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A NOVEL LC–MS/MS-BASED METHOD FOR THE DIAGNOSIS OF ADA2 DEFICIENCY FROM DRIED PLASMA SPOTS

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BACKGROUND-AIM
Adenosine Deaminase 2 Deficiency (DADA2) (OMIM: 607575) is a monogenic autoinflammatory disease caused by loss of function homozygous or heterozygous mutations in ADA 2 gene (previously CECR1, Cat Eye Syndrome Chromosome Region 1. A timely diagnosis is crucial to start Anti-TNF therapies that are efficacious in controlling the disease. The confirmation of DADA2 is based on DNA sequencing and enzymatic assay. It is thus very important to have robust and reliable assays that can be rapidly utilized in specialized laboratories that can centralize samples from other centers.

METHODS
In this paper we show a novel enzymatic assay based on liquid chromatography-tandem mass spectrometry that allows the accurate determination of the ADA2 enzyme activity starting from very small amounts of plasma spotted on filter paper (dried plasma spot). ADA2 activity was determined in dried plasma spots (DPS) from 44 healthy donors, 18 DADA2 patients and 4 carriers.

RESULTS
ADA2 activity, expressed as mean ± SD, was 2.63 ± 1.7 mU/mL in healthy controls, 0.02 ± 0.03 mU/mL in DADA2 patients and 0.025 ± 0.18 mU/mL in carriers.

CONCLUSIONS
The method allows to significantly distinguish healthy controls from affected patients and carriers and could be of help in implementing the diagnostic workflow of DADA2.

STUDY OF TRACE ELEMENTS AND PROTEINS IN CHILDREN WITH PROTEIN ENERGY MALNUTRITION

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BACKGROUND-AIM
WHO has described malnutrition as a “global problem”, having an adverse effect on the survival, health performance, and progression of the population group. It is highly prevalent in developing countries among children below the age of 5 years; with severe forms occurring in 1-10% and underweight observed in 20-40%. The aim of the study was to evaluate copper and zinc levels in children with protein-energy malnutrition.

METHODS
Serum zinc and copper were determined in thirty (30) malnourished pre-school-age children (age, 1-5 years) and thirty (30) age-and sex-matched apparently healthy well-nourished controls to evaluate the effect of protein-energy malnutrition on serum zinc and copper. Serum zinc and copper concentrations were estimated by the Atomic Absorption Spectrophotometer.
RESULTS
Mean serum zinc and copper concentrations were significantly reduced (p<0.05) in malnourished as compared to well-nourished children. Serum total protein concentration was significantly lower (p<0.05) in malnourished children than in the controls, and comparable (p>0.05) to those found in kwashiorkor and marasmus.

CONCLUSIONS
This study shows that malnourished children are deficient in serum zinc and copper. For an effective management of protein-energy malnutrition, zinc and copper supplementation should be part of the treatment regimen. However, in order to prevent zinc and copper deficiency and its health implications in pre-school age children, food fortification should be promoted.

METROLOGICAL EVALUATION OF THE ABBOTT OPTIUM AND ABBOTT OPTIUM NEO POCT SYSTEMS FOR THE DETERMINATION OF GLUCOSE IN NEONATAL BLOOD

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BACKGROUND-AIM
Glucose level is a critical value that requires immediate action, especially in neonatal units. Determination by POCT devices speeds up the process and reduces time.

Before implementation in a clinical unit outside the laboratory, such as neonatology, the usefulness of POCT systems should be evaluated and ratified.

The objective was the metrological evaluation of the Abbott Optium and Optium Neo POCTs and their suitability for neonatology.

METHODS
CLSI (EP7-A2) and SEQC recommendations were followed.
We studied: Imprecision (intraserial: DSis and CVis; interserial: DSid and CVid), trueness, systematic error (absolute [ES] and relative [ESrel]), uncertainty (typical [u] and expanded [U]), with MediSense 0.04% glucose control material. An accuracy study was carried out with 63 neonatal samples. Glucose values are determined by both glucometers and by reference method (hexokinase). To compare serum and whole blood concentrations, whole blood data are transformed according to recommendations. Student’s t test, absolute difference analysis (Di) and relative difference analysis (RD), regression analysis and Pearson’s r were calculated.

The ANCOVA method was used to study the influence of haematocrit (HTO).

RESULTS
Accuracy and ES:
-Optium: DSis=2.93; CVis=6.04; DSid= 4.30; CVid=8.70; ES=9.40; ESrel=23.50; u= 4.30; U=8.60; ET=32.20.
-OptiumNeo: DSis=3.99; CVis=9.58; DSid=2; CVid=4.88; ES=1; ESrel=2.50; u=2; U=4 ET=7.38

Student’s t-test: p=0.000 in both cases.
Analysis of differences:
Optium: Di 95% CI [-17; -24] and DR 95% CI [-14; -19].
Optium Neo: Di 95% CI [-8; -14] and RD 95% CI [-6; -11].
Correlation:
Optium: r=0.959.
Optium Neo: r=0.967.
Linear regression:
Optium: slope = 1.067; 95% CI [0.987; 1.148]; intercept = 13.186; 95% CI [3.409; 22.964].
Optium Neo: slope = 1.077; 95% CI [1.005; 1.150]; intercept = 2.509; 95% CI [-6.289; 11.300].
ANOVA: POCT system influences glucose score, controlling for the effect of HTO (p=0.004).

CONCLUSIONS
The study shows that the Optium Neo glucometer is more suitable for glucose measurement in neonates, and it is implemented in the unit.
Metrological evaluation is a basic step in the implementation of any new POCT method, especially in neonatology, where the sample is influenced by the HTO.

STATUS OF VITAMIN D AND VITAMIN \(B_{12}\) IN THE PEDIATRIC NEPALESE POPULATION

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BACKGROUND-AIM
Vitamin D helps in the absorption of calcium for the development of bone and teeth, as well as vitamin \(B_{12}\) is important in body growth, production of red blood cells and neurological development. Hypovitaminosis leads to improper physical and mental development in children. This study aims to determine the status of vitamin D and Vitamin \(B_{12}\) in Nepalese children.

METHODS
This study was carried out among 240 children attending the Modern Diagnostic Laboratory and Research Center, Kathmandu, Nepal. Serum Vitamin D and Vitamin \(B_{12}\) were measured by the Advia Centaur Xp immunoassay. The Shapiro-wilk test was used for the test of normality distribution. Student’s t-test, One-way Anova test, Mann-Whitney U test, and Kruskal Wallis test were used for comparison between different groups.

RESULTS
The mean Vitamin D level in the study population was 16.6±10.59 ng/dl while the median (P25- P75) was 335 (266 - 466.75) ng/L. The prevalence of hypovitaminosis D was 92.1% while hypovitaminosis \(B_{12}\) was 4.2% in the age group between (1-18) years. Females showed significantly lower Vitamin D concentrations than the males. Adolescent populations have lower vitamin D concentrations in serum than developing children.

CONCLUSIONS
The prevalence of hypovitaminosis in children was found to be higher in the study population. It leads to the loss of bone density and contributes to osteoporosis and bone fracture in children. Vitamin D supplementation in children should be targeted to minimize the risk related to bone disease.
THE ROLE OF LABORATORY BIOMARKERS IN THE DIAGNOSIS AND MANAGEMENT OF MULTISYSTEM INFLAMMATORY SYNDROME IN CHILDREN (MIS-C)

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BACKGROUND-AIM
Multisystem Inflammatory Syndrome in Children (MIS-C) associated with Coronavirus Disease (COVID-19) is a serious condition among paediatric patients, usually under 19 years, with previous exposure to SARS-CoV-2. Clinical manifestations consist of persistent fever along with weakness, abdominal pain, vomiting and/or diarrhea and skin rashes.

