Metabolomics in Newborns

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Contents

Abstract

Metabolomics is the quantitative analysis of a large number of low molecular weight metabolites that are intermediate or final products of all the metabolic pathways in a living organism. Any metabolic profiles detectable in a human biological fluid are caused by the interaction between gene expression and the environment. The metabolomics approach offers the possibility to identify variations in metabolite profile that can be used to discriminate disease. This is particularly important for neonatal and pediatric studies especially for severe ill patient diagnosis and early identification. This property is of a great clinical importance in view of the newer definitions of health and disease. This review emphasizes the workflow of a typical metabolomics study and summarizes the latest results obtained in neonatal studies with particular interest in prematurity, intrauterine growth retardation, inborn errors of metabolism, perinatal asphyxia, sepsis, necrotizing enterocolitis, kidney disease, bronchopulmonary dysplasia, and cardiac malformation and dysfunction.

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1. INTRODUCTION

The primary target of neonatology is to guarantee the health care of newborns using all the available resources to increase the survival rate of all especially those with critical conditions. In this scenario, different areas of research emerged with the focus of better understanding the pathogenic mechanisms of disease [1]. This research has enabled the adoption of new standards in early diagnosis, promoted the development of adequate and affordable therapies, as well as improved prognosis. An important scientific contribution in this endeavor comes from the field of metabolomics, defined by Nicholson *et al.* as the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiologic stimuli or genetic modification [2]. Characterization of the metabolic profile is achieved by measurement of metabolites produced in response to external stimuli or internal messages. According to Nicholson, metabolomics is a web which connects genetics and environment and evaluates the complete biological profile of a living system during its metabolic modifications over time. As can be expected, metabolites can be found in a wide variety of biologic fluids including blood or cord blood, saliva, urine, cerebrospinal fluid, amniotic fluid (AF), and feces. The development of specialized analytical platforms such as ^{1}H-nuclear magnetic resonance (^{1}H NMR), gas chromatography (GC), and liquid chromatography (LC) coupled with mass spectrometry (MS) is paramount to provide a comprehensive metabolic snapshot of the specimen at that specific moment in time [3]. Metabolites are molecules with a unique structure and biologic role. These include carbohydrates, amino acids, small peptides, fatty acids, lipids, purines, pyrimidines, as well as other organic molecules. Metabolomics is classified based on the approach used [4–6]. Targeted metabolomics is a study in which a specific class of molecules is investigated. Untargeted metabolomics is a study to identify as many metabolites as possible and compare them between classes of samples. The latter provides a global overview of metabolism; however, identification of unknown molecules remains a major challenge [7–9].

2. DATA ANALYSIS IN METABOLOMICS

Following testing, data analysis needs to be performed in order to describe the dataset produced by the metabolic investigation. The techniques used to decode high-throughput metabolomics data are essentially

adapted from other omic approaches. Univariate methodologies, such as ANOVA or Wilcoxon test, are frequently used to show the greatest response of the measured metabolites among several conditions [10]. Unfortunately, univariate methods fail to differentiate among groups if there are slight variances in single molecule level. Therefore, the use of multivariate statistical analysis (MVA) captures modification of single metabolites and identifies the correlation between the most important molecules. The most used MVA techniques in metabolomics are principal component analysis (PCA) and partial least squares regression (PLS) including discriminant analysis (PLS-DA).

PCA is a statistical method for element reduction through an orthogonal transformation. It will compress the original features to new relevant random vector. It is an unsupervised method and can be used to identify specific structures in a dataset such as clusters, anomalies, or trends that exist between observations [11]. The analyzed data are projected along directions that allow the maximum possible variance, ie, the principal components. The first principal component is defined by the set of variables that describe most of the variance. The second describes the main component orthogonal to the first. However, most of the predictive models rely on the supervised method. By far, the two most popular methods for supervised pattern recognition include PLS-DA combined with orthogonal signal correction and logistic regression [12,13]. DA is useful to determine the number of relationships between grouping variables and feature variables as well as establish discriminant function that will facilitate discrimination between groups. PLS-DA selects different types of sample data to build a training set model which can then be used to predict the category about unknown samples. From a supervised analysis it is possible to find the set of important variables on the projection (VIP). These variables can be considered the main metabolites responsible for separation among the groups. However, identification and interpretation of metabolomic results are difficult. Although statistical tools such as PCA and DA support biomarker discovery, VIP may be responsible for separation among groups but may not necessarily be most suitable as biomarkers. Thus, the study of the metabolic correlation networks seems an interesting approach to improve visual investigation of datasets and identification of new biomarkers [14]. Because metabolic pathways constitute a chain of reactions occurring in an organism, analysis of these pathways is indicated graphically as nodes which symbolize compounds and edges which symbolize reactions. By studying metabolic networks for each group of samples, associations among VIP can be found.

This is a crucial step for identification of routes connecting the compounds of interest within the metabolic pathways. Because a large number of routes can exist between two compounds, a huge number of VIP are needed. Currently, several metabolic databases are available that help for reconstruction of metabolic pathways such as MetaMapR; however, such databases are far from being the norm for metabolomic studies [15].

3. METABOLOMICS IN NEONATOLOGY

Metabolomics represents an important tool in biomedical research because it offers new approach for studying individual response to pharmacologic stimuli, thus leading to personalized therapy. It is also possible to compare physiologic and pathophysiologic metabolites to define limits between these conditions and intercept abnormal metabolic trajectories before irreparable or fatal consequences. Time is a precious resource in neonatal medicine especially in the intensive care unit setting. Metabolomics provides new data which may influence clinical intervention in acute cases. Similarly, these data may enable early assessment of metabolic alterations that potentially lead to long-term pathologic consequences. Barker was the first to hypothesize that alterations during fetal life may increase the rate of development or likelihood of chronic disease in adults [1]. Life constitutes a biological system in constant evolution with different stimuli that imprint and influence physiologic processes. As an investigative tool, metabolomics provides a perfect opportunity to effectively explore this hypothesis between early life and later disease development. The most common disorders in neonatology include prematurity, intrauterine growth retardation (IUGR), neonatal sepsis, necrotizing enterocolitis (NEC), bronchopulmonary dysplasia (BDP), perinatal asphyxia, cardiac malformation and dysfunction, kidney diseases (KDs), and metabolic screening. Each is a daily challenge for neonatologists. A better understanding of the biochemical processes inherent to these conditions will be a step toward improving life expectancy in these children (Table 1).

3.1 Prematurity

The World Health Organization has defined preterm infants as those born before 37 weeks or 259 days of gestation [50]. Because this is a generic definition, classification which distinguishes preterm infants by gestational age has been developed [51]. Neonates born between 32 and 33 weeks of gestation are considered moderate/mild preterm. The ones born at 34–36 of

Table 1 Discriminant Metabolites

Disease	Study	Patients	Sample	Method	Metabolites
Prematurity	Menon *et al.* [16]	25 Preterm vs 25 term	Amniotic fluid	LC-MS/ GC-MS	Increased: progesterone, pantothenol, bile acids, androsterone sulfate Decreased: squalene, lathosterol, cortisol, cortisone, hydroxypregnenolone disulfate, pregnanediol disulfate, androsteroid monosulfate, estriol-3-sulfate
	Graça *et al.* [17]	17 Preterm	Amniotic fluid	UPLC-MS	Increased: hexose(probably glucose) Decreased: leucine/isoleucine, histidine, methionine, phenylalanine, valine
	Tea *et al.* [18]	8 Preterm vs 8 controls	Arterial and venous umbilical cord blood	^{1}H NMR	Increased: formate, glutamine, glutamate Decreased: HDL, VLDL, LDL, pyruvate, glucose, acetone, albumin-lysyl, glucose (venous umbilical cord plasma), alanine, tyrosine, valine, isoleucine, leucine, threonine, and 3-methyl-histidine (arterial umbilical cord plasma)
	Romero *et al.* [19]	33 Preterm vs 40 term	Amniotic fluid	GC-MS/ LC-MS	Increased: urocanic acid, dulcitol, 1-methyladenine, butanoic acid, beta hydroxyphenylethylamine, vitamin B6, salicylamide, oleic acid Decreased: alanine, galactose, urea, pyroglutamic acid, 3-hydroxybutanoic acid, proline, palmitate, glycine, mannose, octadecanoic acid, glutamine, inositol, butanedioic acid
	Graça *et al.* [20]	12 Preterm	Amniotic fluid	^{1}H NMR	Increased: allantoin Decreased: myoinositol, alanine, citrate

