



New diagnostic possibilities in systemic neonatal infections: metabolomics

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ABSTRACT

Systemic neonatal infection is a serious complication in preterm and term infants and is defined as a complex clinical syndrome caused by bacteria, fungi and virus. Sepsis remains among the leading causes of death in both developed and underdeveloped countries above all in the neonatal period. Earlier diagnosis may offer the ability to initiate treatment to prevent adverse outcomes. There have been many studies on various diagnostic haematological markers like acute phase reactants, C-reactive protein, procalcitonin, interleukins and presepsin. However, there is still no single test that satisfies the criteria as being the ideal marker for the early diagnosis of neonatal sepsis. In this regard, metabolomic analysis seems to be a promising method for determining metabolic variations correlated with systemic neonatal infections.

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Introduction

Sepsis is a clinical syndrome characterized by a series of immunological, metabolic, haemodynamic and respiratory alterations secondary to an infective process caused by an abnormal systemic inflammatory response syndrome (SIRS) in the organism. Systemic neonatal infections are an important long-term cause of mortality and morbidity for preterm and hospitalized newborn babies. In the United States the annual incidence of serious sepsis has been estimated at 0.3% and neonatal mortality is 10.3%, with most deaths occurring within 48 hours from the onset of the infection [1]. Mortality rates vary depending on the organisms considered and the data of the National Institute of Child Health and Human Development Neonatal Research Network report mortality rates between 10 and 20% for gram positive infections, 36% for gram negative ones and 32% for fungal infections [2]. In the field of systemic neonatal infections, besides those caused by bacteria and fungi, also to be considered are those caused by viruses that can be transmitted from mother to child (CMV, HSV1, HSV2) and those caused by viruses capable of causing bronchiolitis and respiratory pathologies with viremia (such as the respiratory syncytial virus) [3]. The perinatal period is a time at risk for exposure to various virulent organisms that may come from the uterus, the birth canal, hospitalization, invasive procedures and family members and visitors. The high

incidence and seriousness of neonatal systemic infections are to be attributed to the reciprocal influence of different factors such as immaturity of the immune defence mechanisms, especially weak in preterms, and the complex interaction between the infecting pathogen and the host. Sepsis is a clinical condition caused by infectious microorganisms and induced by mediators of inflammation that cause alteration of the immune, inflammatory and coagulative equilibrium [4]. Neonatal sepsis concerns infections with onset within the first 28 days of life for a term neonate and within 4 weeks after the predicted birth date for preterms; in this period early diagnosis is complicated by the presence of non-infective conditions similar to those of sepsis, especially in low birth weight preterms and by the lack of optimal diagnostic tests. Despite the many and extensive investigations that have been performed in the last decades, there is still no single test that satisfies the criteria as being the ideal marker for the early diagnosis of neonatal sepsis. Since neonatal sepsis is a complex disorder involving different organs which leads to wide variations in the organism's metabolites, analysis of the entire metabolome is a promising method for determining metabolic variations correlated with sepsis.

Systemic infections and metabolomics

Systemic infections are among the major causes of neonatal mortality and long-term disability in very low birth weight (VLBW) children. Although biomolecular knowledge on the pathophysiology of sepsis has improved over the years, for this pathology no markers making early diagnosis possible have yet been identified nor have prognostic biomarkers of high sensitivity and specificity been

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Table 1
Metabolomic studies that have analyzed the metabolic profiles of septic patients.

Author	Population study	Sample	Metabolomic analysis	Metabolite results
Mickiewicz et al. 2013 [13]	60 septic shock vs 40 SIRS vs 40 control pediatric patients	Serum	¹ H-NMR	2-hydroxybutyrate, 2-hydroxyisovalerate, lactate, glucose, 2-oxoisocaproate, creatine, creatinine, histidine, and phenylalanine
Fanos et al. 2013 [12]	12 HCMV infected vs 11 control newborns	Urine	¹ H-NMR	Myoinositol, glycine, 3-hydroxybutyrate, 3-aminoisobutyrate, creatine, taurine and betaine
Schmerler et al. 2012 [11]	74 SIRS vs 69 septic vs 16 control human adults	Blood	LC-GC/MS	Acylcarnitines and glycerophosphatidylcholines
Izquierdo-García et al. 2011 [14]	14 septic vs 14 control rats	Lung tissue, bronchoalveolar lavage fluid and serum	¹ H-NMR	Alanine, creatine, phosphoethanolamine and myoinositol
Liu et al. 2010 [10]	40 septic vs control rats	Plasma	UPLC-Q-TOF-MS	Hypoxanthine, indoxyl sulfate, glucuronic acid, gluconic acid, proline, uracil, nitrotyrosine, uric acid and trihydroxy cholanolic acid
Lin et al. 2009 [9]	40 septic vs 20 control rats	Serum	¹ H-NMR	Lactate, alanine, acetate, acetoacetate, hydroxybutyrate and formate

¹H NMR, ¹H-nuclear magnetic resonance; HCMV, human cytomegalovirus; SIRS, systemic inflammatory response syndrome; UPLC-Q-TOF-MS, ultra performance liquid chromatography quadrupole time-of-flight mass spectrometry; LC, liquid chromatography; GC/MS, gas chromatography/mass spectrometry.

