**Význam laktátu v diagnostice
mitochondriálních onemocnění u dětí**

**Role of lactate in diagnostics
of mitochondrial disorders in children**

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2011

Doktorské studijní programy v biomedicíně
Univerzita Karlova v Praze a Akademie věd České republiky

Obor: Biochemie a patobiochemie

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ABBREVIATIONS

A	acetoacetate
ADP	adenosine diphosphate
ATP	adenosine triphosphate
ATP6	ATP synthase 6
ATP12	ATP synthase, mitochondrial F_1 complex, assembly factor 2
B	3-hydroxybutyrate
BCS1	*S. cerevisiae* bcs1 protein
COX I, II	cytochrome c oxidase subunit I, II
COX 10, 15	cytochrome c oxidase assembly protein 10, 15
CPEO	chronic progressive external ophtalmoplegia
CSF	cerebrospinal fluid
CT	computer tomography
cyt	cytochrome
DGUOK	deoxyguanosine kinase
DNA	deoxyribonucleic acid
DIDMOAD	diabetes insipidus, diabetes mellitus, optic atrophy, deafness
FAD	flavin adenine dinucleotide
IMM	inner mitochondrial membrane
IF	impact factor
KSS	Kearns-Sayre syndrom
L	lactate
L/P	lactate/pyruvate ratio
LDH	lactate dehydrogenase
LHON	Leber hereditary optic neuropathy
LRPPRC	leucine-rich PPR motif-containing protein
MELAS	mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes
MERRF	myoclonic epilepsy associated with ragged-red fibers
MRI	magnetic resonance imaging
MTCYB	cytochrome b of complex III
mtDNA	mitochondrial deoxyribonucleic acid
NAD^+	nicotinamide adenine dinucleotide
NARP	neuropathy, ataxia, and retinitis pigmentosa
ND 3, 5, 6	complex I, subunit 3, 5, 6
nDNA	nuclear deoxyribonucleic acid
OGTT	oral glucose tolerance test
OMM	outer mitochondrial membrane

OXPHOS	oxidative phosphorylation
P	pyruvate
PC	pyruvate carboxylase
PDHA1	pyruvate dehydrogenase complex, E1-alpha polypeptide 1
PDHB	pyruvate dehydrogenase complex, beta polypeptide
PDHc	pyruvate dehydrogenase complex
PDHE3	dihydrolipoamide dehydrogenase (DLD), pyruvate dehydrogenase component E3
PEO	progressive external ophtalmoplegia
POLG1	DNA polymerase gamma
ROS	reactive oxygen species
SDHA	succinate dehydrogenase, subunit A
SDHC	succinate dehydrogenase, subunit C
SDHD	succinate dehydrogenase, subunit D
SANDO	sensory ataxic neuropathy, dysarthria, ophthalmoplegia
SUCLA2	succinate-coenzyme-A ligase
SURF1	Surfeit 1
TIM	translocase of the inner mitochondrial membrane
TK2	mitochondrial thimidine kinase
TMEM70	transmembrane protein 70
TOM	translocase of the outer mitochondria membrane
tRNA	transfer RNA
UCP	uncoupling protein
UQCRB	ubiquinone-binding protein
UV	ultraviolet light

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1. ABSTRACT / ABSTRAKT

The lactate level assesment in various body fluids plays an important role in the diagnostics of mitochondrial disorders in children. However, the interpretation of lactate level is often difficult due to its unspecificity and variability even in particular mitochondrial disorders. Three specific aims have been stated in this PhD Thesis: 1. To analyse the role of lactate examination in the differential diagnosis between children with mitochondrial disorders and children with other diseases. 2. To study the lactate level differences in various mitochondrial syndromes. 3. To characterise the clinical and laboratory data of neonates with mitochondrial disorders and to suggest new diagnostic algorhytms. Clinical and laboratory data from patients hospitalized in the Department of Pediatrics were collected. Laboratory methods were provided in the cooperation with the Mitochondrial laboratory of the Department of Pediatrics and Institute of Inherited Metabolic Disorders. The study with lactate levels in 107 patients documented that brief seizures lasting less than 2 minutes did not increase lactate concentration in the CSF. CSF-lactate was a relialable marker in differential diagnosis in the children with mitochondrial disorders against children with epilepsy. 2. The severity of particular phenotype is more important for the severity of the lactic acidosis than the particular syndrome. 3. The diagnostic algorhytms for neonates with suspicion of mitochondrial disorder were proposed. It is without any doubt that lactate in an important biochemical marker of children with mitochondrial disorder. This PhD Thesis evaluated some aspects of the role of lactate in mitochondrial disorders in order to help better interprete its values.