Continued

Table 1 Discriminant Metabolites—cont'd

Disease	Study	Patients	Sample	Method	Metabolites
IUGR	Barberini *et al.* [21]	23 IUGR vs 10 controls	Urine	GC-MS	Increased: inositol
	Dessì *et al.* [22–24]	12 IUGR vs 17 controls	Urine	^{1}H NMR	Increased: myoinositol, creatinine, creatine, citrate, urea, glycine
	Sanz-Cortés *et al.* [25]	23 Early IUGR and 56 late IUGR vs 79 controls	Umbilical vein blood plasma	^{1}H NMR	Increased: glutamine, creatine (early IUGR) Decreased: phenylalanine, tyrosine (early IUGR), valine, leucine (late IUGR)
	Cosmi *et al.* [26]	8 IUGR twins vs 16 controls	Umbilical vein blood plasma	LC-HRMS	Increased: phenylalanine, sphingosine, glycerophosphocholine, choline Decreased: valine, tryptophan, isoleucine, proline
	Favretto *et al.* [27]	22 IUGR vs 21 controls	Cord blood	LC-HRMS	Increased: proline, valine, isoleucine, glutamate, methionine, dopamine, phenylalanine, tryptophan, histidine, uric acid, caffeine, 5-methyl-2-undeceno acid, L-thyronine, hexadecanedioic acid Decreased: kynurenine
	Dessì *et al.* [28]	26 IUGR vs 30 controls	Urine	^{1}H NMR	Increased: myoinositol, sarcosine, creatine, creatinine
Inborn errors of metabolism	Reinecke *et al.* [29]	140 Infants	Urine	GC/MS	Characterizing metabolites: 255 endogenous and 46 exogenous related to carbohydrate, amino acids, and fatty acids
	Sahoo *et al.* [30]	235 IEM infants	Dried blood samples	LC-MS/MS	Characterizing metabolites: related to acylcarnitine, fatty acid oxidation metabolism, electron transport chain, inosine monophosphate synthesis, pyrimidine metabolism, tricarboxylic acid cycle, and glycolysis.

	Aygen *et al.* [31]	989 Infant	Urine	^{1}H NMR	Characterizing metabolites: 2-oxoisovaleric acid, 2-hydroxyisovaleric acid, 2-hydroxyphenylacetic acid, 2-oxoisocaproic acid, 2-hydroxyisocaproic acid, 2-phenyllactic acid, 2-hydroxybutyric acid, 3-hydroxyglutaric acid, 3-hydroxyisovaleric acid, 3-hydroxypropionic acid, 3-hydroxyvaleric acid, 3-methyl-2-oxovaleric acid, 3-methylcrotonylglycine, 3-phenyllactic acid, 4-hydroxyphenylacetic acid, 4-hydroxyphenyllactic acid, 5-aminolevulinic acid, acetoacetic acid, acetone, allo-isoleucine, citrulline, D-galactonic acid, D-sorbitol, E-glutaconic acid, ethylmalonic acid, galactitol, glutaric acid, isovalerylglycine, L-leucine, L-isoleucine, L-pyroglutamic acid, methylmalonic acid, *N*-acetylaspartic acid, neopterin, orotic acid, phenylacetic acid, L-phenylalanine, phenylpyruvic acid, propionic acid, propionylglycine, uracil, uridine, valine, and xanthine
Perinatal asphyxia	Chu *et al.* [32]	254 Neonates	Urine	GC-MS	Increased in asphyxia with good outcome: ethylmalonate, 3-hydroxy-3-methylglutarate, 2-hydroxyglutarate, 2-oxoglutarate Decreased in asphyxia with poor outcome: glutarate, methylmalonate, 3-hydroxybutyrate, orotate

Continued

Table 1 Discriminant Metabolites—cont'd

Disease	Study	Patients	Sample	Method	Metabolites
	Walsh *et al.* [33]	31 HIE infants vs 40 asphyxiated infants without encephalopathy vs 71 controls	Cord blood	LC-MS/MS	Metabolites characterizing asphyxia: glycerophospholipids and taurine Metabolites characterizing HIE: alanine, asparagine, isoleucine, methionine, phenylalanine, proline, tyrosine, valine
	Reinke *et al.* [34]	25 HIE infants vs 34, asphyxiated infants without encephalopathy vs 59 controls	Cord blood	^{1}H NMR	Increased: acetone, betaine, creatinine, glucose, 3-hydroxybutyrate, and *O*-phosphocholine
	Fanos *et al.* [35–38]	3 Infants	Urine	GC/MS	Characterizing metabolites: glycine, valine, maleic acid, and sorbitol
	Longini *et al.* [39]	14 Neonates	Urine	^{1}H NMR	Increased: threonine and 3-hydroxyisovalerate, lactate, glucose, TMAO; Decreased: acetate, succinate, dimethylamine, citrate, dimethylglycine, creatine, creatinine, betaine, cis aconitate, trans aconitate, urea, formate
Sepsis	Dessì *et al.* [22–24]	1 Fungal septic newborn vs 13 controls	Urine	GC-MS	Increased: *N*-glycine, D-serine, L-threonine, D-glucose, maltose Decreased: citric acid, hexadecanoic acid, octadecanoic acid

	Fanos *et al.* [35–38]	9 Septic newborns vs 16 controls	Urine	^{1}H NMR/ GC-MS	Increased: lactose, maltose, glucose Decreased: ribitol, ribonic acid, pseudouridine, 2,3,4-trihydroxybutyric acid, 2-ketogluconic acid, 3,4-dihydroxybutanoic acid, and 3,4,5-trihydroxypentanoic acid
	Mickiewicz *et al.* [40]	60 Septic newborns vs 40 controls	Serum	^{1}H NMR	Increased: lactate, glucose, creatinine, 2-oxoisocaproate, 2-hydroxyisovalerate, 2-hydroxybutyrate Decreased: threonine, acetate, 2-aminobutyrate, adipate
	Fanos *et al.* [41,42]	12 HCMV septic newborns vs 11 controls	Urine	^{1}H NMR	Increased: myoinositol, glycine, 3-hydroxybutyrate, 3-aminoisobutyrate, creatine, taurine, betaine
NEC	Morrow *et al.* [43]	11 NEC preterm vs 21 controls	Urine	^{1}H NMR	Increased: alanine, histidine Decreased: alanine, histidine
	Ioannou *et al.* [44]	17 NEC preterm vs 24 controls	Plasma	HPLC	Decreased: citrulline
	Garner *et al.* [45]	6 NEC vs 7 controls	Feces	HS-SPME-GC-MS	Decreased: VOCs, 2-ethylhexyl acetic ester, decanoic acid ethyl ester, dodecanoic acid ethyl ester, hexadecanoic acid ethyl ester
Kidney diseases	Beger *et al.* [46]	40 Children	Urine	LC-MS/ MS	Characterizing metabolites: homovanillic acid sulfate