Although this syndrome is rare and has variable expressivity, some children can evolve from hypotension and cardiogenic shock to multiple organ dysfunction. Laboratory abnormalities include elevation of acute phase reactants and myocardial dysfunction; similar to those observed in Kawasaki disease or toxic shock syndrome, requiring a differential diagnosis.

METHODS
An 11-year-old male attended the emergency department due to fever (>38°C) of 6 days of evolution, myalgias, abdominal pain and maculopapular lesions, after being with a COVID-19 positive friend a week ago. No other medical history. RT-PCR respiratory viruses (SARS-CoV-2, RSV, H. influenzae), blood and urine cultures: negative.

RESULTS
Elevation of inflammatory markers (C-reactive protein 282 mg/L, ferritin 1103 ng/mL, IL-6 152 pg/mL, fibrinogen 667 mg/dL, PCT 6.56 ng/mL); cardiac markers (NT-proBNP 966 pg/mL); D-dimer 1120 ng/mL and renal and liver function tests (creatinine 1.04 mg/dL and GPT 44 U/L). Decrease in sodium (130 mEq/L), albumin (3.5 g/dL), lymphopenia (0.52×10³/µL) y thrombopenia (71×10³/µL). RT-PCR was repeated for SARS-CoV-2 resulting negative, but positive IgG antibodies.

CONCLUSIONS
Given the suspicion of MIS-C, he was admitted to the Paediatric-ICU and conservative fluid therapy and antibiotic therapy (cefotaxime) were initiated. Intravenous immunoglobulin and methylprednisolone were also administered, along with respiratory support. After 9 days hospitalized, heart, renal and liver functions progressively improved, he became afebrile and was discharged.

Since MIS-C symptoms are not specific, the interest of this case lies in the importance of laboratory results for its diagnosis and in the fact that biochemical findings correlate with clinical manifestations of the syndrome. Although the etiological relationship is not yet completely understood, most patients have positive IgG serology and increased inflammatory markers suggesting immune dysregulation by SARS-CoV-2.
VARIATION OF THE SERUM N/GLYCOsyLATION DURING THE PREGNANCY OF A MPI-CDG PATIENT

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BACKGROUND-AIM

Congenital Disorders of Glycosylation (CDG) are a rapidly expanding family of rare inborn errors of metabolism. The first cases were reported 40 years ago (Jaeken et al. 1980) and today more than 130 different CDG have been reported (Jaeken 2020). MPI-CDG is characterized by a deficiency in mannose-6-phosphate isomerase due to autosomal recessive mutations in the MPI gene coding for the phosphomannose isomerase (Niehues et al. 1998). As a consequence, the biosynthesis of GDP-mannose and the lipid-linked oligosaccharide (LLO) precursors pools, necessary for the biosynthesis of N-glycans, are reduced leading to a defect of N-glycosylation (Ichikawa et al. 2014). MPI-CDG is mainly characterized by hepatic fibrosis, enteropathy (Pedersen and Tygstrup 1980), hyperinsulinism (de Lonlay et al. 1999), venous thrombosis, and bleeding episodes with an absence of neurological symptoms (Damen et al. 2004). MPI-CDG is the one of the only CDG where clinical manifestations are improved by oral D-mannose supplementation (Westphal et al. 2001). Symptoms usually improve with age, and normal pregnancies have already been observed in MPI-CDG patients (Helander et al. 2014; Girard et al. 2020). We studied the glycosylation of a MPI-CDG patient during pregnancy without mannose supplementation using three different methods.

METHODS

For the first time the glycosylation of a patient with a MPI-CDG during pregnancy without mannose supplementation is monitored using the carbohydrate deficient transferrin (CDT) assay, transferrin isoelectrofocusing (IEF), and mass spectrometry of total serum N-glycans.

RESULTS

A general improvement of the glycosylation profile of the patient due to a better transfer of the glycan precursors as well as an increase of the triantennary glycans (and sialylation) was observed.

CONCLUSIONS

In the absence of mannose supplementation, the previously observed glycosylation abnormality of the MPI-CDG patient was corrected. The molecular mechanism underlying this N-glycosylation rescue during MPI-CDG pregnancy further needs to be investigated.

HYPERTENSION, HYPOKALAEMIA AND METABOLIC ALKALOSIS IN A NEONATE

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BACKGROUND-AIM

Liddle syndrome is an autosomal dominant disorder arising from mutations of the genes encoding for the alpha, beta and gamma epithelial sodium channel (ENaC) subunits. This leads to refractory hypertension, hypokalaemia, metabolic alkalosis, hyporeninaemia and hypoaldosteronism through over-activation of the ENaC.
We report on an 11-year-old male of Ethiopian descent who at the age of 5 days was admitted to the neonatal ICU with severe dehydration and acute renal failure that resolved following treatment. He subsequently developed uncontrolled hypertension requiring readmission and was managed on multiple antihypertensive drugs and potassium supplements, with successful blood pressure control achieved following addition of amiloride.

METHODS
Electrolyte measurements were performed using ion selective electrodes in an Abbott Architect ci8200 instrument. Molecular testing was undertaken in this patient. All the exons and exon-intron boundaries of the alpha- [SCNN1A (GenBank NM_001038.5)], beta- [SCNN1B (GenBank NM_000336.2)] and gamma- ENaC (epithelial sodium channel) subunits’ [SCNN1G (GenBank NM_001039.3)] genes were amplified by PCR (New England Biolabs, Ipswich, MA, USA). Fragments were sequenced using the Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 according to the manufacturer’s instructions.

RESULTS
Biochemistry test results revealed hypokalaemia, hypernatraemia and metabolic alkalosis associated with suppressed aldosterone and renin concentrations. Initial genetic testing, limited to c.1815G>A (pR563Q) of the SCNN1B gene, was negative. Further testing involved sequencing of SCNN1A and SCNN1B and revealed compound heterozygous mutations in each subunit. The c.1000G>A and c.1987A>G mutations in SCNN1A were detected, while the c7G>A and c.1325G>T were identified in SCNN1B.

CONCLUSIONS
The clinical and laboratory features were in keeping with Liddle syndrome, a rare disease that can be easily missed or overlooked in paediatric patients. To our knowledge this is the youngest patient diagnosed with Liddle syndrome, and the first study of its kind in which compound heterozygous mutations resulted in severe disease.

THIOL/DISULFIDE HOMEOSTASIS IN MEDICATION-NAIVE CHILDREN AND ADOLESCENTS WITH OBSESSIVE–COMPULSIVE DISORDER

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BACKGROUND-AIM
Obsessive–compulsive disorder (OCD) causes significant psychic distress and affects children’s social and academic functioning. Approximately 80% of OCD cases begin in childhood. Earlier onset is associated with more severe obsessive compulsive symptoms, poorer treatment response, and a more unfavorable clinical course. A particular oxidative stress marker, thiol/disulfide homeostasis, using a new, comparatively inexpensive, easily calculated, easily accessible, repeatable, and fully automated method was investigated between pediatric patients diagnosed with OCD and a healthy control group in this study.

METHODS
This study is the first to address this subject in pediatric patients with OCD and aims to contribute to our knowledge of the etiopathogenesis and treatment of pediatric OCD. The study included children with OCD (n = 35, 52.2%) (drug free, comorbidity free) between 11 and 18 years of age and age- and sex-matched healthy controls (n = 32, 47.8%). K-SADS-PL DSM-5 and the
Children’s Yale–Brown Obsessive–Compulsive Scale (CY-BOCS) were administered, and a sociodemographic data form was completed for all children. Serum native and total thiol levels were assessed by the automated colorimetric method (Rel Assay Diagnostics, Turkey).