Vyšetření hladin laktátu v krvi, likvoru a v moči zastává důležitou roli v diagnostice mitochondriálního onemocnění u dětí. Interpretace výsledků je však často obtížná pro nespecifičnost a variabilitu i u jednotlivých mitochondriálních poruch. V disertační práci byly stanoveny tři specifické cíle: 1. Analyzovat význam vyšetření laktátu v diferenciální diagnóze mezi dětmi s mitochondriálním onemocněním a dětmi s jinými chorobami. 2. Charakterizovat hladiny laktátů u různých mitochondriálních syndromů. 3. Popsat klinické a laboratorní data novorozenců s mitochondriálním onemocněním a navrhnout diagnostické algoritmy pro tuto věkovou skupinu. Byl zpracován klinický průběh a výsledky dětí vyšetřovaných na Klinice dětského a dorostového lékařství (KDDL). Laboratorní vyšetření byly provedeny v spolupráci s Mitochondriální laboratoří KDDL a s Ústavem dědičných a metabolických poruch. 1. Výsledky studie s vyšetřením laktátu u 107 dětí prokázaly, že krátké křeče trvající méně než 2 minuty nezvýšily koncentraci laktátu v likvoru. Laktát v likvoru tak byl spolehlivým markerem v diferenciální diagnostice dětí s mitochondriální poruchou od dětí s epilepsií. 2. Závažnost příslušného fenotypu je pro tíži laktátové acidózy důležitější než vlastní syndrom. 3. Byly navrženy diagnostické algoritmy pro novorozence s podezřením na mitochondriální onemocnění. Laktát je důležitým biochemickým markerem dětí s mitochondriálním onemocněním. Tato disertační práce analyzuje některé aspekty jeho vyšetření s cílem zlepšit interpretaci získaných hodnot.

2. INTRODUCTION

Mitochondrial research has started about 170 years ago. The earliest records on intracellular structures that probably represent mitochondria go back to the 1840s. Mitochondria were probably first recognized by A. Kölliker in the middle of the 19th century (Kölliker 1856). The name originates from the Greek "mitos" (μίτος, thread) and „chondros" (χονδρίον, granule) (Ernster and Schatz 1981).

Early studies on cell respiration and oxidative phosphorylation were performed from the early 1910s. In guinea-pig liver extracts, Warburg reported in 1913 that cell oxidative reactions are associated with insoluble cellular structures. In 1925, Keilin described the cytochromes. This discovery led to the definition of the respiratory chain as a sequence of catalysts comprising the dehydrogenases on one end and "respiratory enzyme" (Atmungsferment according to Warburg) on the other (Keilin 1925). Mitochondrial research was initially focused on studying of fundamental metabolic and bioenergetic functions (oxidative phosphorylation, OXPHOS), Krebs cycle, heme biosynthesis, β-oxidation of fatty acids, and the metabolism of certain amino acids (Ernster and Schatz 1981). Several other research areas have grown important: e.g. targeting and import of proteins into mitochondria (Neupert and Herrmann 2007), biogenesis of Fe/S clusters (Lill and Kispal 2000), or the role of mitochondria in controlling apoptosis in higher eukaryotes (Jiang and Wang 2004). Mutations in mitochondrial DNA and other mitochondrial defects are to higher and higher extent linked to a wide range of human diseases: numerous neuropathies and myopathies (Wallace 2005), premature ageing of mice (Trifunovic et al. 2004) and many others.