Continued

Table 1 Discriminant Metabolites—cont'd

Disease	Study	Patients	Sample	Method	Metabolites
	Atzori *et al.* [47]	21 Children affected by nephrouropathies vs 19 controls	Urine	^{1}H NMR	Characterizing metabolites: alterations of amino acids, purine and pyrimidine, and the citric acid cycle
	Fanos *et al.* [35–38]	3 Infants	Urine	GC/MS	Characterizing metabolites: glucose, asparagine, gluconic acid, aspartic acid, ornithine, and lysine
Bronchopulmonary dysplasia	Lussu *et al.* [48]	1° Group: 6 BDP newborns vs 13 controls 2° Group: 3 BDP newborns vs 9 controls	Urine	^{1}H NMR	Characterizing metabolites: glycine, ascorbate, citrate, lactate
	Fanos *et al.* [35–38]	18 BDP newborns vs 18 controls	Urine	^{1}H NMR	Increased: lactate, taurine, TMAO, myoinositol Decreased: citrate, gluconate
	Carraro *et al.* [49]	40 Adolescent ex BDP patients	Exhaled breath condensate	Orbitrap LC/MS	Increased in ex BDP: lysophosphatidylcholine, platelet-activating factor, the unsaturated phosphatidylcholines, and plasmenyl-phosphatidylserine Decreased in BDP: hydroxyeicosapentaenoic acid

gestation are considered late preterm. Very preterm births are infants born at a gestational age between 28 and 31 weeks. Those born earlier than 28 weeks of gestation are defined as extremely preterm. A recent survey has identified that increased preterm labor is a problem in several countries [52]. In the United States the preterm birth rate is 12–13%, while in Europe and other developed countries it is 5–9%. Preterm delivery remains a major contributor of neonatal mortality (75%) and morbidity (high risk of neurodevelopmental impairment, respiratory, and gastrointestinal complications). However, the survival rate has grown for neonates born at 25 and 26 weeks of gestation (survival rate for 26 weeks: 78%, http://www.epicure.ac.uk/overview/survival/) [53]. The condition of prematurity is related essentially to three etiological risk factors: (1) medically induced preterm birth; (2) maternal complications (pregnancy hypertension and vascular disorder, medical acute illness or chronic conditions, obstetrical complication, antepartum bleeding, maternal age >35 years, multiple pregnancies); and (3) fetal problems (intrauterine growth restriction, unstable fetal condition, fetal anomaly). In 2014, Menon *et al.* [16] reported on spontaneous preterm delivery (<34 weeks) using metabolomics to identify differences with term births. The study was performed in African Americans, an ethnic group particularly at risk of preterm birth. Using AF as the source material, the study showed that the liver was one of the main organs affected by preterm birth. Metabolites of interest included those associated with xenobiotic detoxification, CoA metabolism, steroids, fatty acids, and inflammation markers. Gestational age effects were also noted in bile acid metabolites, likely reflecting maternal liver involvement.

Twenty years earlier, Bock *et al.* [54] analyzed AF collected from pregnant females of different gestational ages and different maternal-fetal complications. Using NMR spectroscopy, several metabolites, choline, glucose, glycine, and lactate, were associated second and third trimester pregnancy. A metabolic fingerprint was reported by Graça *et al.* [20,55], who analyzed AF collected from pregnant women who had preterm delivery and those with preterm premature rupture of membranes. The presence of alanine, allantoin, citrate, and myoinositol characterized the former, whereas glutamine, methionine, and threonine characterized the latter group.

In a subsequent study, Graça *et al.* [17] evaluated AF and urine from women in the second semester of pregnancy who delivered preterm. LC/MS analysis revealed decreased amino acids (leucine and isoleucine, methionine, histidine, valine, and phenylalanine) and increased hexose in women with preterm labor. These results may reflect the key role that

the placenta plays in amino acid transport. Substantial contributions to AF metabolomic profiling came from research work of Romero *et al.* [19,56], who conducted two retrospective cross-sectional studies in three groups of pregnant females with spontaneous preterm labor and intact membranes. The first group delivered at term, the second delivered preterm without intraamniotic infection/inflammation (IAI), and the third delivered preterm with IAI. Using metabolomics, the accuracy in establishing patients in each clinical group was 96.3%. In the subsequent study, the rate was 88.5%. Together, these studies provided the framework for creation of a preliminary human AF metabolome.

Metabolic differences in urine have also been examined in term and preterm infants. Using ^{1}H NMR, Atzori *et al.* [56] reported that hippurate, tryptophan, phenylalanine, malate, tyrosine, hydroxybutyrate, *N*-acetylglutamate, and proline discriminated these groups. Tea *et al.* [18] examined maternal-fetal alterations in metabolic exchange mediated by placenta. Plasma was collected immediately after birth from umbilical vein, umbilical artery, and maternal blood of mothers delivering very low birth weight and controls who delivered normoponderal full-term neonates. ^{1}H NMR revealed a clear distinction between maternal and cord plasma of all samples by PCA. Maternal plasma from mothers in the former group had decreased acetate and increased lipids, pyruvate, glutamine, valine, and threonine. Both arterial and venous cord plasma of females with very low birth weight babies had decreased lipoproteins, glucose, pyruvate, and albumin. The simple and less-invasive mode of specimen collection allows for potential intervention to improve maternal-fetal management of preterm risk.

3.2 Intrauterine Growth Retardation

The term intrauterine growth retardation (IUGR) indicates a fetus which fails to reach its potential growth, ie, having a birth weight below the 10th percentile for gestational age and an abdominal circumference under the 2.5th percentile [57]. This condition affects 4–8% of neonates born in industrialized countries, but 6–30% in developing countries [58–60]. In addition to lower birth weight, IUGR contributes to increased risk of mortality, birth hypoxia, neonatal complications, and permanent neurologic damage. Currently, diagnosis of IUGR is based on ultrasound assessment of uterine artery Doppler velocimetry performed at 22–24 weeks gestation. Although surrogate markers have been proposed, they do not reach sufficient accuracy needed for diagnosis [61,62]. A long-term complication

associated with IUGR may be increased risk of developing metabolic syndrome [63,64], a suggestion first proposed by Barker [65]. Metabolomics offers a unique opportunity to assess if early changes in metabolism predispose to later changes in adulthood. In animal studies, metabolomics revealed that IUGR and early impairment of glucose metabolism may lead to the development of type 2 diabetes and alterations in fatty acid metabolism in adults [66,67]. These results were later confirmed in human newborns. In 2011, Dessì *et al.* first studied the metabolic profile of IUGR infants [28]. Using urine, the study reported that IUGR infants were metabolically impaired with respect to three biochemical pathways. These pathways included arginine and proline metabolism, the urea cycle, and glycine, serine, and threonine metabolism. Discriminant metabolites were myoinositol, sarcosine, creatine, and creatinine. Among these, myoinositol and creatinine were significantly increased. Interestingly, increased myoinositol in plasma and urine appears associated with adult glucose intolerance and insulin resistance and as such could become a valid indicator during fetal development [68]. Another metabolomic study on IUGR was conducted by Favretto *et al.* [27]. Cord blood LC-MS analysis revealed that phenylalanine, tryptophan, and methionine were increased [27].

Subsequent metabolomic studies on IUGR could lead to new diagnostic standards for this condition pre- and postnatally [21,22,25,26]. Despite apparent differences between these studies, many discriminant metabolites were found to be associated with the tricarboxylic acid cycle including citrate, glutamine, phenylalanine, leucine, and phenylalanine (Table 1).

3.3 Inborn Errors of Metabolism

Inborn errors of metabolism (IEM) are described as a class of genetic disorders in which the function of different enzymes is lost leading to several pathological conditions. The disorders include defect in enzymes that catalyze the metabolism of carbohydrates, amino acids, fatty acids, nucleic acids, cholesterol, bile acid, steroids, vitamin D, and the urea cycle. In most cases, complications are caused by accumulation of toxins that can give rise to a broad spectrum of clinical symptoms that can potentially lead to death [69]. Early diagnosis is essential in order to implement appropriate therapeutic intervention to avoid disease exacerbation and complications thereof. A milestone in the field of IEM was development of liquid (LC) and gas chromatography (GC) coupled with tandem mass spectrometry (MS/MS). This powerful analytical platform in combination with multivariate

statistical tools provides a robust technology to screen biologic specimens such as plasma and urine. For example, an untargeted metabolomic study was performed in respiratory chain deficiency (RCD) [29]. Using MS, 255 endogenous and 46 exogenous molecules were identified in urine. The ability to identify a large number of potential markers provides a unique opportunity to develop a metabolic "signature" consistent with or diagnostic of RCD. A step forward in IEM analysis was made by Sahoo *et al.* [30]. This study, which reconstructed the metabolic map by adding missing reactions and pathways involved in acylcarnitine (AC) metabolism and fatty acid oxidation, identified defects in carbohydrate, amino acid, and lipid metabolism as more frequent. Recently, Aygen and coworkers [31] highlighted the potential use of NMR due to its reproducibility, rapidity, and ease of sample preparation. They also reported on the use of this technology for identification of novel peaks of interest as well as monitoring therapy. This large study involved 989 neonatal urine samples from 14 clinical centers throughout Turkey. NMR spectra were subjected to untargeted and targeted analyses. For untargeted analysis, PCA and hierarchical multimodel/soft independent modeling for class analogy (HMM/SIMCA) were performed to identify statistical outliers. Targeted analysis was used to establish distributions for 65 metabolites and to identify IEM. Outliers were also tested against reference metabolites in suspected IEM. The authors proposed that this technology allowed for the identification of IEM metabolic profiles as well as the construction of a healthy neonate database which could subsequently identify statistical outliers in disease states. Future use of NMR may include identification of unknown pathologic peaks, correlation with clinical parameters, follow-up assessment, as well as monitoring metabolic phenotype over time. This approach provides a new tool for managing and treating asymptomatic newborns with suspected IEM [70].