**RESULTS**

The total thiol \( (p = 0.025) \) and disulfide \( (p = 0.001) \) levels and the disulfide/native thiol \( (p = 0.001) \) and disulfide/total thiol ratios \( (p = 0.001) \) were significantly different between the groups. Also, in the patient group, biochemical analysis revealed that the disulfide level \( (p = 0.05) \) and the disulfide/native thiol \( (p = 0.034) \) and disulfide/total thiol ratios \( (p = 0.039) \) differed significantly according to the presence of a family history of psychiatric disorders.

**CONCLUSIONS**

The results of our study show that thiol/disulfide homeostasis may affect the etiopathogenesis of oxidative stress in pediatric OCD patients. Moreover, these preliminary findings point to increased oxidative stress levels in pediatric OCD patients with family histories of psychiatric disorders.

**AN ANALYTICAL AND DIAGNOSTIC COMPARISON OF A GC-MS AND LC-MS/MS ANALYSIS APPLIED IN THE IDENTIFICATION OF AMINO ACID-RELATED INHERITED METABOLIC DISEASES**

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**BACKGROUND-AIM**

The timely, efficient diagnosis of inherited metabolic diseases (IMDs) via “hyphenated” mass spectrometry applications has become standard practice. The aim of this study was to compare a gas chromatography-mass spectrometry (GC-MS) based amino acid analysis with an automated liquid chromatography tandem mass spectrometry (LC-MS/MS) application in the diagnosis of amino acid-related IMDs. This included comparing the analytical time and diagnostic capacity of the two methods by analyzing 14 anonymized urine samples (provided by an external proficiency scheme) of patients with confirmed amino acid IMDs.

**METHODS**

The EZ:faast™ GC-MS kit for amino acids was validated and utilized in the manual preparation of specimens which were subsequently analyzed on a 6890 GC coupled to a 5973 MS (Agilent Technologies). The MassChrom® Amino Acid kit was utilized to validate an automated sample preparation by using the Hamilton Microlab STAR liquid handling robot prior to analysis on LC-MS/MS (Agilent 1290 Infinity II coupled to Agilent 6470 LC/TQ-ESI). The preparation times per batches (an average of 40 samples, diagnosed with various amino acid-related disorders as well as other non-amino acid-related IMDs, a negative control, a blank and calibrators) were compared (as shown in figure 1) and diagnostic accuracy were assessed for the anonymized patient samples.

**RESULTS**

The average preparation time per batch prior to GC-MS and LC-MS/MS analysis was 120 minutes and 20 minutes, respectively, with less sample volume required for the automated method (25 μl for LC-MS/MS compared to 100 μl for GC-MS). The instrument acquisition time was 12 minutes for the GC-MS method and 20 minutes for the LC-MS/MS application. With regard to diagnostic proficiency, 13 of the 14 amino acid related conditions could be identified via LC-MS/MS. The GC-MS application could identify 8 of the same 14 conditions as this method could not accurately measure amino acids aberrations associated with citrullinemia type 1, ornithine transcarbamylase deficiency, cystinuria, homocystinuria, HHH syndrome, molybdenum cofactor deficiency and hypophosphatasia.
CONCLUSIONS

Although the LC-MS/MS acquisition time was longer, the application proved to be superior to the GC-MS analysis with regard to diagnostic accuracy and the option to automate with a reduced sample volume. The LC-MS/MS approach is ideal from a diagnostic perspective due to its high throughput capacity.

Figure 1: Dual experimental design of amino acid analysis on GC-MS and LC-MS/MS of anonymised samples

BURNING MUSCLE – A CLINICAL CASE OF HEATSTROKE WITH RHABDOMYOLYSIS

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BACKGROUND-AIM

Heatstroke is a life-threatening injury requiring neurocritical care, characterized by central nervous system dysfunction, multiorgan failure, and extreme hyperthermia, in the setting of exposure to hot weather or extreme physical exertion.

AIM: Case report presentation.

METHODS

CLINICAL REPORT: A 16-year-old boy was admitted in the Paediatrics Emergency Room for malaise, tiredness and increase of his dyskinesia crisis with 1-day evolution, after spending a day at a river beach. The past history comprised psychomotor development retardation, and movement disturbance with dyskinetic crises. He had been hospitalized twice with dyskinetic crises complicated by acute kidney injury (AKI) and rhabdomyolysis. Clinical observation revealed dehydration, high fever and upper limb dyskinesia. Laboratory analysis of the patient’s blood revealed concentrations of leucocytes 35,4x10^9/L, creatinine (Cr) 1.61mg/ dL, creatine kinase (CK) 4549U/L, troponin T (TnT) 32ng/L, hypernatremia and hypokalaemia. A SARS-CoV-2 RT-PCR test was negative. Being diagnosed with hypernatremic dehydration and prerenal AKI owing to a heatstroke with rhabdomyolysis, an aggressive fluid therapy was inducted.

RESULTS

The next day, despite the good clinical evolution and the resolution of dyskinesias, the patient was still proposed for hospitalization due to persisting critical blood test results. On the 2nd day of hospitalization, in spite of the surveillance and the aggressive fluid therapy support, there was an abrupt clinical and analytical worsening (Cr 3.89 mg/dL; Urea 125 mg/dL; CK >200,000 U/L; TnT >823 ng/L; lactate dehydrogenase 7,712 U/L; elevated transaminases; having developed cardiogenic and disruptive shock there was the need for invasive ventilation, and the progressive worsening of the AKI...
demanded continuous venovenous hemodialysis. The patient was transferred to the intensive care unit, where he still is 15 days after admission.

CONCLUSIONS
This clinical case illustrates the importance of an accurate and prompt response from the Clinical Pathology Laboratory when facing extremely high values which require successive dilutions. The accuracy of these results is essential for the patient’s follow up. As Pathologists, our role is to keep updated and adapt to the needs of the patients and clinicians even in the most difficult situations.

DIAGNOSIS OF CYSTIC FIBROSIS IN INDIAN CHILDREN: EXPERIENCE OF SWEAT CHLORIDE ESTIMATIONS FROM A SINGLE CENTRE

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BACKGROUND-AIM
Cystic fibrosis (CF) is an inherited disorder. Because of its varied presentation involving lungs, pancreas, and reproductive organs, it appears in the differential diagnosis of many pediatric diseases. Diagnosis of CF by consensus criteria includes identification of clinical features or positive family history and laboratory confirmation by identification of genetic mutations or positive sweat chloride results (two separate occasions). Over 1500 mutations in the CFTR gene make molecular diagnosis difficult. In Indians, the commonest mutation (ΔF508) occurs in lower frequency, and also rare and novel mutations exist. Sweat chloride analysis is therefore a handy alternative tool for diagnosis. CF is not as very rare as thought and is probably under-diagnosed in Indian children. In this paper, we report our experiences with sweat chloride analysis in children of the eastern region of India using a low cost indigenous method.

METHODS
Analysis was performed in infants and children (since June 2012) and rarely in symptomatic adults. Briefly, steps involve pilocarpine iontophoresis on the arms/legs of the patient for inducing local sweat production, collection in pre-weighed filter paper (minimum 100 mg) for 30 mins, estimation of chloride by titration with mercuric chloride. The method has a sensitivity of 10 mEq/L. Values of > 60 mEq/L are likely to be diagnostic of CF. The method has side effects such as inadequate collection, and minor burns in a minority of patients. Precautions to prevent contamination or evaporation of sweat, prevents false positives.

RESULTS
In our cohort of 956 patients (564 males) with a median age of 4 years and 4 months (range: 29d – 51 years), an average sweat collection of 170 ±4 mg was reported. Inadequate collection (164/956) and burns (20/956) were recorded. The common causes for referral were recurrent cold/ cough with respiratory distress, recurrent pneumonia, failure to thrive, pancreatitis and history of meconium ileus. 88/956 patients were diagnosed with CF (two positive sweat chloride findings). Gene analysis of 72 common mutations in 25 cases revealed that ΔF508 mutation, though the most common, occurred in a low frequency in our population.