Mitochondria are highly dynamic and remarkably plastic structures. They have a great ability to change their shape, fuse with one another and then separate again (Soubannier and McBride 2009). They are not present in the form of many individual organells in the cell but they constitute dynamic mitochondrial networks (Bereiter-Hahn and Voth 1994; Soubannier and McBride 2009). The structure and function of mitochondria also differ in particular tissues depending on the energetic demands of such tissue (Benard et al. 2006). The system of oxidative phosphorylation (OXPHOS) serves as a major energy-supplying process, which utilises redox

equivalents (NADH and FADH$_2$) raised by substrates oxidation to ATP. Mitochondria and OXPHOS are functionally controlled by two genomes: nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) as the only exception in the biology of man. Mitochondrial disorders represent a clinically, biochemically and genetically heterogeneous group of diseases associated with dysfunction of the oxidative phosphorylation system (OXPHOS). In these disorders, organs with the highest energy demand (brain, heart and skeletal muscles) are predominantly affected (Wallace 1999). The first mitochondrial disorder was recognized in 1962, when Swedish endocrinologist Rolf Luft described a young woman with severe hypermetabolism not due to thyroid dysfunction (Luft et al. 1962). More than 350 mutations, structural changes and deletions in mtDNA are known at present. These are responsible for more than 30 defined mitochondrial syndromes and diseases. (Tuppen et al. 2010). Mutations in nuclear DNA have been found in other more than 80 mitochondrial diseases (Spinazzola and Zeviani 2007). More than 119 defined diseases of mitochondrial energetic metabolism are thus known. (OMIM; www.ncbi.nlm.nih.gov/omim). Elliot and colleagues (Elliott et al. 2008) showed that the prevalence of pathogenic mtDNA mutations is at least 1 to 200. However, the prevalence of mitochondrial disorders, which includes both of mtDNA and nDNA disorders, is estimated to 1 to 5,000 (Skladal et al. 2003; Thorburn 2004; Taylor and Turnbull 2005).

The clinical manifestation of mitochondrial diseases is very heterogenous. The presentation usually depends on generalised or tissue-specific decrease in ATP production. Some mitochondrial disorders affect a single organ (e.g., the eye in Leber hereditary optic neuropathy), but many involve multiple organ systems. Virtually any organs may be impaired, but the organs with highest energetic demands are typically involved, including brain, muscle, heart and liver (Scaglia et al. 2004; Bohm et al. 2006). Mitochondrial diseases may manifest at any age (Leonard and Schapira 2000). Clinical spectrum covers prenatal complications, acute neonatal metabolic disroder, manifestation in childhood but also in adult age.
   The clinical presentation and course of patients with mitochondrial syndromes are extremely diverse, even among patients with identical enzymatic or genetic defects. The range of clinical manifestations

of mitochondrial disorders is broad and includes almost all CNS functions, visual and hearing, heart and skeletal muscle, gastrointestinal tract, kidneys, endocrine glands and haematological changes. Many mitochondrial disorders may manifest as a characteristic cluster of clinical features (DiMauro and Schon 2001; Munnich and Rustin 2001). These include e.g. Kearns-Sayre syndrome (KSS), chronic progressive external ophtalmoplegia (CPEO) (Moraes et al. 1989), mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS) (Hirano et al. 1992), myoclonic epilepsy with ragged-red fibres (MERRF) (Hammans et al. 1993), neurogenic weakness with ataxia and retinitis pigmentosa (NARP) (Holt et al. 1990), or Leigh syndrome (Ciafaloni et al. 1993).

Mitochondrial diseases may manifest at any age (Leonard and Schapira 2000). Clinical spectrum covers prenatal complications, acute neonatal metabolic disroder, manifestation in childhood but also in adult age. Until recently it was generally thought that the mitochondrial disorders due to mutations in nuclear genes manifest in childhood and disorders due to mtDNA mutations demonstrate in late childhood or adult life (Skladal et al. 2003; Bohm et al. 2006). However, recent advances have shown that many mtDNA disorders present in childhood, and many nuclear genetic mitochondrial disorders manifest in adult life (Finsterer 2006A).

For a diagnostics of mitochondrial disorders it is necessary to take in account the particular family and personal history, the course of the disease, the comprehensive clinical examination, results of specialized examinations (especially cardiology, visual fundus examination, brain imaging, EMG,), the basic but also laboratory analyses in body fluids, and examination of bioptic samples of muscle, skin, and eventually liver. The definitive diagnosis is confirmed by the molecular-genetic examination.

The most prominent biochemical marker of mitochondrial disorders is elevated lactic acid level in body fluids.

Lactate and mitochondrial disorders

Lactate (2-hydroxypropanoic acid) is the end product of anaerobic glucose metabolism. It is further utilized either via oxidation within the citric acid cycle, depending on the presence of oxygen and/or

well functional oxidative phosphorylation, or *via* gluconeogenesis within the Cori cycle. Lactate thus represents the link between aerobic and anaerobic metabolism (Thomas 1998).