3.4 Perinatal Asphyxia

Perinatal asphyxia is a condition attributed to hypoxia and/or ischemia around the time of birth. Annually, this pathologic state affects 4 million neonates worldwide resulting in 1 million mortalities. Although some successfully recover, other survivors have permanent neurologic conditions with organ impairment [71–73]. This condition is routinely assessed by Sarnat staging based on clinical presentation [74]. Although metabolomics has been suggested as an alternative approach, the identification of metabolite signatures consistent with hypoxia/asphyxia has been poorly explored

[35,75–78]. One study performed in 2006 [32] examined urine metabolic profiles in neonates with clinical evidence of severe asphyxia at birth. High-throughput MS revealed eight organic acids associated with neurodevelopment. Ethylmalonate, 3-hydroxy-3-methylglutarate, 2-hydroxyglutarate, and 2-oxoglutarate were associated with good neurologic outcome, whereas glutarate, methylmalonate, 3-hydroxy-butyrate, and orotate were associated with poor outcome. In a subsequent study [33], LC-MS/MS was used to examine cord blood from infants with hypoxic ischemic encephalopathy (HIE) ($n=31$), those with biochemical or clinical risk of asphyxia without encephalopathy ($n=40$), and controls ($n=71$). All the samples were matched for clinical parameters. A total of 148 metabolites were subsequently identified and quantified. Metabolites were characterized as ACs, glycerophospholipids, sphingolipids, amino acids, and biogenic amines. MVA identified of three clusters of metabolite classes that were significantly altered, ie, glycerophospholipids in asphyxia, amino acids in HIE, and ACs in both. More recently, Reinke *et al.* [34] characterized umbilical cord serum using NMR. Fifty-nine neonates were enrolled and stratified by disease severity (25 infants with HIE, including 13 mild, 6 moderate, and 6 severe cases, and 34 infants who were asphyxiated but did not present clinical neurological signs), together with 1:1 matched healthy controls. A total of 37 metabolites classified as organic compounds, alcohols, amino acids, ketones, biogenic amines, and sugars were identified. Using MVA, significant differences were found for 18 metabolites in asphyxia and 13 in HIE. Twelve metabolites were common to both. These included alanine, choline, creatine, glycerol, isoleucine, lactate, leucine, myoinositol, pyruvate, phenylalanine, succinate, and valine (significantly increased in asphyxia and HIE). Acetone, betaine, creatinine, glucose, 3-hydroxybutyrate, and *O*-phosphocholine were exclusively increased in asphyxia. Methionine was the only metabolite increased in HIE [34]. A small metabolomics study investigated the effect of perinatal asphyxia on newborns ($n=6$) vs healthy controls ($n=8$) [39]. The purpose of this study was to generate a global low molecular weight metabolic profile. ^{1}H NMR spectroscopy analysis coupled with PCA MVA was used to identify the main metabolic differences between these two groups. These metabolites included, in order of importance, acetate, glucose and TMAO, dimethylglycine, dimethylamine, creatine, glucose, succinate, threonine and 3-hydroxyisovalerate, formate, urea, and aconitate. Despite their identification, their role remains unclear. Because hypoxia results in depletion of aerobic metabolism, it is likely that metabolites involved in this process,

ie, Krebs citric acid cycle intermediates, should be impacted. However, only two studies implicated these factors [34,39]. It is likely, however, that the lack of consistent data among these research groups is reflective of inter-individual differences, ie, the metabolic profile and resilience of each neonate [36].

3.5 Sepsis

Sepsis is a clinical syndrome characterized by immunologic, metabolic, hemodynamic, and respiratory alterations secondary to a condition known as systemic inflammatory response syndrome (SIRS) [23]. Septicemia and infectious diseases, ie, meningitis, respiratory infection, diarrhea, and neonatal tetanus, are main causes of mortality in neonates [79]. Time of onset is an important clinical distinction [80]. Early-onset sepsis is mainly due to bacteria acquired before and during delivery. Late-onset sepsis occurs when pathogens are acquired after delivery. Incidence varies geographically [81–84]. In Asia, the incidence varies from 7.1 to 38 per 1000 live births. In Africa, it varies from 6.5 to 23. In the United States, the annual incidence of severe sepsis has been estimated at 0.3% and neonatal mortality is 10.3% with most deaths occurring within 48 h from onset [85]. Differences in developing vs developed countries reflect the responsible microorganism. The majority of infections from gram negative bacteria include *Klebsiella* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella*. For gram negatives, these include *Staphylococcus aureus*, *Coagulase Negative Staphylococci (CONS)*, and Streptococci species such as *Pneumoniae*, *Pyogenes*, and *Group B Streptococcus*. Mortality is correlated to microorganism type. The National Institute of Child Health and Human Development Neonatal Research Network reports mortality rates between 10% and 20% for gram positive, 36% for gram negative, and 32% for fungal infections [86]. Other factors influencing outcome include infection with viruses associated with maternal-fetal transmission (CMV, HSV1, HSV2) and those responsible of bronchiolitis and respiratory involvement with viremia (ie, RSV) as well as fungi. The latter represents the third leading cause of increased length of stay in Neonatal Intensive Care Units (NICU) among very low birth weight neonates. Risk factors for fungal-related sepsis include antibiotic therapy, parenteral nutrition, presence of a central venous catheter, and abdominal surgery [87]. The most common pathogenic agents of fungal sepsis are *C. albicans* and *C. parapsilosis*. Unfortunately, measurement of biochemical parameters (C-reactive protein, white blood cell count, fibrinogen, D-dimer, platelet count, procalcitonin, serum amyloid A protein, interleukins, and presepsin)

lacks the sensitivity and specificity needed for these patients [88,89]. Fortunately, the advent of "omic" technologies may allow for increased diagnostic support. For example, a recent study examined the urine metabolome of a newborn with fungal infection vs healthy baby controls [24]. The septic urine contained increased *N*-glycine, D-serine, and L-threonine and decreased hexadecanoic, octadecanoic, and citric acids. These profiles suggest a hypermetabolic state in response to acute stress. Carbohydrate, lipid, and protein metabolism may also play a prevalent role. In one metabolomics study, lactate, glucose, and myoinositol were increased in sepsis [90]. Unfortunately, most studies were conducted in animal models [91,92]. Mickiewicz *et al.* [40] used metabolomics to evaluate its utility in the diagnosis and prognosis of septic patients including newborns in Pediatric Intensive Care Units. Patients were divided as those with septic shock of bacterial origin (gram positive, gram negative, and both) ($n=60$), those with SIRS ($n=40$), and healthy controls ($n=40$). NMR spectroscopy revealed clear metabolic distinctions. In sepsis, lactate, glucose, creatinine, 2-oxoisocaproate, 2-hydroxyisovalerate, and 2-hydroxybutyrate were increased vs controls. Glucose and 2-hydroxybutyrate as well as glycerol were increased more so in sepsis vs SIRS. Decreased threonine, acetate, 2-aminobutyrate, and adipate were noted in sepsis vs controls. Comparison of sepsis vs SIRS found that threonine, taurine, suberate, serine, pyruvate, ornithine, methionine, lactate, isoleucine, hypoxanthine, glycine, glutamate, alanine, and adipate were decreased. Fanos *et al.* [37] used a combined ^{1}H NMR and GC-MS approach with MVA to examine the urine metabolome in sepsis. This study found increased lactose, maltose, and glucose and decreased ribitol, ribonic acid, pseudouridine, 2,3,4-trihydroxybutyric, 2-ketogluconic, 3,4-dihydroxybutanoic, and 3,4,5-trihydroxypentanoic acids in septic patients vs controls. Together, these studies clearly provide the framework for additional investigation of metabolomics as a viable option in the assessment of bacterial sepsis.