CONCLUSIONS
Availability of sweat chloride analysis serves as an invaluable tool to rule out CF. A low cost method adopted in our centre diagnoses CF in children from various socioeconomic backgrounds. This may eventually help to treat and manage CF and avoid severe malnutrition and early mortality due to delayed diagnosis.
BIOCHEMICAL AND LIVER BIOMARKERS MONITORING IN CHILEAN HEREDITARY TYROSINEMIA TYPE-1 PATIENTS UNDER NTBC TREATMENT AND PROTEIN-RESTRICTED DIET

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BACKGROUND-AIM
Tyrosinemia type 1 (Tyr-1) is an inborn error of metabolism caused by defects in tyrosine metabolism and characterized by accumulation of tyrosine and the toxic metabolite, succinylacetone (SA). Treatment of Tyr-1 patients is based on nitisinone (NTBC) administration and tyrosine- and phenylalanine-restricted diet. To prevent liver complications such as hepatocarcinogenesis, permanent monitoring of NTBC concentration in blood, SA levels, alpha-fetoprotein (αFP) and liver function biomarkers are recommended, to be measured in each clinical control, by the current consensus guidelines. Also, the routine monitoring of these parameters allows to improve the NTBC dosage in the patients. The aim of present study was to evaluate the optimal therapeutic range of NTBC in Tyr-1 Patients.

METHODS
In a one-year evaluation we retrospectively analyzed the following parameters: NTBC levels (plasma and DBS), urinary SA, liver biomarkers and amino acids levels in 43 samples from fifteen Tyr-1 patients.

RESULTS
First, we globally observed the status of each laboratory parameter allowing us to describe general adherence to treatment and look for associations between treatment and pathophysiological biomarkers. We found that 45% tested samples were within the recommended range for blood NTBC concentration being the median for all samples of 21.3 μmol/L. Eighty-nine percent of samples showed a SA value <0.5 mmol/mol creatinine. We established a conversion factor for NTBC concentration in plasma and DBS samples of 2.57 and determined an optimal range of NTBC management in DBS that could hinder SA excretion and maintain αFP levels under controlled levels. This optimal target range for NTBC concentration in DBS was established within 14.5-24.9 μmol/L. Also, we explored a possible correlation of NTBC range levels with nutritional follow-up parameters (tyrosine and phenylalanine) and liver biomarkers, showing that for αFP, NTBC concentrations trends to be negatively associated.

CONCLUSIONS
Our observational study revealed that a lower target range for NTBC in DBS than previously described would be optimal to maintain controlled biochemical and liver parameters. We encourage the use of DBS samples for monitoring of NTBC in Tyr-1 patients to facilitate metabolic control and ensure an adequate management and prognosis of disease.

FUNDAMENTAL ROLE OF THE CLINICAL LABORATORY IN DETECTION OF HOMOZYGOUS HEMOGLOBIN S (HBS) AS A CAUSE OF SICKLE CELL DISEASE

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BACKGROUND-AIM
Hemoglobinopathies are monogenic diseases, with autosomal recessive inheritance, that affect the structure or synthesis of globin chains of hemoglobin. Specifically, hemoglobin S results from the substitution of valine for glutamic acid at position 6 of
β-globin. This mutation results in HbS with reduced solubility and, when PO2 falls, it precipitates deforming the red blood cells, becoming them “sickle”, more fragile and rigid, which occludes microcirculation.

Heterozygous people (sickle cell trait) are asymptomatic and have normal blood count values due to the presence of HbA. In contrast, homozygous HbSS (sickle cell anemia) is characterized by severe hemolytic anemia, which appears within a few months of birth when HbS replaces fetal Hb, usually increased helping to decrease sickle cell effects. These patients present acute exacerbations with vaso-occlusive, aplastic and hemolytic crises, acute chest syndrome and increased susceptibility to infections. HbS can also be combined with other variant hemoglobins. For laboratory diagnosis, it is advisable to use 2 different methods. Treatment mainly consists of antibiotic prophylaxis and vaccination, with administration of hydroxyurea to increase HbF. Hematopoietic stem cell transplantation is the only effective therapy, but due to the associated risks it is restricted to critically ill patients.

METHODS
Biochemical analysis was performed on autoanalyzers Cobas-8000 (Roche®) and complete haemogram on autoanalyzers with flow cytometry SysmexXN-10®. Hemoglobin variants study is extended by High Performance Liquid Chromatography (HPLC, D-10® Bio-Rad).

RESULTS
We present the case of an one-year-old female from Nigeria that goes to the emergency center for fever and vomiting of 36 hours of evolution.

In the complete blood count, these highlights were observed: hemoglobin, 8.3 g/dL [reference interval 11-14], MCV, 73.4 fL [73-91], MCH, 23.2 pg [24-31] and reticulocytes, 9.3% [0.6-2.1]; platelets and leukocytes values were within the reference range. Examination of erythrocyte morphology in peripheral blood smear (May-Grünwald-Giemsa stain) shows a marked anisopoikilocytosis, target cells and abundant sickle cells (Figure 1).

In the serum biochemistry, these highlights were observed: an increase in LDH, 516 U/L [120-300], a decrease in haptoglobin 0.25 g/L, [0.3-2.0], with bilirubin and the rest of iron profile within the reference range. Glucose-6-phosphate dehydrogenase activity is normal at 410.8 mU/10^9 erythrocytes [221-570], dismissing its deficiency as a cause of hemolytic anemia. HPLC results show: a variant HbS of 75% [0], elevated HbF, 22.4% [<1] and normal HbA2, 2.6% [2.2-3.7] with absence of HbA. The presence of homozygous HbS is confirmed by capillary electrophoresis (Capillarys-2® Sebia) revealing a migration peak in zone 5(S) of 78%. Therefore, the diagnosis of homozygous hemoglobinopathy S or sickle cell anemia is confirmed. The parents were studied, both presenting heterozygous HbS.

CONCLUSIONS
Therefore, although HbS has a higher incidence in Africa, the Middle-East and the Mediterranean basin, migration has forced methods for detecting hemoglobinopathies. Neonatal screening is essential for a correct diagnosis, early treatment and genetic counseling, with the Clinical Laboratory playing a crucial role. In this case, since the girl was born in a country where newborn screening is not available, it could not be detected earlier. However, thanks to the complete blood count analysis, the peripheral blood smear and the hemoglobin fractions, it was possible to detect this case of sickle cell anemia allowing its subsequent treatment.

EPSTEIN-BARR VIRUS-INDUCED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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BACKGROUND-AIM
Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening syndrome provoked by excessive immune activation. It most frequently affects infants, but the disease is also observed in all ages. HLH can be a primary (Medelian
inherited condition) or secondary (apparently non-Medelian inherited condition) disorder triggered by a variety of
events that disrupt immune homeostasis as: infections, malignancies and autoimmune or autoinflammatory
disorders.

In agreement with that, HLH can be a rare complication of Epstein-Barr virus (EBV) infection. EBV-HLH carries a high
mortality rate, however quick diagnosis and appropriate treatment can subside the disease. Clinicopathological char-
acteristics of the syndrome are clinically fever, splenomegaly, cytopenia, histological evidence of hemophagocytosis and
extremely high serum levels of ferritin, lactate dehydrogenase (LDH) and soluble CD25 (sCD25) or soluble interleukin-2
(IL-2) receptor.

METHODS

A fourteen-year-old girl is admitted to the hospital with high fever and mild jaundice. During examination splenomegaly
and cytopenias are evident. It also highlights extremely high levels of ferritin and sCD25. LDH, bilirubin, creatinine,
procalcitonin, lactate, transaminases and triglycerides are also elevated. On the other hand, microbiology service informs
a positive EBV-PCR in plasma and bone marrow.