Lactate metabolism is regulated very precisely; therefore, in healthy children, the lactate concentration in body fluids only rarely exceeds the reference range (Benoist et al. 2003; Robinson 2006). The plasma lactate level reflects the equilibrium between its production and its comsuption by different tissues. Elevated blood lactate concentrations can be found in cases of inappropriately high accumulation or disturbed utilization (Thomas 1998).

Lactate is generated by the reduction of pyruvate. Lactate level thus depends on 1) pyruvate level and 2) shift of concentration equilibrium of lactate and pyruvate. The blood lactate-to-pyruvate (L/P) molar ratio reflects the equilibrium between product and substrate of the reaction catalyzed by lactate dehydrogenase. The L/P ratio is correlated with the cytoplasmic $NADH/NAD^+$ ratio and is used as a surrogate measure of the cytosolic oxido-reduction state (Robinson 2006). All circumstances leading to intracellular increase of the pyruvate concentration, the formation of H^+, and the decrease of NAD^+, will raise the concentration of lactate (Stacpoole 1993).

The cumulative total of lactate produced per day glycolytically is between 70 and 110 g for the human body. From red cells and skin come 33.5 g each with another approximately 20 g from skeletal muscle, brain and a small amount from intestinal mucosa (Robinson 2006). The capacity for homeostatic maintenance of lactate is dependent mainly on rates of gluconeogesis in gluconeogenic tissues with some oxidative removal by muscle tissue (Ahlborg and Felig 1982).

Accumulation of lactate in blood to levels higher than 5 mmol/l (Thomas 1998), or more than 7 mmol/l (Vassault 2008) leads to lactic acidosis. The hyperlactataemia and lactic acidosis should be thus distinguished, although due to some simplification this is not always done in clinical praxis. The hyperlactataemia should then represent a condition characterized by an increased lactate concentration and a blood pH≥7.33-7.34 in conjunction with normal (physiological) acid-base status or alkalosis. On the other hand, lactic acidosis occurs with the blood pH≤7.33-7.34, when H^+ accumulation together with increased lactate synthesis cannot be compensated (Thomas 1998).

The different and even more precise terminology is used according to some authors. Hulin discriminates between acidosis, where pH in blood is in normal range, and acidaemia, with pH lower than 7.34. According to this statement, theoretically three possible states of increased lactate could be classified: 1) hyperlactataemia with a mild increase of lactate with no change of blood pH, 2) lactic acidosis as a condition with increased lactate level, which would lead to pH shift, but this is compensated by blood buffer mechanisms, and 3) lactic acidaemia with increased lactate level leading to acidic pH (Maasová 1998).

Increased lactate level is a very common biochemical finding in paediatrics. Lactic acidosis is found both in patients with inherited metabolic diseases and in those with various acquired conditions. It is known to be a presenting feature of many inborn errors of metabolism: organic acidurias, urea-cycle defects (mainly citrulinaemia), fatty acid oxidation defects, disorders of liver glycogen metabolism, disorders of liver gluconeogenesis, the PDHc deficiency, or Krebs-cycle defects; and dysfunction of the oxidative phosphorylation. The latest are considered to be the most common causes of congenital forms of lactic acidosis (Stacpoole et al. 1997).

The differential diagnosis of congenital lactic acidosis was presented by Zeman et al. 1998 (Zeman et al. 1998). They published a retrospective study of 230 children with suspected congenital lactic acidosis. Younger children manifested mainly the CNS involvement, the older children manifested the myopathic symptoms. The inherited metabolic disorder was diagnosed in 49 children, i.e. 21% of the group. The mitochondrial disorder was diagnosed in 23 children. 10 children had an isolated complex IV deficiency, two children had the combined complex I and IV deficiency, three children had the PDHc deficit, and one child had a point mutation in mtDNA with ATP synthase deficit. Seven children had the beta-oxidation disorder (4x MCAD a 3x LCHAD). The liver glycogen storage metabolism was proved in 13 children. Three had organic aciduria, two of them urea cycle deficit (OTC, citrulinaemia) and fructose metabolism disorder was found in three children. The low free and total carnitine level was found in five children as a result of valproate treatment (Zeman et al. 1998).