Metabolomics may also be used for sepsis involving viruses. Fanos *et al.* [41] analyzed urine of neonates infected by cytomegalovirus (CMV). ^{1}H NMR combined with MVA demonstrated clear differences in infected vs normal controls. Metabolites of distinction included myoinositol, glycine, 3-hydroxybutyrate, 3-aminoisobutyrate, creatine, taurine, and betaine.

3.6 Necrotizing Enterocolitis

NEC is an acute inflammatory neonatal bowel disease characterized by necrosis of intestinal mucosa that extends to the deepest layers, mostly involving the proximal ileum and the colon [93]. While its etiology is unknown, the

condition represents the most common gastrointestinal emergency in the neonate, affecting 1 out of every 1000 live births in the United States.

Preterms are mostly affected. Although 50% recover fully with appropriate bowel rest and antibiotic treatment, others evolve to more severe disease requiring surgical intervention. In the latter, survivors have higher risk of long-term consequences not only of gastrointestinal nature but also neurodevelopmental [94]. Preterms are more susceptible to developing NEC due to its pathogenesis. Three main events are implicated in onset of mucosal necrosis. These include recent bowel colonization, the presence of food, and an event that disrupts the mucosa barrier. Unfortunately, the pathogenesis of NEC remains poorly understood. Metabolomics may, however, provide a new avenue for exploration into the biochemical mechanisms associated with this complex disease. Investigating the role of bacteria in NEC, Morrow *et al.* [43] combined metagenomics (stool bacterial 16S rRNA) and metabolomics (urine ^{1}H NMR) to evaluate 32 infants <29 weeks gestation. This study concluded that early dysbiosis was strongly implicated in NEC. In those with earlier onset NEC, a Firmicutes dysbiosis preceded necrosis, whereas a Proteobacteria dysbiosis was consistent with later onset. Interestingly, Propionibacterium was not evident in any of the NEC cases. Urine metabolomics revealed that alanine was associated with NEC preceded by Firmicutes dysbiosis. Histidine was inversely related to NEC preceded by Proteobacteria dysbiosis. Increased urine alanine/histidine ratio was predictive for NEC in 78% of cases.

In another study, Garner *et al.* [45] evaluated volatile organic compounds (VOC) in the feces of preterm babies with NEC vs controls. Overall, VOC were decreased in NEC. In fact, 2-ethylhexyl acetic ester, decanoic acid ethyl ester, dodecanoic acid ethyl ester, and hexadecanoic acid ethyl ester were completely missing. This pilot study suggested the VOC may be used for identification of NEC.

Because decreased plasma arginine was associated with NEC in neonates, attention has also focused on its precursor, citrulline [44,95]. This hypothesis was expanded to include the association of amino acid depletion with NEC in premature infants [96,97]. The authors proposed that citrulline may be a potential biomarker of intestinal function and recovery in NEC.

3.7 Kidney Disease

KD is a major cause of illness among newborns. Although one-third of these patients develop end-stage renal disease requiring replacement therapy,

research on this topic is scarce [98]. It should be noted, however, that substantial progress has been made for improving newborn survival rate in general. Unfortunately, preterm newborns as a group are specifically impacted by increased mortality and morbidity associated with KD. This finding is likely associated with reduced kidney function during the first two years of life. As such, metabolomics may play a novel role in identifying markers associated with increased risk for both acute and chronic forms of KD.

In 2008, Beger *et al.* [46] studied 40 children undergoing cardiac surgery. Urine was collected prior to surgery and at 4 and 12 h postsurgery. LC-MS/MS and unsupervised MVA revealed differences in metabolite profile in postsurgical urines from patients who subsequently developed acute kidney injury (AKI). This study identified homovanillic acid sulfate as a marker strongly associated with disease development, ie, 90% sensitivity and 95% specificity.

In a subsequent study, Atzori *et al.* [47] characterized urine metabolite patterns associated with nephropathy. ^{1}H NMR with MVA metabolomics identified alterations in purine and pyrimidine metabolism as well as the citric acid cycle. In a more recent study, Fanos *et al.* [36] investigated the urine metabolite profile in three newborns with perinatal asphyxia treated with hypothermia. GC/MS with MVA revealed that these newborns were located in different part of the multivariate space due to their different metabolic profiles, ie, one died and two survived. One survivor developed AKI with discriminant metabolites identified as glucose, asparagine, gluconic acid, aspartic acid, ornithine, and lysine. Discriminant metabolites associated with perinatal asphyxia were glycine, valine, maleic acid, and sorbitol. This study, while limited, may provide a new approach to identify early KD and potentially improve outcome in these patients [42,99].

3.8 Bronchopulmonary Dysplasia

BDP is one of the main causes of lung illness in premature babies. It is described as persistence of oxygen dependence for at least 28 days of life and is associated with lung symptoms and radiographic anomalies [100]. The current classification is based on three degrees of severity, ie, mild, moderate, and severe, with respect to duration of oxygen treatment and ventilatory support [101]. Predisposing factors are increased prematurity and low birth weight [102]. In 2013 Lussu *et al.* [48] suggested that the metabolic profiles of newborns with BDP may likely be different than full-term babies. Subsequently, a congenital predisposition for developing BDP in

preterm newborns was identified by Fanos *et al.* [38]. ^{1}H NMR with MVA was conducted on urine from preterm newborns <29 weeks gestation. Discriminant metabolites associated with BDP were myoinositol, lactate, taurine, and TMAO. Discriminant metabolites associated with the control group were citrate and gluconate. It should be noted, however, that congenital refers to epigenetical, ie, genetic plus intrauterine epigenetics, in these cases. In a recent study, Carraro *et al.* [49] investigated adolescents born prematurely with BDP to assess if a metabolic "fingerprint" persisted. Examination of exhaled breath condensate and urine by MS with MVA revealed that lysophosphatidylcholine, platelet-activating factor, unsaturated phosphatidylcholines, [PC(18:1(9Z)/4:0], and plasmenyl-phosphatidylserine characterized the BPD group and hydroxyeicosapentaenoic acid and phosphatidylserine characterized the control group. These findings were suggestive of impaired energy metabolism combined with the presence of oxidative and inflammatory factors in BDP.

3.9 Cardiac Malformation and Dysfunction

Cardiovascular disease represents a worldwide health problem, and it is one of the most common causes of death. In Europe, 4.3 million people die from cardiovascular disease each year and account for 48% of deaths overall (54% females and 43% males) [103]. Nowadays, cardiovascular disease can no longer be a considered a disease of exclusive onset in adulthood. A fetal-neonatal origin should also be considered, a relationship first studied by Barker in 1995 [66]. Preterm neonates with low gestational age and reduced fetal growth have increased risk of cardiovascular disease [104]. Electrocardiography has revealed that the QT interval corrected for heart rate (QTc) was significantly prolonged in extremely preterm infants and correlated to both gestational age and birth weight [105]. QTc is a recognized risk factor for developing potential life-threatening ventricular arrhythmia. As such, these findings highlight the importance of monitoring in this susceptible population. Endothelial dysfunction is one of the earliest clinically detectable stages of cardiovascular disease preceding atheroma formation and coronary heart disease [106]. Low birth weight neonates exhibit endothelial dysfunction which may persist in adulthood [107]. Endothelial dysfunction has been proven to be an existing condition in extremely preterms. The triad composed by preterm labor, gestational age, and birth weight influences early circulatory dysfunction and, as such, may be predictive of increased cardiovascular risk [108]. It is speculated that extreme prematurity

contributes to imbalanced production of nitric oxide and vasodilatory substances and/or release of vasoconstrictor compounds such as asymmetric dimethylarginine (ADMA). For example, blood ADMA was decreased in ex-preterms vs healthy full-term controls and inversely correlated to gestational age and birth weight [109]. Metabolomics may play a role in the investigation of these endothelial and cardiovascular changes in neonates. Decreased blood albumin-lysyl in preterm babies could be suggestive of oxidative stress and thus contribute to cardiac and vascular complications including hypertension, atherosclerosis, ischemic heart disease, cardiomyopathy, and congestive heart failure [18,110]. Additional studies were performed by Atzori *et al.* [111], who investigated patent ductus arteriosus (PDA), one of the most common congenital abnormalities in preterms [111]. Persistent PDA is diagnosed when it fails to close after 72 h. In this pilot study, ^{1}H NMR was used to analyze urine from a small group of neonates that included four full-term infants, four preterms without PDA, and six preterms with PDA. Metabolomics distinguished these three subgroups at day 4 of life. Using these preliminary findings, it may be possible to predict and monitor PDA, thereby avoiding unnecessary drug prophylaxis in the future [112,113].

