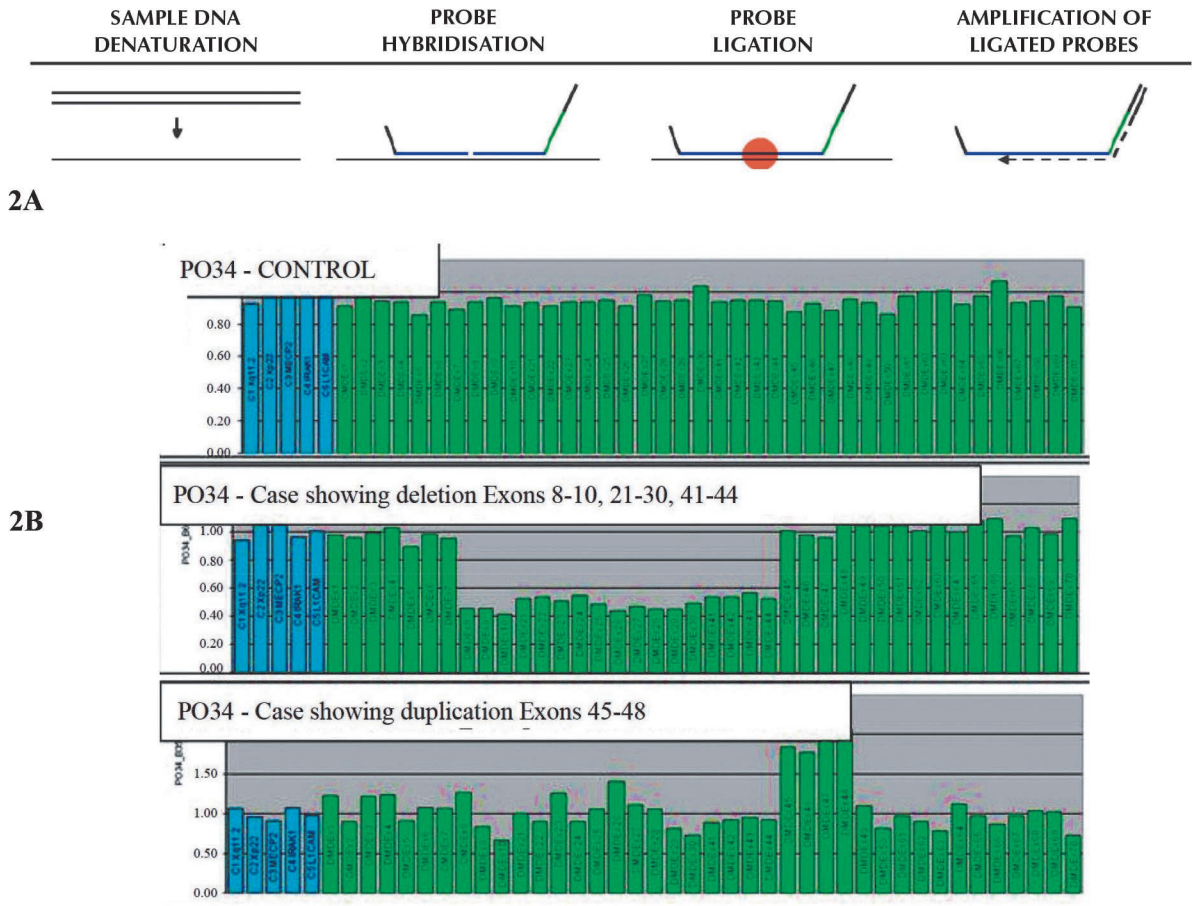


Figure 2: MLPA reaction (A) results showing deletion and duplication (B).

2A. Reproduced from MRC-Holland - Products - Details - P034 DMD mix 1
2B. Reproduced from Sakthivel Murugan S.M., et al 2013

An Update on Blood Lead Levels in Children

Dr Hafsa Majid
Chemical Pathology

Childhood lead poisoning is one of the most common and entirely preventable diseases of toxic environmental origin. It remains a problem of enormous importance for child health and development worldwide and lead poisoning accounts for about 0.6 per cent of the global burden of disease. The condition is worse especially in developing countries, as due to limited available resources there focus remains on infectious diseases. Lead plays no essential role in the human body and is ubiquitous in the environment leading to clinical and subclinical toxicity primarily through ingestion and inhalation.