RESULTS

According to laboratory results and after having discarded other causes of hepatic failure, the patient is diagnosed of
EBV-HLH and is admitted to intensive care unit where is included in HLH treatment protocol.

Ciclosporin-A, as other immunomodulators, has strong activity against proliferation of cytotoxic T/NK-cells and mac-
rophages, and it also has an inhibitory activity against the cytokine storm that induces and maintains HLH. Unfortu-
nately, despite the treatment with Ciclosporin-A the patient deceased after some days.

CONCLUSIONS

HLH diagnosis is a medical emergency owing to its high morbimortality rates. Due to that, a rapid diagnose and a correct
treatment to diminish hyperinflammatory state is essential.

The laboratory has a very important role in the diagnosis and monitoring of that disorder with many parameters as ferritin
and CD25 and cytomorphologic bone marrow studies.
RESULTS

74 neonates were identified, 54% were girls. In terms of maturity, 41% were preterm, 35% were term and the rest had no data. For gestational weight/age adequacy, 34% were underweight, 50% were adequate weight, 1% were macrosome and the rest had no data.

Culture was positive in 56 cases (76%). Of these, 46% were blood cultures, 20% urine cultures, 12.5% catheter tip cultures, 12.5% umbilical exudate, 7% stool cultures and 2% others.

The microbiological agent identified was *S. epidermidis* (48%), *E. coli* (30%), *K. pneumoniae* (14%) and minority others (8%).

Laboratory values were: PCT = 4.4 ng/mL (SD: 11.4); CRP = 12.4 mg/L (SD: 25.8); leukocyte count = 17032 /μL (SD: 7872) and polymorphonuclear cells (PMN) = 54 % (SD: 17).

Student’s t was significant (p<0.017) for PCT, with a mean difference of 10.9 ng/mL and a 95% confidence interval (95%CI) of (2.1-19.8). The area under the curve (AUC), with 95% CI95 was: AUCPCT = 0.788 (0.598-0.978), AUCPCR = 0.571 (0.409-0.733), AUCLeucocytes = 0.496 (0.332-0.660) and AUCPMN = 0.627 (0.475-0.779). ROC analysis showed differences for PCT (p<0.05).

CONCLUSIONS

Our casuistry shows a higher sensitivity of culture (75%). The most frequent germ (*S. epidermidis*) differs from other series (more common *E. coli*).

PCT is the only biomarker with diagnostic validity in sepsis, with a Se of 73.4% and a PPV of 95.7%.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY PREDISPOSES TO OXIDATIVE STRESS AND HYPERBILIRUBINEMIA IN NEWBORNS

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BACKGROUND-AIM

Glucose-6-phosphate dehydrogenase deficiency (G6PDD), the most common enzyme deficiency in humans is an inborn error of metabolism. This study is to determine the involvement of Glucose-6-phosphate dehydrogenase deficiency as a factor that predisposes red blood cell to lipid peroxidation and oxidative stress causing a spectrum of diseases including neonatal hyperbilirubinaemia.

METHODS

Glucose-6-phosphate dehydrogenase status was determined using the methaemoglobin reduction method while venous blood samples were collected from 25 Glucose-6-phosphate dehydrogenase deficient newborns (G6PDD). Also, venous blood samples were collected from 25 newborns that were not Glucose-6-phosphate dehydrogenase deficient (G6PD) to serve as control. Oxidative indices (Superoxide dismutase, Malnoaldehyde, Glutathione peroxidase and Total bilirubin) were determined in the venous blood using spectrophotometric techniques.

RESULTS

The results shows that there were significant reductions in blood mean ± standard error of mean values of Superoxide dismutase and Glutathione peroxidase (4.20±0.17 IU/L and 82.32IU/L) in G6PDD when compared with G6PD (8.97±0.25 IU/L and 142.30 IU/L). Also, for malonaldehyde peroxidase and total bilirubin, the results show that there were significant increases in blood mean ± standard error of mean values in G6PDD (1.68±1.03 IU/L and 164.32μmol/L) when compared with G6PD (1.09±0.05 IU/L and 80.14 μmol/L).
CONCLUSIONS
The implication of the result is that Glucose-6-phosphate dehydrogenase deficiency especially in neonates promotes lipid peroxidation and oxidative stress thereby making red blood cell more susceptible to haemolysis as indicated by hyperbilirubinaemia in G6PDD.

MATERNAL AND NEONATAL VITAMIN B_{12} DEFICIENCY DETECTED THROUGH EXPANDED NEWBORN SCREENING IN SOUTHERN SPAIN

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BACKGROUND-AIM
When undiagnosed, infant vitamin B_{12} deficiency can result in anemia, failure to thrive, developmental regression and neurological deficits. It is most commonly caused by maternal vitamin B_{12} deficiency. Biochemically, vitamin B_{12} deficiency leads to an accumulation of total homocysteine (tHcy), methylmalonic acid (MMA), and propionylcarnitine (C_{3}). Although vitamin B_{12} deficiency is not a primary target of expanded newborn screening (NBS) programs, markers for methylmalonic and propionic acidemias (C_{3} and C_{3}/C_{2}) may identify vitamin B_{12}-deficient newborns.

Causes of maternal vitamin B_{12} deficiency include adherence to a diet that excludes or has limited amounts of animal products, pernicious anemia, and previous gastric bypass. Unrecognized neonatal vitamin B_{12} deficiency worsens if the infant is breastfed without vitamin B_{12} supplementation. Clinical presentation of vitamin B_{12} deficiency is often nonspecific which can lead to a delay in diagnosis and treatment. Irreversible neurologic damage results from prolonged vitamin B_{12} deficiency; however, the extent and degree of disability depends on the severity and duration of the deficiency. The aim of this study is to describe the cases detected with suspected vitamin B_{12} deficiency over 10 years in our NBS program.

METHODS
Amino acid and acylcarnitine levels were determined from single dried blood-spot samples from 368,152 newborns, between April 2010 and December 2018, using tandem mass spectrometry (MS/MS) and a commercial reagent kit (MassChrom, Chromsystems, Germany). A new sample was requested if there was an increase in C_{3} and/or C_{3}/C_{2}, having previously established the cutoff point at p99.9 of the healthy population (C_{3}<3.87 µmol/L, C_{3}/C_{2}<0.17). All cases with persistently high levels were studied further, both mother and child, evaluating CBC, acylcarnitines, homocysteine and vitamin B_{12} levels in plasma, and organic acids in urine samples. Mothers were also tested for gastric parietal cells (GPC) and intrinsic factor (IF) serum antibodies.

RESULTS
Increased C_{3} and/or C_{3}/C_{2} levels were persistently elevated levels in 84 cases. Further biochemical studies of these showed: 69 vitamin B_{12} deficiencies, 3 inborn errors of vitamin B_{12} metabolism (1 newborn with TCBlR defect and 2 sisters with TCN1/CUBN defect) and 12 false positive cases. The ratio C_{3}/C_{2} (64/69) was a more sensitive marker than C_{3} (31/69) for the detection of vitamin B_{12} deficiency.

Most newborns were exclusively breastfed at diagnosis (50/69). One of the mothers had previously undergone a partial gastrectomy and only one mother was strict-vegetarian. 24 cases of probable maternal pernicious anemia were detected (anti-GPC titer over 1:80 and/or anti-IF antibody positive). Newborns of mothers with pernicious anemia had a significantly more severe deficit than the rest of newborns (tHcy: 40.6 µmol/L vs 16.6 µmol, p<0.0001; urine MMA: 321 mmol/mol Crea vs 68 mmol/mol Crea, p=0.01). All confirmed cases (children and mothers) were treated with oral or intramuscular vitamin B_{12} and did not present significant hematological or neurological complications during follow-up.
CONCLUSIONS
Identification of newborns with nutritional vitamin B12 deficiency (with a high frequency in our population of 1:5,335) is an additional benefit of NBS programs. The sensitivity of MS/MS for NBS screening vitamin B12 deficiency is still unknown, but the inclusion of the ratio C3/C2 as a primary marker increases the program’s sensitivity. If a deficiency is suspected, then both the mother and the infant should be promptly evaluated for vitamin B12 deficiency. Maternal screening for pernicious anemia is cost effective as it identifies the cause in 35% of cases, which are also the most serious. NBS programs should consider newborns diagnosed with confirmed vitamin B12 deficiency to be true-positive cases.