It is far known that patients with primary disorders of the citric cycle, and oxidative phosphorylation may develop abnormal lactate accumulation. Oxidation of NADH and $FADH_2$ produced by the PDHc reaction and the tricarboxylic acid cycle is hampered by impaired electron transfer through the respiratory chain. The consequent rise in the intramitochondrial $NADH/NAD^+$ ratio inhibits PDHc activity. Pyruvate oxidation decreases, and pyruvate is converted to lactate, resulting in an increased lactate/pyruvate ratio. Another important consequence is the decrease of the ATP production (Stacpoole et al. 1997).

The majority of proven mitochondrial oxidative disorders present with a raised blood or CSF lactate. It is a common presenting feature in some mitochondrial disorders (MTDs), intermittent in others and for some of MTDs, it is not a presenting feature at all (Robinson 1993; Scaglia et al. 2004; Bohm et al. 2006; Robinson 2006). The lactate level analysis thus plays a major role in the diagnostics of mitochondrial disorders on the biochemical level (Morava et al. 2006).

In general, the more severe the defect is, the more likely it is to display an increased lactate in body fluids (Robinson et al. 1990). Increased blood lactate was found in 85% and increased blood alanine in 65% of 180 children with COX deficiency. The CSF lactate level was elevated in 81% of examined cases (Bohm et al. 2006). Only 60% of 113 patients with mitochondrial disorders had elevated blood lactate sampled on >1 occasion in paper published by Scaglia and colleagues (Scaglia et al. 2004).

A normal level of blood lactate in a child with clinical suspicion of mitochondrial disorder thus does not exclude mitochondrial etiology of his disease. (Stern 1994; Magner et al. 2011A). Repeated blood lactate assessment is thus important in children for whom clinical suspicion of mitochondrial disorder exists but who have presented normal lactate levels in previous analyses.

There are some studies indicating that lactic acidosis occurs predominantly in patients with mitochondrial diseased based rather on mtDNA than nuclear DNA defects. This is illustrated by study of Bohm et al. who found elevated lactate levels regularly in patients with combined respiratory chain defects and known mtDNA mutations (79 patients) (Bohm et al. 2006). On the other hand, there are mitochondrial diseases, such as NARP and its infantile variant maternally inherited Leigh syndrome, in which blood lactate is often normal or mildly increased at best (DiMauro et al. 2004). There are also literary evidences, that lactic acidosis can be present also in

syndromes caused by specific nuclear genes mutations. For syndromes caused by specific nuclear genes mutations. For example in the study of (Cizkova et al. 2008), lactic acidosis was present in all 27 patients with mutation in the nuclear gene TMEM70 coding an assembly protein for ATP synthase. Anyway, presence of lactic acidosis/elevated lactate concentrations is relative more constant in some syndromes than others. For example, severe infantile lactic acidosis (>5 mmol/l) is typical for patients with severe manifestation of PDHA1 and DLD deficiencies, severe nDNA or mtDNA defects of complex I, severe type B pyruvate carboxylase deficiency and SURF1 defects accompanied by COX deficiency (Robinson 2006). Degree of lactate level elevation is also dependent on severity of syndrome manifestation in every single patient.

3. AIMS OF THE THESIS

The heterogeneity of mitochondrial disorders is a challenge for routine diagnosis. Because of the highly variable phenotypes, the clinical suspicion of a mitochondrial disorder is often delayed, or the disease remains undiagnosed. The golden diagnostic examination standard upon suspicion of mitochondrial disorder remains the skeletal muscle biopsy with histological and biochemical analysis. However, muscle biopsy represents an invasive procedure, with an additional risk for patients with a dysfunction in oxidative phosphorylation, severe muscle hypotonia, cardiac symptoms, and CNS abnormalities. Therefore it is very important to evaluate the chance for succesful diagnosis prior to deciding on muscle biopsy.

To fulfill this statement it is necessary to characterise the natural course of the mitochondrial disorders in childhood. The aim of this PhD Thesis is a brief contribution in to this effort with special focus on importance of lactate analyses in body fluids.

Three specific aims have been stated in this work:

A.   To analyse the role of lactate examination in the differential diagnosis between children with mitochondrial disorders and children with other diseases.

The mitochondrial disorders and some neurological or inherited metabolic disorders may present a very similar clinical manifestation. Especially the discrimination from children with epilepsy is very important as a substantial part of mitochondrial children manifests seizures. Lactate as a possible biomarker thus could help to indicate the muscle biopsy in proper cases and prevent its unnecessary misuse.