4. CONCLUSIONS

Metabolomics is a rapidly evolving science and has many applications in neonatal medicine. This novel technology may provide unique insight into disease conditions associated with especially high morbidity and mortality namely prematurity, perinatal asphyxia, and neonatal sepsis. While preliminary, these studies have generated substantial interest in the field of metabolomics as a diagnostic tool. It is clear that significant research remains to be done to substantiate these early findings and further elucidate the role of metabolomics in neonatal disease.

REFERENCES

[1] D.J. Barker, Fetal origins of coronary heart disease, BMJ 311 (1995) 171–174.
[2] J.K. Nicholson, J.C. Lindon, E. Holmes, 'Metabonomics': understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data, Xenobiotica 11 (1999) 1181–1189.
[3] J. Van der Greef, A.K. Smilde, Symbiosis of chemometrics and metabolomics: past, present, and future, J. Chemom. 19 (2005) 376–386.
[4] S. Collino, F.P. Martin, S. Rezzi, Clinical metabolomics paves the way towards future healthcare strategies, Br. J. Clin. Pharmacol. 75 (2013) 619–629.

[5] O. Fiehn, Metabolomics—the link between genotypes and phenotypes, Plant Mol. Biol. 48 (2002) 155–171.
[6] M. Mussap, R. Antonucci, A. Noto, The role of metabolomics in neonatal and pediatric laboratory medicine, Clin. Chim. Acta 426 (2013) 127–138.
[7] J. Bartel, J. Krumsiek, F.J. Theis, Statistical methods for the analysis of high-thrughput metabolomics data, Comput. Struct. Biotechnol. J. 4 (2013) e201301009.
[8] A. Weljie, J. Newton, P. Mercier, Targeted profiling: quantitative analysis of ^{1}H-NMR metabolomics data, Anal. Chem. 78 (2006) 4430–4442.
[9] H.J. Issaq, Q.N. Van, T.J. Waybright, Analytical and statistical approaches to metabolomics research, J. Sep. Sci. 32 (2009) 2183–2199.
[10] E. Saccenti, H.C.J. Hoefsloot, A.K. Smilde, Reflections on univariate and multivariate analysis of metabolomics data, Metabolomics 10 (2014) 361–374.
[11] J. Trygg, E. Holmes, T. Lundstedt, Chemometrics in metabolomics, J. Proteome Res. 6 (2007) 469–479.
[12] M. Barker, W. Rayens, Partial least squares for discrimination, J. Chemom. 17 (2003) 166–173. B.M. Beckwith-Hall, J.T. Brindle, R.H. Barton, Application of orthogonal signal correction to minimise the effects of physical and biological variation in high resolution ^{1}H-NMR spectra of biofluids, Analyst 127 (2002) 1283–1288.
[13] D.K. Barupal, P.K. Haldiya, G. Wohlgemuth, T. Kind, S.L. Kothari, MetaMapp: mapping and visualizing metabolomic data by integrating information from biochemical pathways and chemical and mass spectral similarity, BMC Bioinf. 13 (2012) 99.
[14] D. Grapov, K. Wanichthanarak, O. Fiehn, MetaMapR: pathway independent metabolomics network analysis incorporating unknowns, Bioinformatics 31 (2015) 2757–2760. Pii:btv194.
[15] A. Noto, V. Fanos, L. Barberini, The urinary metabolomics profile of an Italian autistic children population and their unaffected siblings, J. Matern. Fetal Neonatal Med. 2 (2014) 46–52.
[16] R. Menon, J. Jones, P.R. Gunst, Amniotic fluid metabolomic analysis in spontaneous preterm birth, Reprod. Sci. 21 (2014) 791–803.
[17] G. Graça, B.J. Goodfellow, A.S. Barros, UPLC-MS metabolic profiling of second trimester amniotic fluid and maternal urine and comparison with NMR spectral profiling for the identification of pregnancy disorder biomarkers, Mol. Biosyst. 8 (2012) 1243–1254.
[18] I. Tea, G. Le Gall, A. Küster, 1H-NMR-based metabolic profiling of maternal and umbilical cord blood indicates altered materno-foetal nutrient exchange in preterm infants, PLoS One 7 (2012) e29947.
[19] R. Romero, S. Mazaki-Tovi, E. Vaisbuch, Metabolomics in premature labor: a novel approach to identify patients at risk for preterm delivery, J. Matern. Fetal Neonatal Med. 23 (2010) 1344–1359.
[20] G. Graça, I.F. Duarte, A.S. Barros, Impact of prenatal disorders on the metabolic profile of second trimester amniotic fluid: a nuclear magnetic resonance metabonomic study, J. Proteome Res. 9 (2010) 6016–6024.
[21] L. Barberini, A. Noto, C. Fattuoni, Urinary metabolomics (GC-MS) reveals that low and high birth weight infants share elevated inositol concentrations at birth, J. Matern. Fetal Neonatal Med. 27 (2014) 20–26.
[22] A. Dessì, F.C. Marincola, M.G. Pattumelli, Investigation of the ^{1}H-NMR based urine metabolomic profiles of IUGR, LGA and AGA newborns on the first day of life, J. Matern. Fetal Neonatal Med. 27 (2014) 13–19.
[23] A. Dessì, G. Corsello, M. Stronati, New diagnostic possibilities in systemic neonatal infections: metabolomics, Early Hum. Dev. 90 (2014) S19–S21.
[24] A. Dessì, B. Liori, P. Caboni, Monitoring neonatal fungal infection with metabolomics, J. Matern. Fetal Neonatal Med. 27 (2014) 34–38.