MAPLE SYRUP URINE DISEASE (MSUD): A CASE REPORT DETECTABLE BY NEWBORN SCREENING

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BACKGROUND-AIM
Maple Syrup Urine Disease (MSUD) is an inborn error of metabolism caused by a deficiency of the branched-chain-2-keto acid dehydrogenase (BCKDH) leading to the accumulation of branched-chain amino acids (BCAA) leucine, isoleucine, valine and alloisoleucine (pathognomonic markers). Clinical manifestations are predominantly neurological, starting with feeding difficulties, progressing to lethargy and coma. This autosomal recessive disease can be detected by newborn screening (NBS), allowing its early detection before clinical features are present and reducing irreversible sequelae for the patient.

METHODS
MSUD NBS is based on the quantification of BCAA by tandem mass spectrometry (HPLC-MS/MS). The MS/MS method used does not differentiate isobaric amino acids (leucine, isoleucine and alloisoleucine); therefore, confirmation with HPLC is necessary. Urine organic acid analysis (GS-MS) supports the diagnosis.

RESULTS
We report the case of a newborn of 39 weeks gestation who was admitted to the pediatric-ICU due to altered NBS with elevated BCAA (Xle 1282.6 µM [<240], Val 686.1 µM [<237]) and informative ratios (Xle/Phe=24 [<4.2]; Xle/Ala=6 [<1]; Val/Phe=12.8 [<4.2]) was suspicious for MSUD. No family history of MSUD was noted, the child had non-consanguineous parents, and no alteration in behaviour was observed. A plasma sample was analysed, confirming, by HPLC, elevations of concentrations of leucine, 2792.1 nmol/mL [<150], alloisoleucine, 582.4 nmol/mL and ammonium, 93 µmol/L [<35]. Elevated urinary organic acids specific to MSUD were also found: 2-OH- isovaleric, 2-ketoisocaproic, 2-keto-3-methylvaleric; along with ketone bodies and lactic acid. At the onset of neurological symptoms (hypertonia and irritability), with leucine levels being the most neurotoxic BCAA, the child was treated with carnitine, thiamine, dietary protein restriction and hemodiafiltration. Molecular genetic study showed a BCKDHA mutation.

CONCLUSIONS
The patient was monitored by controlling plasma BCAA until levels as close as possible to normal values were achieved. After discharge, this type of patient must follow lifelong semisynthetic diet with reduced BCAA intake and periodic controls. However, thanks to laboratory analysis, the amino acid disorder could be detected before the neonate was symptomatic. This case supports the importance of including MSUD in NBS programs.
OPTIMIZATION OF ALPHA-GALACTOSIDASE A ENZYME ACTIVITY ASSAY

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BACKGROUND-AIM
Identification of deficient alpha-galactosidase A enzyme activity in dried blood spots (DBS) is the most efficient and reliable method of newborn screening for Fabry disease. The incidence of Fabry disease is estimated at 1:50,000 males. Laboratories engaged in screening for lysosomal storage diseases carry out testing by measurement of enzymatic activities in DBS using either tandem mass spectrometry or fluorimetry. The aim of the study was optimization of the fluorimetric assay for alpha-galactosidase A activity.

METHODS
Blood was collected onto filter paper and allowed to dry. For long-term storage, the DBS samples were stored at -20°C and protected from moisture. Collection and handling of all samples were consistent with the Declaration of Helsinki of the World Health Organization.

A filter paper disk with a diameter of 3.2 mm was punched from each DBS sample, placed in a 96-well microplate and incubated with 50 μl of 0.1 M acetate buffer (pH 4.5) containing 2.5 mM 4-methylumbelliferyl-α-d-galactopyranoside and 0.1 M N-acetylgalactosamine for 18 h at 37°C. To stop the reaction, 150 μl of 0.2 M glycine-carbonate buffer (pH 10.5) was added. The samples were centrifuged at 1000 x g for 2 min at 4°C. Fluorescence (excitation 365 nm; emission 450 nm) was measured on a Wallac 1420 Multilabel Counter (Victor-2), PerkinElmer. Readings were corrected for blanks, and compared with a 4-methylumbelliferone calibration curve. Data are presented as mean±SD.

RESULTS
Measured alpha-galactosidase A activity in DBS from patient with Fabry disease was lower than the detection limit of the assay. There was no overlap among the results of Fabry patient (n=1; not detected activity) and controls (n=60; 21,7±9,4 μmol/ l/h).

For an accurate measurement of fluorescence, a transparent solution is needed. The signal to noise ratio could be improved by centrifugation of a 96-well microplate. This is a reason for the lower rate of screen false-positives using the modified fluorimetric assay.

CONCLUSIONS
The present modification of fluorimetric method could be used for the screening of Fabry disease.

ESTABLISHMENT OF REFERENCE INTERVALS FOR SERUM CREATININE IN PAKISTANI CHILDREN USING A DATA MINING APPROACH

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BACKGROUND-AIM
Substantial gaps regarding the influence of age, sex, and race on Serum Creatinine (Cr) activity exist. However, the ethical and practical challenges due to physiological dynamics with age restrict the establishment of pediatric reference intervals (RIs) using direct approaches. Data mining of laboratory information systems has been identified as a solution to these limitations. The objective of this study is to establish RIs for Cr in Pakistani children using an indirect data mining approach.
METHODS
This study was conducted at the Section of Clinical Chemistry, Department of Pathology and Laboratory Medicine, Aga Khan University Hospital (AKUH) in collaboration with the Department of Pediatrics, University Hospital Erlangen, Germany. Cr was analyzed on Siemens Advia 1800 analyzer using the rate-Jaffe reaction. For both inpatients and outpatients aged 1 to 17 years who presented between January 2013 and December 2018, including patients from intensive care units and specialty units, creatinine concentration data were retrieved from the laboratory information system. RIs were calculated using a previously validated indirect algorithm developed by the German Society of Clinical Chemistry and Laboratory Medicine’s Working Group on Guide Limits.

RESULTS
96,104 samples were analyzed during the study period. After exclusion of patients with multiple samples during the study period, RIs were calculated for 22,966 males and 18,525 females with stratification into fine-grained age groups. These RIs demonstrate the complex age- and sex related dynamics in Cr during physiological development.

CONCLUSIONS
The population specific RIs serve to allow an accurate understanding of the fluctuations in analyte activity with increasing age and aid clinical decision making.

DATA MINING TO CONSTRUCT CLINICALLY RELEVANT AND CONTINUOUS PERCENTILES FOR BIOCHEMICAL MARKERS IN CHILDREN

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BACKGROUND-AIM
Adequate reference intervals are relevant to minimize clinical uncertainty based on erroneous classification of laboratory data. However, obtaining sufficient sample sizes from healthy voluntary individuals for establishing valid reference intervals is particularly challenging in pediatric populations. Here we demonstrate how routine laboratory data enable construction of continuous reference percentiles that compare favourably with published conventional reference intervals.

METHODS
Anonymized blood sample test results requested by primary health care physicians were extracted from our laboratory database for several biochemical markers that vary with age in childhood. Duplicate identities were removed, the chronologically first result per individual was retained and likely outliers were excluded by ROUT-algorithm during polynomial nonlinear regression, as implemented in GraphPad Prism 9.2. Reference percentiles were modelled using the LMS algorithm provided in the ‘gamlss’ package in R.