found [5]. In recent years, several single biochemical parameters of sepsis have been proposed such as C-reactive protein (CRP), white cell count, fibrinogen, D-dimer, platelet count, procalcitonin (PCT), serum amyloid protein, interleukins and presepsin [6,7]. However, certain recently developed investigative techniques provide a holistic vision of the biological system in its entirety. Among these, metabolomics (the most recent of the omic sciences) appears to be the most promising. It is defined as the study of the complex system of metabolites and, besides taking into consideration one or a few metabolites and their relative metabolic processes, it is capable of examining the entire metabolic profile of an individual. Recent studies show the efficacy of metabolomics in providing significant information useful in arriving at an early diagnosis of several neonatal pathologies [8]. The need for a highly sensitive and predictive test in the neonatal period for identifying and correctly classifying the highest possible number of septic neonates make metabolomics a method of great diagnostic potential. Works on metabolomics concerning sepsis conducted on animals and humans have recently been published (Table 1). It is interesting to see in the different studies that among the metabolites that characterize septic patients there are clear analogies. Firstly, both in studies on rats [9,10] and human patients [11–13] increases in metabolites in septic patients compared to controls (in plasma and urine) which are part of the oxidative metabolism of fatty acids (such as hydroxybutyrate, acylcarnitines and acetoacetate) have been found. It is known that fatty acids, once released from adipose tissue and transported into the cytoplasm of hepatic and muscle cells, are activated in the form of esters of coenzyme A. The latter, by means of a complex mechanism of transesterification with carnitine, cross the mitochondrial membrane where they enter the beta oxidation cycle with the formation of acetyl-CoA. The latter is used by the liver for the synthesis of the ketone bodies (3-hydroxybutyrate and acetoacetate) while in muscle tissue it directly enters the cycle of the tricarboxylic acids with freeing of CO₂ and H₂O and formation of ATP. It has been observed that conditions of stress of the organism that cause an increase in lipolysis and/or protein catabolism to satisfy the need for a surplus of energy, as in the case of sepsis, determine an increase in renal excretion of carnitine and other metabolites involved in the oxidative metabolism of fatty acids. In several works [9,12–14], lactate, together with glucose and myoinositol content in the blood and urine of septic patients compared to controls has also been found to be higher. These results can be explained by taking into account that sepsis corresponds

to a systemic inflammation caused by the anomalous presence of bacterial or viral antigens and involves different mechanisms, such as hypoxia and oxidative stress. At the outset of the infection, inhibition of glycogen synthesis is observed and this leads to a major availability of global glucose and greater cell absorption [15]. Glucose absorption appears increased in organs containing a large population of phagocytic cells (liver, spleen, intestine, lungs). Sepsis is also capable of significantly modifying cytoplasmic glycolysis at the transcriptional level. In particular, the key enzymes of glycolysis and those of the mitochondrial respiratory chain are temporarily under-expressed. It has been seen that in the muscle of septic rats the activity of pyruvate dehydrogenase is reduced, at the same time with an increase in the activity of pyruvate dehydrogenase kinase (PDK), its inhibitor. The net result of all these changes is the reduction of the pyruvate that enters the mitochondria while the conversion of pyruvate into lactate is accelerated. This is confirmed by the fact that in patients with septic shock there is an increased use of glucose and an increase in the production of lactate at the anaerobic level [10,14]. Briefly stated, alterations in the glucose metabolism in critical conditions can be seen as a redistribution of glucose consumption from the mitochondrial oxidative phosphorylation to other metabolic pathways, such as the production of lactate and the pentose phosphate pathway. All this would explain the same metabolic pattern found in several septic patients assessed by means of metabolomics. Still few are the works published in the literature on metabolomics and systemic infections in pediatric patients and in particular in neonates. In a quite recent study by Mickiewicz et al. [13] it is demonstrated that the metabolomic approach may be useful in the diagnosis and prognosis of septic shock in pediatric intensive care units (PICUs). The authors examined serum samples collected from 60 patients with septic shock (by Gram- and/or Gram+), 40 patients with SIRS and 40 healthy children by nuclear magnetic resonance spectroscopy spectra. Some of the metabolite concentrations identified in serum samples changed markedly, thus indicating their influence on the separation between patient groups. These metabolites represent a composite biopattern of the pediatric metabolic response to septic shock and might be considered as the basis for a biomarker panel for the diagnosis of septic shock and its mortality in PICUs. In another work by Fanos et al. [12], urine samples of neonates infected by human cytomegalovirus (HCMV) were analyzed by means of ¹H-nuclear magnetic resonance spectroscopy combined with multivariate statistical analysis. The authors suggest

the use of metabolomics as a new and useful instrument also in the study of congenital HCMV infections. In the literature we can also find preliminary data on metabolomics and viral bronchiolitis in the neonate [16]. As concerns bacterial pneumonia infections, it is interesting to see that thanks to the use of metabolomics it has been possible to differentiate the urine metabolic profile in pneumonia caused by pneumococcus from the metabolic profiles of viral pneumonia and other bacterial forms [17].

Conclusions

Present-day methods and procedures for the diagnosis of systemic neonatal infections are hindered by low sensitivity and long response times. They are inadequate to satisfy the need to proceed to a rapid, timely and efficacious therapeutic treatment. Metabolomics is showing promise of becoming a most effective method in neonatology, especially because it not only allows early diagnosis of different pathologies and their monitoring, but is even capable of predicting their onset and the capacity to respond to therapy, thus making possible a tailored management of complex pediatric disorders. Taking into account all the studies published up to now, metabolomics appears to be a promising and useful instrument also in the diagnosis of sepsis. However, it will be necessary in the future to confirm these results by means of further studies so as to arrive at a more detailed picture from the metabolic standpoint, especially in systemic neonatal infections.

Conflict of interest

The authors declare that they do not have conflict of interest.

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