[25] M. Sanz-Cortés, R.J. Carbajo, F. Crispi, Metabolomic profile of umbilical cord blood plasma from early and late intrauterine growth restricted (IUGR) neonates with and without signs of brain vasodilation, PLoS One 8 (2013) e80121.
[26] E. Cosmi, S. Visentin, D. Favretto, Selective intrauterine growth restriction in monochorionic twin pregnancies: markers of endothelial damage and metabolomic profile, Twin Res. Hum. Genet. 16 (2013) 816–826.
[27] D. Favretto, E. Cosmi, E. Ragazzi, Cord blood metabolomic profiling in intrauterine growth restriction, Anal. Bioanal. Chem. 402 (2012) 1109–1121.
[28] A. Dessì, L. Atzori, A. Noto, Metabolomics in newborns with intrauterine growth retardation (IUGR): urine reveals markers of metabolic syndrome, J. Matern. Fetal Neonatal Med. 24 (2011) 35–39.
[29] C.J. Reinecke, G. Koekemoer, F.H. Van der Westhuizen, Metabolomics of urinary organic acids in respiratory chain deficiencies in children, Metabolomics 8 (2012) 264–283.
[30] S. Sahoo, L. Franzson, J.J. Jonsson, I. Thiele, A compendium of inborn errors of metabolism mapped onto the human metabolic network, Mol. Biosyst. 8 (2012) 2545–2558.
[31] S. Aygen, U. Dürr, P. Hegele, NMR-based screening for inborn errors of metabolism: initial results from a study on Turkish neonates, JIMD Rep. 16 (2014) 101–111.
[32] C.Y. Chu, X. Xiao, X.G. Zhou, Metabolomic and bioinformatic analyses in asphyxiated neonates, Clin. Biochem. 39 (2006) 203–209.
[33] B.H. Walsh, D.I. Broadhurst, R. Mandal, The metabolomic profile of umbilical cord blood in neonatal hypoxic ischaemic encephalopathy, PLoS One 7 (2012) e50520.
[34] S.N. Reinke, B.H. Walsh, G.B. Boylan, ^{1}H-NMR derived metabolomic profile of neonatal asphyxia in umbilical cord serum: implications for hypoxic ischemic encephalopathy, J. Proteome Res. 12 (2013) 4230–4239.
[35] V. Fanos, A. Noto, T. Xanthos, Metabolomics network characterization of resuscitation after normocapnic hypoxia in a newborn piglet model supports the hypothesis that room air is better, Biomed. Res. Int. 2014 (2014) 731620. http://dx.doi.org/10.1155/2014/731620.
[36] V. Fanos, A. Noto, P. Caboni, Urine metabolomic profiling in neonatal nephrology, Clin. Biochem. 47 (2014) 708–710.
[37] V. Fanos, P. Caboni, G. Corsello, Urinary (1)H-NMR and GC-MS metabolomics predicts early and late onset neonatal sepsis, Early Hum. Dev. 90 (2014) S78–S83.
[38] V. Fanos, M.C. Pintus, M. Lussu, Urinary metabolomics of bronchopulmonary dysplasia (BPD): preliminary data at birth suggest it is a congenital disease, J. Matern. Fetal Neonatal Med. 27 (2014) 39–45.
[39] M. Longini, S. Giglio, S. Perrone, Proton nuclear magnetic resonance spectroscopy of urine samples in preterm asphyctic newborn: a metabolomic approach, Clin. Chim. Acta 15 (2015) 250–256.
[40] B. Mickiewicz, H.J. Vogel, H.R. Wong, Metabolomics as a novel approach for early diagnosis of pediatric septic shock and its mortality, Am. J. Respir. Crit. Care Med. 187 (2013) 967–976.
[41] V. Fanos, E. Locci, A. Noto, Urinary metabolomics in newborns infected by human cytomegalovirus: a preliminary investigation, Early Hum. Dev. 89 (2013) S58–S61.
[42] V. Fanos, C. Fanni, G. Ottonello, Metabolomics in adult and pediatric nephrology, Molecules 18 (2013) 4844–4857.
[43] A.L. Morrow, A.J. Lagomarcino, K.R. Schibler, Early microbial and metabolomic signatures predict later onset of necrotizing enterocolitis in preterm infants, Microbiome 1 (2013) 13.
[44] H.P. Ioannou, E. Diamanti, K. Piretzi, Plasma citrulline levels in preterm neonates with necrotizing enterocolitis, Early Hum. Dev. 88 (2012) 563–566.

[45] C.E. Garner, A.K. Ewer, K. Elasouad, Analysis of faecal volatile organic compounds in preterm infants who develop necrotising enterocolitis: a pilot study, J. Pediatr. Gastroenterol. Nutr. 49 (2009) 559–565.
[46] R.D. Beger, R.D. Holland, J. Sun, Metabonomics of acute kidney injury in children after cardiac surgery, Pediatr. Nephrol. 23 (2008) 977–984.
[47] L. Atzori, R. Antonucci, L. Barberini, ^{1}H-NMR-based metabolic profiling of urine from children with nephrouropathies, Front. Biosci. (Elite Ed.) 2 (2010) 725–732.
[48] M. Lussu, M.C. Pintus, G. Palmas, Metabolomics in bronchopulmonary dysplasia: preliminary results, Early Hum. Dev. 89 (2013) 87.
[49] S. Carraro, G. Giordano, P. Pirillo, Airway metabolic anomalies in adolescents with bronchopulmonary dysplasia: new insights from the metabolomic approach, J. Pediatr. 166 (2015) 234–239.
[50] World Health Organization, The Prevention of Perinatal Mortality and Morbidity: Report 457, WHO Technical Report Series, Geneva, Switzerland, 1970.
[51] J.M. Moutquin, Classification and heterogeneity of preterm birth, BJOG 110 (2003) 30–33.
[52] R.L. Goldenberg, J.F. Culhane, J.D. Iams, Epidemiology and causes of preterm birth, Lancet 371 (2008) 75–84. Elsevier.
[53] S.B. Effer, J.M. Moutquin, D. Farine, Neonatal survival rates in 860 singleton live births at 24 and 25 weeks gestational age. A Canadian multicentre study, BJOG 7 (2002) 740–745.
[54] J.L. Bock, Metabolic profiling of amniotic fluid by proton nuclear magnetic resonance spectroscopy: correlation with fetal maturation and other clinical variables, Clin. Chem. 40 (1994) 56–61.
[55] G. Graça, I.F. Duarte, A.S. Barros, (1)H-NMR based metabonomics of human amniotic fluid for the metabolic characterization of fetus malformations, J. Proteome Res. 8 (2009) 4144–4150.
[56] L. Atzori, R. Antonucci, L. Barberini, 1H-NMR-based metabolomic analysis of urinefrom preterm and term neonates, Front. Biosci. (Elite Ed.) 3 (2011) 1005–1012.
[57] B. Diderholm, Perinatal energy metabolism with reference to IUGR & SGA: studies in pregnant women & newborn infants, Indian J. Med. Res. 130 (2009) 612e7.
[58] V. Fanos, M. Puddu, A. Reali, Perinatal nutrient restriction reduces nephron endowment increasing renal morbidity in adulthood: a review, Early Hum. Dev. 86 (2010) 37–42.
[59] A. Dessì, F.C. Marincola, V. Fanos, Metabolomics and the great obstetrical syndromes—GDM, PET, and IUGR, Best Pract. Res. Clin. Obstet. Gynaecol. 29 (2015) 156–164.
[60] E. Hafner, M. Metzenbauer, I. Stümpflen, First trimester placental and myometrial blood perfusion measured by 3D power Doppler in normal and unfavourable outcome pregnancies, Placenta 31 (2010) 756e63.
[61] A. Conde-Agudelo, A.T. Papageorghiou, S.H. Kennedy, Novel biomarkers for predicting intrauterine growth restriction: a systematic review and meta-analysis, BJOG 120 (2013) 681–694.
[62] J.E. Harding, The nutritional basis of the foetal origin of adult disease, Int. J. Epidemiol. 30 (2001) 15–23.
[63] C.N. Hales, D.J. Barker, Type 2 (non insulin dependent) diabetes mellitus: the thrifty phenotype hypothesis, Diabetologia 35 (2003) 395e601.
[64] A. Dessì, G. Ottonello, V. Fanos, Physiopathology of intrauterine growth retardation: from classic data to metabolomics, J. Matern. Fetal Neonatal Med. 25 (2012) 13e8.
[65] D.J. Barker, Fetal origins of coronary heart disease, BMJ 311 (1995) 171e4.
[66] P.M. Nissen, C. Nebel, N. Oksbjerg, Metabolomics reveals relationship between plasma inositols and birth weight: possible markers for fetal programming of type 2 diabetes, J. Biomed. Biotechnol. 2011 (2011) 378268.