RESULTS
Continuous LMS-percentile-curves constructed from routine laboratory data and recalculated continuous reference intervals from The Nordic Reference Interval Project (NORIP) cohort are presented with overlays of published reference upper and lower limits (Fig. 1). Agreement between the models was generally good. The application of continuous reference percentiles in the same way as in standard pediatric growth charts allows to adequately account for covariation with age, especially for parameters with substantial age-dependent dynamics.
CONCLUSIONS
Data mining provided comparable reference limits to arbitrarily age-partitioned nonparametric reference intervals. Continuous reference percentiles allow for quantitative and longitudinal benchmarking of patient blood test result in terms of z-scores, adjusted for both sex and age. The clinical utility of this framework reduces the potential for missing pathological results as well as for unnecessary follow-up of biochemically healthy children due to misclassifications arising from discretely age-partitioned reference intervals.

A NOVEL PIPELINE FOR THE ESTIMATION OF CONTINUOUS PEDIATRIC REFERENCE INTERVALS USING REAL-WORLD DATA

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BACKGROUND-AIM
Reference intervals are essential for the interpretation of laboratory test results in medicine. Especially in pediatrics, many analytes show pronounced dynamics during physiological development from birth to adulthood. To adequately assess pediatric test results, continuous reference curves are needed. However, ethical and practical challenges restrict the creation of age-dependent reference intervals using population-based methods. While indirect methods allow the establishment of reference intervals using test results obtained during patient care, available algorithms do not account for continuous change with age. Here, we propose an automated data-mining approach leveraging routine measurements to establish continuous reference intervals and percentile charts for biomarkers of interest.
METHODS
We developed a pipeline for the generation of continuous reference curves utilizing real-world data and a recently published indirect method (refineR) in combination with generalized additive models for location, scale and shape (GAMLSS). First, the input data is divided into very fine-grained subgroups, i.e., one group for each day of age. Second, we apply the indirect method to each group and use the estimated model to assign weights to each input data point, which reflect a data point’s probability of originating from a physiological sample. Finally, we use GAMLSS to create continuous reference curves. The presented pipeline was applied to three important biomarkers with extensive pediatric dynamics (hemoglobin, alkaline phosphatase, and creatinine) using data obtained during patient care.

RESULTS
The calculated percentile charts for hemoglobin, alkaline phosphatase (Fig. 1B) and creatinine from birth to 18 years of age are in accordance with previously established reference intervals demonstrating that the presented automated pipeline correctly models age-dependency and generates valid continuous reference curves.

CONCLUSIONS
The presented pipeline enables the generation of precise percentile charts using real-world data. Percentile charts accurately capture the pronounced age-dependent dynamics that occur in many biomarkers, especially in neonates and during puberty, facilitating the interpretation of test results and ultimately improving patient care.

Figure 1 A) Novel pipeline for the generation of continuous reference intervals using real-world data. B) Age-dependent percentile chart for alkaline phosphatase (ALP) for boys, calculated using the presented pipeline. The chart shows the 2.5th and 97.5th percentiles (i.e. the reference interval; red lines), the 10th and 90th percentiles (orange lines), the 25th and 75th percentiles (green lines) and the 50th percentile (median; blue line). To account for the stronger dynamics in infants, the x-axis is scaled in months during the first year of life and afterwards in years.

IMPLEMENTING A NEW METHOD FOR MEASUREMENT OF AMINO ACIDS IN BLOOD PLASMA

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BACKGROUND-AIM
Amino acids (AA) are an important biomarker for detecting and monitoring diseases called aminoacidopathies and for monitoring the dietary compliance and nutritional status of patients. Due to the considerable age of the system we use and the frequent need for its repair, a switch to a new method was needed.
METHODS
Our current method includes a Series 200 HPLC (Perkin Elmer, USA) coupled with a 3200 Qtrap® (AB Sciex, Canada) tandem mass spectrometry system (MS/MS) and a TRAQ kit (Sciex, USA) for sample preparation. This method was compared with the ACQUITY UPLC I-class system coupled with a Xevo®-TQ-S micro MS/MS system (Waters, USA). Samples were prepared with the MassChrom® Amino Acid Analysis in plasma/serum (Chromsystems, Germany) kit. We assessed agreement and correlation between methods by analyzing plasma of 166 pediatric patients and using the Bland Altman method and a linear regression model respectively to analyze results. To assess precision and accuracy we analyzed proprietary controls and calculated relative standard deviation (RSD) and relative error (RE), respectively. The calculations were based on comparison of measurements of 14 AA. The cut-off values for acceptability of parameters were 15% for bias, RSD or RE, and the value of $R^2$ was set at 0.70.

RESULTS
The agreement between methods was sufficient for all except lysine (Lys) (-26.4%) and threonine (Thr) (-20.3%). Correlation was high, with measurements for 7/14 AA having $R^2$ above 0.90, 4/14 having $R^2$ above 0.80, and 3/14 having $R^2$ above 0.73. Precision was sufficient, except for Lys in low concentration range (22.5% RSD). The accuracy of the new kit is sufficient except for arginine measured in the low concentration range (-20.2%).

CONCLUSIONS
Results obtained with both methods correlate well and the agreement between them is sufficient, with the exception of Lys and Thr, for which new reference ranges were calculated. Precision and accuracy of the new method are sufficient. The discrepancies in both parameters can be explained by lower concentrations, as small differences in measurements result in higher RSD or RE, without having an impact on the interpretation of the results. The new kit requires no derivatization of AA; furthermore, a calibration is performed with each run, improving its accuracy. After adjusting reference values, the new method is suitable for routine use.

HIGH RESOLUTION MASS SPECTROMETRY METABOLOMICS COMBINED WITH MACHINE LEARNING IS A USEFUL APPROACH FOR RISK STRATIFICATION IN NEUROBLASTOMA

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BACKGROUND-AIM
Neuroblastoma (NB) is the most common extra-cranial malignant tumor in children and its outcome is unfavorable in a high percentage of cases. Risk assessment at diagnosis is crucial to address patients to different treatment protocols.

METHODS
We investigated the ability to characterize NB patients by means of High-Resolution Mass Spectrometry (HRMS)-based metabolomic data in combination with Machine Learning techniques. Plasma samples from 60 NB patients at the onset of disease (30 high risk and 30 low risk NB) and paired controls were subjected to metabolomic analysis combining different analytical approaches, using a Vanquish Horizon UHPLC System coupled to an Orbitrap Q-exactive Plus Mass Spectrometer (Thermo Scientific, Milan, Italy). Accurate mass retention time database libraries were used for accurate compound identification. Machine learning techniques were used to discover the most important metabolites capable of predicting the clinical risk.
RESULTS
Our methodology was able to distinguish NB patients from healthy subjects and to stratify them based on the
differential expression of a hundred metabolites. Key metabolites of L-DOPA catabolism were identified in both low
risk and high-risk NB patients, as well as other metabolites involved in several different metabolic pathways.

CONCLUSIONS
The results of our work demonstrate, for the first time, that HRMS metabolomics has the potential to characterize NB
patients starting from a small (50 uL) amount of plasma.

CANNABIDIOL DETERMINATION IN PERIPHERAL BLOOD USING VOLUMETRIC
ABSORPTIVE MICROSAWMING (VAMS) AND LC-MS/MS: A USEFUL TOOL
FOR TDM OF EPILEPSY PATIENTS

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BACKGROUND-AIM
Interest in cannabis-based therapies has recently increased, due to the availability of cannabidiol (CBD) for the treatment of
epilepsy without psychoactive effects. Therapeutic drug monitoring can prevent drug interactions and minimize drug
toxicity. The aim of this work is to evaluate volumetric absorptive microsampling (VAMS) from capillary blood as an
alternative strategy for therapeutic drug monitoring (TDM) in patients treated with the newly available GW-puri
fied form of cannabidiol (Epidiolex®).