B.   To study the lactate level differences in various mitochondrial syndromes.

As lactate level may vary from one mitochondrial syndrome to the other, the aim was to analyse the lactate levels in children with various mitochondrial diseases.

C. To characterise the clinical and laboratory data of neonates with mitochondrial disorders and to suggest new diagnostic algorhytms.

The diagnostics of neonates with metabolic disorders is especially demanding. The symptoms as hypotonia, refusal to eat, failure to thrive, cardiorespiratory failure, are often unspecific. We will try to prepare new diagnostic algorhytms for the diagnostics of mitochondrial disorders in neonatal period

4. METHODS AND MATERIAL

4.1. Patients

During my 5-year stay at the Department of Pediatrics of Charles University I had a possibility to see app. 1000 children with various metabolic disorders. A significant part of them was admitted with a suspicion on mitochondrial disorder. 107 children ranging in the age from 1 month to 13 years were included in the crucial study of the PhD thesis characterising the role of lactate in differential diagnosis of mitochondrial disorders. In this study patients were divided into five groups according to their primary diagnosis.

Group I consisted of 24 children with mitochondrial encephalopathy resulting from isolated or combined deficiency of respiratory chain complexes or ATP synthase; the ages of the children in this group ranged from 1 month to 13 years (M/F 16/8). Mutations in nuclear or mitochondrial genes were identified in half of the patients in this group. All children in group I, but none from any other groups, fulfilled the diagnostic criteria for mitochondrial diseases published by Walker and colleagues. (Walker et al. 1996) Six children had complex I deficiency (NADH:ubiquinone oxidoreductase); eight had complex IV deficiency (cytochrome *c* oxidase), including two with mutations in the SCO2 gene and three with mutations in the SURF1 gene; four children had NARP syndrome (Neurogenic muscle weakness, Ataxia, Retinitis Pigmentosa) with complex V deficiency (ATP synthase); six children had combined respiratory chain complex deficiencies including one patient with MELAS syndrome (Mitochondrial myopathy, Encephalopathy, Lactic Acidosis and Stroke-like episodes), one with MERRF syndrome (Myoclonic Epilepsy, Ragged-Red Fibers), and one with Alpers-Huttenlocher syndrome due to mutations in POLG1 gene. In seven children, the samples for biochemical analysis were obtained after an attack of brief seizures. The duration of seizures was less than 3 min and lumbar puncture was performed between 30 min and 72 h after the seizures. No seizures were observed in the other 17 children.

Group II consisted of 32 children with epilepsy (1 month to 6 years, M/F 23/9). Patients with epilepsy underwent lumbar puncture for purposes of the broad-differential diagnosis mostly due to poorly compensated seizures. No metabolic disorders were recognized.

In 15 children, the samples for biochemical analysis were obtained 3.0 ± 0.6 h after seizures (range between 1 and 8 h). The duration of seizures in the study group was relatively short, ranging from 0.5 min to 2 min. Seizures persisted for more than 30 min, fulfilling status epilepticus criteria, in only one patient. The other 17 children in Group II were without recent history of seizures at the time of analysis.

Group III consisted of 23 children with psychomotor retardation (5 months to 6 years, M/F 15/8) with developmental quotient below 50 points. For children up to 1 year, a delay in achievement of psychomotor milestones greater than three months was considered as a criterion of psychomotor retardation. These patients without history of seizures were ascertained for the suspicion of various hereditary metabolic disorders, none of which were confirmed.

Group IV consisted of 12 children with bacterial meningitis (1 month to 2 years, M/F 4/8) due to Streptococcus pneumoniae (5x), Streptococcus agalactiae (2x), Neisseria meningitidis B (2x), Neisseria meningitidis C (1x), and Escherichia coli (2x), respectively. Control group V was represented by 16 children with acute febrile illnesses or meningism without any laboratory findings of neuroinfection (3 months to 2 years, M/F 9/7).