The American Academy of Pediatrics and Centers for Disease Control and Prevention (CDC) recommend that blood lead screening be done of all children at the ages of approximately one and two years. The levels are evaluated according to cutoffs defined by CDC, which are now updated after every four years based on recent evidence of lead related health hazards and United States NHANES surveys reporting lead exposure in children. Recently recommended CDC action lead level of children for the period of 2012-2016 are <5ug/dl. But mounting evidence now is showing that blood lead levels (BLLs) that were considered previously to be safe

can compromise health and injure multiple organs, even in the absence of overt symptoms. Recent research indicates that lead is associated with neurobehavioural damage at blood levels of 5 µg/dl and even lower. So there appears to be no threshold level below which lead causes no injury to the developing human brain.

The gravest consequence of low level or subclinical lead toxicity in childhood is damage to the developing brain and nervous system and as the human brain has little capacity for repair, so the hazardous effects are untreatable and irreversible. The immune, reproductive and cardiovascular systems are also adversely affected by relatively low levels of exposure to lead. The Joint FAO/WHO Expert Committee on Food Additives reevaluated lead in June, 2010 and withdrew the provisional tolerable weekly intake guideline value on the grounds that it was inadequate to protect against IQ loss. Researchers are proposing even lower action lead levels then recommended by CDC and in few countries government agencies have already lowered their BLL cutoffs as well as recommendations regarding selective screening to mandatory screening for all children, for example Germany; action BLL for children are now lowered to <3.5ug/dl. Avoidance of lead exposure remains the primary preventive strategy for reducing adverse health effects of lead poisoning. So approaches to reduce lead exposure should by adopted, on local as well as public level. Exposure sources should be identified and eliminated or controlled to reduce this trace metal's environmental toxicity. Along with it action BLL should be redefined for our population and awareness should be raised regarding this issue at all levels; among public health officials, healthcare providers as well as general public.

Blood Lead Levels Testing at AKUH Clinical Laboratory

Blood lead levels are analyzed to detect and confirm exposure to lead. It may also be ordered to monitor the effectiveness of treatment and to confirm that lead levels are decreasing over time. At AKUH Clinical Laboratory lead is analysed by Graphite Furnace Atomic Absorption Spectrometry. For analysis 4 cc whole blood in Lithium Heparin tube is needed. Please note the sample should not be centrifuged or plasma must not be separated as whole blood is required.

Quiz Answers:

1. Hypophosphatemic rickets or X linked Hypophosphatemic (XLH) rickets, usually presents with typical signs of rickets in young children.

2. To confirm Hypophosphatemic rickets, as it is accompanied by hypophosphatemia and low TmP/GFR hence TmP/GFR calculation will confirm his suspicion.

3. The TmP/GFR estimate the ratio of phosphorus tubule maximum (TmP) to glomerular filtration rate (GFR), to evaluate renal phosphate transport. Ratio is calculated by following formula: Tmp/GFR= SPO4 – [(UPO4×SCr)/ UCr]

4. The XLH rickets is caused by various mutations in the PHEX gene (Xp22.1) and is transmitted as an X-linked dominant trait with complete penetrance, but variable expressivity. PHEX encodes an endopeptidase expressed predominantly in bone and teeth that regulates fibroblast growth factor 23 (FGF-23) synthesis. PHEX mutations lead to increased circulating levels of FGF-23, a phosphate-regulating hormone (phosphatonin), that leads to reduced renal phosphate reabsorption and consequently abnormal bone mineralization. Eventually there is decreased proximal renal tubular resorption of phosphate, resulting in renal phosphate wasting and hypophosphatemia due to these circulating phosphatonins. The principle phosphatonin in hereditary hypophosphatemic rickets is FGF-23.