METHODS
A fast ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) coupled to an online
sample preparation system analysis was carried out on a Thermo Scientific Ultimate 3000 LC system coupled to a TSQ
Quantiva triple quadrupole for the quantification of cannabidiol (CBD) and, in addition, delta-9-tetrahydrocannabinol
(D9-THC). After validation using European Medicine Agency (EMA) guidelines, the method was applied to samples
obtained by finger prick of five pediatric patients treated with Epidiolex® for Dravet syndrome and the results were
compared to those obtained from venous blood and plasma.

RESULTS
The method is linear in the range of 1–800 ug/L for both CBD and THC with intra- and inter-day precisions ranging from
5% to 14% and accuracies from -13% to +14% starting from 30 uL of sample. Stability in VAMS is ensured for up to 4 weeks
at 25°C thus allowing simple delivery. There was no difference (p = 0.69) between concentrations of CBD measured from
VAMS sampled from capillary or venous blood (range: 52.19–330.14 or 72.15–383.45 ug/L) and those obtained from
plasma (range: 64.3–374.09 ug/L).

CONCLUSIONS
This proof-of-concept study suggests that VAMS allows monitoring of CBD plasma levels and can offer valuable support
for personalized therapy in refractory epilepsy.
CAN BILIRUBIN BE DETECTED IN URINE OF NEWBORNS WITH JAUNDICE? – A PILOT STUDY

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BACKGROUND-AIM
Community surveillance for neonatal jaundice (hyperlbilirubinemia) is crucial to ensure early recognition and treatment to prevent the neurological complications associated with increased bilirubin concentration. Current available diagnostic and screening tools are resource-intensive and expensive, limit their use in resource-constraint settings. Increased access to low-cost, simple to use point-of-care devices is in the global health priorities to improve newborn health. New insights gained on bilirubin transporters in bilirubin metabolism speculate that a proportion of bilirubin is excreted via the renal system. Urine is a preferred non-invasive sample collection method, and hence, this study aims to determine if bilirubin can be detected in the urine of newborns.

METHODS
Urine and blood samples were collected from a convenience sample of healthy newborns aged < 72 hours at a tertiary hospital’s postnatal ward. Laboratory measurement of urine bilirubin levels was determined using the Liquid chromatography Triple Quadrupole Mass Spectrometry method. Serum bilirubin levels were determined by the hospital core laboratory based on absorption spectrometry, using the BuBc slide of the Vitros Ortho Clinical Diagnostics instrument.

RESULTS
A total of twenty-two newborns were recruited to this pilot study. Seventeen newborns were of gestational age greater than 38 weeks, whereas five were of the early-term category (GA 37-37+6/7 weeks) with an average birth weight of 3.4 Kg. Urine samples tested for bilirubin demonstrated a clear peak for unconjugated bilirubin (UCB) similar to the bile standards used, indicating the presence of UCB in urine with a mean concentration of (8±5) nmol/L. Unconjugated serum bilirubin concentrations ranged from 96 to 290 μmol/L. As the sample size for this pilot study was low, no significant association was established between serum and urine bilirubin concentration.

CONCLUSIONS
This study demonstrates that bilirubin can be detected in the urine of newborns. Further validation of the diagnostic value of urine bilirubin in detecting jaundice using a large sample size is underway to determine the correlation between serum bilirubin and urine bilirubin to develop a urine screening test for hyperbilirubinemia in newborns.
PREVALENCE AND OUTCOME OF CONGENITAL DISORDERS AMONG NEONATES ADMITTED IN AN ACADEMIC HOSPITAL IN ENUGU, NIGERIA

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BACKGROUND - AIMS

Congenital abnormalities (CA) are structural, behavioral, functional and metabolic disorders that occur during intrauterine life and can be identified prenatally, at birth or later in infancy. They have been implicated in the increase in neonatal morbidity and mortality in low-income countries like Nigeria. However, there is poor documentation of these abnormalities in Nigeria. Hence, the aim of this study is to assess the prevalence and mortality rate of congenital disorders in neonates in Enugu, Southeastern Nigeria.

METHODS

This study is a retrospective cross-sectional study. The data was gathered from the records of 285 neonates admitted in the Newborn Intensive Care unit of the University of Nigeria Teaching Hospital Enugu from January 2019 to January 2020. However, 16 out of the 285 were excluded from the study due to incomplete records, leaving 269 (145 males and 124 females) as the study population. The prevalence of various abnormalities and mortality rates were calculated in percentages.

RESULTS

Out of the 269 neonates, 48 (17.8%) had CA with males accounting for the greater percentage (11.5%) while the females accounted for 6.3%. The mortality rate among those with CA was 16.7% (n=8) and mostly males were affected (12.5%; n=6). The most frequent of the 30 different CA recorded was hypoxic ischemic encephalopathy (HIE) (18.8%). However, the overall major cause of admission was preterm birth which accounted for 35% (n=94) followed by neonatal jaundice 14.9% (n=40) while the least frequent cause was low birth weight 7.8% (n=21). Out of the 269 neonates, 49 (18.3%) died (32 males, 17 females), the greater number of them were preterm (42.9%) while the least were those with neonatal jaundice (2%). In neonates with sepsis, the highest mortality rate (9 out of 35; 25.7%) was recorded, followed by preterm newborns (21 out of 94; 22.3%).

CONCLUSION

Prevalence of CA is relatively high with HIE being the most common form. Mortality was recorded mostly in male neonates. There is need to find out the risk factors for these CA prevalent in this study population and to create awareness on ways to reduce them. It is important also to emphasize on the importance of antenatal care looking at the alarming prevalence and mortality rates of neonatal sepsis and prematurity because they are avoidable risk factors.

HAEMOGLOBIN GENOTYPE VARIANTS AMONG SUBJECTS IN ENUGU, SOUTHEAST NIGERIA

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BACKGROUND - AIMS

There has been an increase in sickle cell awareness campaign and obligatory premarital test in Enugu, all in the bid to reduce the frequency of inherited abnormal haemoglobin (Hb). Inherited abnormal haemoglobin is one of the most common gene disorders among Africans; in Nigeria the most common is Sickle Cell disorder. Hence this study evaluates
the frequency of Hb genotype variants to assess the impact of the measures put in place to reduce the frequency of sickle cell disease in Enugu.

METHODS
This is a retrospective cross sectional study using data from two reference laboratories in Enugu (Special Hematology Laboratory of University of Nigeria Teaching Hospital and Spectrum Biomedical Laboratory). The results of all the Hb genotype screening from January 2019 to December 2019 on subjects aged 1-18 years were extracted and analyzed. The subjects comprised of three hundred and fifteen subjects (163 males and 152 females).

RESULTS
Two hundred and three out of 315 (64.4%) subjects were HbAA, 34.3% HbAS, 0.3% HbAC, 1.0% HbSS and 0% HbSC. However, the majority (66.7%) of subjects with HbSS were males. More subjects with HbAA (51.2%) were females while (48.8%) were males. In the group of subjects with HbAS, there were more males (57.4%) than females (42.6%). Also a greater percentage of the subjects with HbSS was observed for males (66.7%) compared to the females (33.3%). The frequency of HbSS was lower in this study group when compared to 5.5% recorded in Ibadan, South Western Nigeria but similar to 1.5% recorded in Uyo Akwa- Ibom, South South Nigeria.

CONCLUSION
The frequency of HbSS in Enugu was low and males were more affected. However, the 34.3% prevalence of HbAS is a high risk that if not taken care of may influence the incidence of HBSS. Therefore, there is need to strengthen the campaign for premarital Hb genotype screening for couples. Also, more awareness should be created to renew people’s consciousness on the burden of sickle cell disease to health.