[67] M.C. Alexandre-Gouabau, F. Courant, G. Le Gall, Offspring metabolomic response to maternal protein restriction in a rat model of intrauterine growth restriction (IUGR), J. Proteome Res. 10 (2011) 3292–3302.
[68] A. Dessì, V. Fanos, Myoinositol: a new marker of intrauterine growth restriction? J. Obstet. Gynaecol. 33 (2013) 776e80.
[69] C. Baumgartner, D. Baumgartner, Biomarker discovery, disease classification, and similarity query processing on high-throughput MS/MS data of inborn errors of metabolism, J. Biomol. Screen. 11 (2006) 90–99.
[70] M. Mussap, V. Fanos, Reducing neonatal mortality and expenditure in the era of health care crisis: is it possible? J. Matern. Fetal Neonatal Med. 25 (2012) 1–3. Early Online: 1–2.
[71] O.D. Saugstad, Reducing global neonatal mortality is possible, Neonatology 99 (2011) 250–257.
[72] H.M. Laila, A.K. Mai, A.E. Nagy, Multi-organ dysfunction in neonates with hypoxic-ischemic encephalopathy, Med. J. Cairo Univ. 78 (2010) 461–467.
[73] P. Shah, S. Riphagen, J. Beyene, Multiorgan dysfunction in infants with post-asphyxial hypoxic-ischaemic encephalopathy, Arch. Dis. Child. 89 (2004) 152–155.
[74] H.B. Sarnat, M.S. Sarnat, Neonatal encephalopathy following fetal distress: a clinical and electroencephalographic study, Arch. Neurol. 33 (1976) 696–705.
[75] R. Solberg, D. Enot, H.P. Deigner, Metabolomic analyses of plasma reveals new insights into asphyxia and resuscitation in pigs, PLoS One 5 (2010) e9606.
[76] D. Barouxis, A. Chalkias, A. Syggelou, Research in human resuscitation: what we learn from animals, J. Matern. Fetal Neonatal Med. 25 (2012) 44–46.
[77] C. Skappak, S. Regush, P.Y. Cheung, Identifying hypoxia in a newborn piglet model using NMR metabolomic profiling, PLoS One 8 (2013) e65035.
[78] C. Fattuoni, F. Palmas, A. Noto, Perinatal asphyxia: a review from a metabolomics perspective, Molecules 20 (2015) 7000–7016.
[79] A. Costello, V. Francis, A. Byrne, The state of the world's newborns, Save the Children Fund, Washington, 2001.
[80] S. Vergnano, M. Sharland, P. Kazembe, C. Mwansambo, Neonatal sepsis: an international perspective, Arch. Dis. Child. Fetal Neonatal Ed. 90 (2005) 220–224.
[81] N.L. Lim, Y.H. Wong, N.Y. Boo, Bacteraemic infections in a neonatal intensive care unit: a nine months survey, Med. J. Malaysia 50 (1995) 59–63.
[82] S.S. Tallur, A.V. Kasturi, S.D. Nadgir, Clinico-bacteriological study of neonatal septicemia in Hubli, Indian J. Pediatr. 67 (2000) 169–174.
[83] A.I. Airede, Neonatal septicaemia in an African city of high altitude, J. Trop. Pediatr. 38 (1992) 189–191.
[84] The WHO Multicentre Study Group, Clinical prediction of serious bacterial infections in young infants in developing countries, Pediatr. Infect. Dis. J. 18 (1999) 23–31.
[85] A.L. Shane, B.J. Stoll, Recent developments and current issues in the epidemiology, diagnosis, and management of bacterial and fungal neonatal sepsis, Am. J. Perinatol. 30 (2013) 131–141.
[86] B.J. Stoll, N. Hansen, A.A. Fararoff, L. Wright, W.A. Carlo, Late-onset sepsis in very low birth weight neonates: the experience of the NICHD neonatal research network, Pediatrics 110 (2002) 285–291.
[87] P. Manzoni, D.K. Benjamin, W. Hope, The management of Candida infections in preterm neonates and the role of micafungin, J. Matern. Fetal Neonatal Med. 24 (2011) 24–27.
[88] F. Raimondi, T. Ferrara, R. Maffucci, Neonatal sepsis: a difficult diagnostic challenge, Clin. Biochem. 44 (2011) 463–464.
[89] M. Mussap, E. Puxeddu, P. Burrai, Soluble CD14 subtype (sCD14-ST) presepsin in critically ill preterm newborns: preliminary reference ranges, J. Matern. Fetal Neonatal Med. 25 (2012) 51–53.

[90] D. Schmerler, S. Neugebauer, K. Ludewig, Targeted metabolomics for discrimination of systemic inflammatory disorders in critically ill patients, J. Lipid Res. 53 (2012) 1369–1375.
[91] Z.Y. Lin, P.B. Xu, S.K. Yan, A metabonomic approach to early prognostic evaluation of experimental sepsis by (1)H NMR and pattern recognition, NMR Biomed. 22 (2009) 601–608.
[92] J.L. Izquierdo-García, N. Nin, J. Ruíz-Cabello, A metabolomic approach for diagnosis of experimental sepsis, Intensive Care Med. 37 (2011) 2023–2032.
[93] M. Puddu, M.A. Marcialis, A. De Magistris, From the "old NEC" to the "new NECs", JPNIM 3 (2014) e030245.
[94] R.L. Moss, L.A. Kalish, C. Duggan, Clinical parameters do not adequately predict outcome in necrotizing enterocolitis: a multi-institutional study, J. Perinatol. 28 (2008) 665–674.
[95] S.A. Zamora, H.J. Amin, D.D. McMillan, Plasma L-arginine concentrations in premature infants with necrotizing enterocolitis, J. Pediatr. 131 (1997) 226–232.
[96] R.M. Becker, G.Y. Wu, J.A. Galanko, Reduced serum amino acid concentrations in infants with necrotizing enterocolitis, J. Pediatr. 137 (2000) 785–793.
[97] M.C. Richir, M.P.C. Siroen, R.M. van Elburg, Low plasma concentrations of arginine and asymmetric dimethylarginine in premature infants with necrotizing enterocolitis, Br. J. Nutr. 97 (2007) 906–911.
[98] A.O. Staples, L.A. Greenbaum, J.M. Smith, Association between clinical risk factors and progression of chronic kidney disease in children, Clin. J. Am. Soc. Nephrol. 5 (2010) 2172–2179.
[99] M.H. Hanna, B.D. Brophy, Metabolomics in pediatric nephrology: emerging concepts, Pediatr. Nephrol. 30 (2015) 881–887.
[100] A.H. Jobe, E. Bancalari, Bronchopulmonary dysplasia, Am. J. Respir. Crit. Care Med. 163 (2001) 1723–1729.
[101] R.A. Ehrenkranz, M.C. Walsh, B.R. Vohr, Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia, Pediatrics 116 (2005) 1353–1360.
[102] B.J. Stoll, N.I. Hansen, E.F. Bell, Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network, Pediatrics 126 (2010) 443–456.
[103] S.C. Smith, A. Collins, R. Ferrari, Our time: a call to save preventable death from cardiovascular disease (heart disease and stroke), J. Am. Coll. Cardiol. 60 (2012) 2343–2348.
[104] G. Mercuro, P.P. Bassareo, G. Flore, Prematurity and low weight at birth as new conditions predisposing to an increased cardiovascular risk, Eur. J. Prev. Cardiol. 20 (2012) 357–367.
[105] P.P. Bassareo, V. Fanos, M. Puddu, Significant QT interval prolongation and long QT in young adult ex-preterm newborns with extremely low birth weight, J. Matern. Fetal Neonatal Med. 24 (2011) 1115–1118.
[106] P.P. Bassareo, V. Fanos, C. Barbanti, Prematurity at birth and increased cardiovascular risk: is a metabolomic approach the right solution? JPNIM 2 (2013) 28–34.
[107] M. Norman, H. Martin, Preterm birth attenuates association between low birth weight and endothelial dysfunction, Circulation 108 (2003) 996–1001.
[108] P.P. Bassareo, V. Fanos, M. Puddu, Reduced brachial flow-mediated vasodilation in young adult ex extremely low birth weight preterm: a condition predictive of increased cardiovascular risk? J. Matern. Fetal Neonatal Med. 23 (2010) 121–124.
[109] P.P. Bassareo, M. Puddu, G. Flore, Could ADMA levels in young adults born preterm predict an early endothelial dysfunction? Int. J. Cardiol. 159 (2012) 217–219.
[110] N.S. Dhalla, R.M. Temsah, T. Netticadan, Role of oxidative stress in cardiovascular diseases, J. Hypertens. 18 (2000) 655–673.

[111] L. Atzori, L. Barberini, M. Lussu, Metabolomics & patent ductus arteriosus diagnosis: is 1H-NMR (nuclear magnetic resonance) spectroscopy of urine at birth predictive as ultrasound? J. Matern. Fetal Neonatal Med. 24 (Suppl. 2) (2011). Proceedings of the 7th International Workshop on Neonatology, Cagliari (Italy) 28–29 October 2011.
[112] V. Fanos, M. Pusceddu, A. Dessì, Should we definitively abandon prophylaxis for patent ductus arteriosus in preterm newborns? Clinics (Sao Paulo) 66 (2011) 2141–2149.
[113] R. Antonucci, P.P. Bassareo, M. Zaffanello, Patent ductus arteriosus in the preterm infant: new insights into pathogenesis and clinical management, J. Matern. Fetal Neonatal Med. 23 (2010) 34–37.