5. 1,25-dihydroxyvitamin D should be tested. Patients with XLH have normal or low serum levels of 1,25-dihydroxyvitamin D3 (also known as calcitriol, the active form of vitamin D), despite having hypophosphatemia, which is a known stimulus of 25-hydroxyvitamin D-1-alpha-hydroxylase activity.

Meeting Report: "Les Confluences" The Society for the Study of Inborn errors of Metabolism (SSIEM) Annual Symposium 2015 in Lyon, France

Dr Noreen Sherazi
Chemical Pathologist

SSIEM is aimed to foster the study of inherited metabolic disorders and related topics. It promotes exchange of ideas between professional workers in different disciplines who are interested in inherited metabolic disease. The origin of SSIEM may be traced to an informal meeting held in England at the Manchester Royal Infirmary in 1962 when an enthusiastic group of biochemists and pediatricians met to discuss phenylketonuria.

The annual symposium of SSIEM 2015 was held in Lyon from 1st till 4th September at Lyon Convention Centre which was located at the Cité Internationale de Lyon between the Parc de la Tête d'Or and the Rhône River. The theme of the symposium was taken from the word "Les Confluences": where Rhône and Saone rivers join in French. In SSIEM, all health professionals work together and when basic research helps clinicians. This symposium was attended by two Chemical Pathologist Dr. Aysha Habib Khan and Dr. Noreen Sherazi and Metabolic Physician Dr. Bushra Afroze from Aga Khan University, Karachi. Abstract regarding frequency of selective screening of organic acidurias and amino acidopathies was selected for poster presentation and two other abstracts were published in abstract book related to quality control and proficiency testing of biochemical genetics laboratory, AKU.

The day one commenced with an attractive scientific program including informative ERNDIM meeting regarding critical errors in proficiency testing followed by Industry Sponsored Symposia on PKU, lunch and poster walk. There was opening ceremony and plenary session on "confluence of research and inborn errors" followed by a refreshing welcome reception towards the end of a hectic day.

Day two started with the parallel session on Organic acidurias/Urea cycle disorders, Amino acids disorders and Mitochondrial disorders where researchers from France, Switzerland and Italy presented their work on various topics including MMA, propionic acidurias, GA type one, OTC deficiency and mutations causing 3-methylglutaconic acidurias etc. A very informative plenary session on "Antenatal manifestations of IEMs discussed fetopathological investigations, diagnostic workup and imaging findings of IEMs in antenatal period after the lunch.

Day three had various plenary sessions on riboflavin and fatty acid oxidation, neurometabolic disorders, vitamins and cofactors and disorders of lysosomal storage. One hour industry sponsored session on "Metabolic decompensation in PA and MMA" was very practical and interactive session. Last day was

also concluded with plenary sessions, hot topics and late breaking news, Garrod award lecture on transcobalamin deficiency and closing remarks with SSIEM Rome 2016 presentation and awards.

Over all the sessions were clinical oriented and research based but as a pathologist these sessions were beneficial in gaining clinical perspective in the area of IEM as it will enhance the understanding of inherited disorders and quality of diagnostic reporting along with the future developments in the field of IEM.

Lyon is internationally known for its gastronomy, which was shared with us during the networking afternoon on day three where we had the opportunity to discover different aspects of the Capitale des Gaules, a UNESCO World Heritage site since 1998.

It was great honor to present the biochemical genetics laboratory two years data on selective screening of organic acidurias and amino acidopathies from AKUH at the international symposium of SSIEM 2015. This was first local data

of IEM diagnosed with locally available expertise in Pakistan. Not only abstract was selected for poster presentation but they have also awarded travel scholarship to Dr. Noreen Sherazi in order to attend the annual symposium 2015. This project was the collaborated efforts of Faculty of Section of Clinical Chemistry, Department of Pathology and Laboratory Medicine, Technical staff of Biochemical genetics unit and Metabolic Physician, Dr. Bushra Afroze from Pediatrics Department AKUH.

hospitals.aku.edu/Karachi/clinical-laboratories