4.2. Biochemical studies

Lactate

Blood samples for lactate analyses were obtained from patients in groups after overnight fasting without venostasis. For lactate measurement, samples were deproteinized immediately by the addition of two volumes of 8% (v/v) perchloric acid. CSF specimen were obtained by lumbar puncture. The CSF specimen were obtained simultaneously with the blood samples; all lumbar punctures were indicated for diagnostic purposes by consultants. All specimen were transported on the wet ice to the laboratory, where the analyses were performed immediately. Lactate levels were measured at the Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague and General University Hospital in Prague (IIMD). A spectrophotometric method

based on the lactate dehydrogenase-catalyzed oxidation of lactate to pyruvate, in which NADH is formed, was used. In this method, the NADH absorbance (measured at 340 nm) is proportional to the lactate concentration in the sample. The analytical variation coefficients at 1.5 and 5.2 mmol/l were 2.4%, and 2.2%, respectively (Noll 1984). Pathological concentrations of metabolites were defined as values higher than: blood lactate >2.3 mmol/l, CSF lactate >2.1 mmol/l. CSF samples with blood contamination were removed from the study with exclusion criterion of more than 50 erythrocytes/3 µl.

Amino acids and organic acids

Amino acids in serum and CSF were analyzed by ion exchange chromatography with ninhydrin detection on an automatic amino acid analyzer (AAA 400, Ingos, Czech Republic) at IIMD. Aliquots of serum (300 µl) were diluted with lithic buffer (composition: citrate, lithium citrate and lithium chloride, pH 4.8), 4-chlorphenylalanine as internal standard and deproteinized by adding sulfosalicylic acid (final volume 500 µl). The analytical CVs at 250 and 500 µmol/l were 6.1%, and 6.0%, respectively (Hyánek 1991).

The profile of organic acids in urine was analyzed by gas chromatography and mass spectrometry (Chalmers 1982).

4.3. Enzymatic and protein studies

The muscle mitochondria gained by biopsy or autopsy were isolated according to Makinen and Lee without use of protease (Makinen et al. 1968) and stored in the liquid nitrogen.

The respiratory chain enzymes activities, NADH-coenzym Q10-oxidoreductase (NQR, complex I), succinyl-coenzyme Q10-oxidoreductase (SQR, complex II), coenzym Q10-cytochrome c oxidoreduktase (QCCR, complex III), and cytochrome *c* oxidase (COX, complex IV) were measured spectrophotometrically (Pelley et al. 1976; Rustin et al. 1994).

Specific oligomycin-sensitive F_1Fo-ATP synthase hydrolytic activity was measured in ATP generating systems as described by (Solaini et al 1984). The steady-state levels of F_1Fo-ATP synthase were determined by blue-native polyacrylamide gel electrophoresis

(BN-PAGE, Schägger and von Jagow 1991) followed by Western blot using monoclonal mouse antibodies against ATP synthase alpha subunit (Mitosciences) (Fornůsková et al 2008).

PDH complex activity and E1 subunit activity were assayed as the release of $^{14}CO_2$ from [^{14}C]pyruvate. The activity of PDH complex in the lymphocytes was measured according to Sheu et al. (1981), and in isolated muscle mitochondria according to Constantin-Teodosiu et al. (1991). The activity of the E1 subunit of the PDH complexes was measured according to Van Laack et al. (1988). The amounts of pyruvate dehydrogenase complex (PDHc) were estimated by Western blot.

The activity of control enzyme citrate synthase was determined spectrophotometrically (Srere 1969). Protein content was determined by the method of Lowry et al. (1951).

4.4. Molecular-genetic analyses

DNA was isolated from muscle and/or blood by method of phenol extraction. The presence of prevalent mutations was analyzed by PCR-RFLP, mitochondrial DNA and selected nuclear genes were studied by method of direct sequenation at the ABI 3100 Avant analysator (Applied Biosystems).

4.5. Statistics

Data was analyzed with Kruskal-Wallis one-way analysis of variance on ranks with Dunn's method used for pair-wise multiple comparison. To compare subgroups, Student's t-test was used. If not specified, P < 0.05 was considered as significant. Statistical analysis was performed using the SigmaStat 3.5 program (Systat Software, Inc.).

4.6. Ethics

All studies with children were approved by the Committees of Medical Ethics at all collaborating institutions. Informed consent was obtained from parents.

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5. RESULTS

5.1. The role of lactate analysis in the differential diagnosis between children with mitochondrial disorders and children with other diseases.

The aim of our study was to ascertain the diagnostic value of lactate and alanine analyses in blood (B) and cerebrospinal fluid (CSF) in children with mitochondrial disorders in comparison to children with epilepsy, psychomotor retardation, meningitis and meningism. To address this problem, we ascertained the diagnostic value of lactate and alanine in B and CSF in children with mitochondrial disorders (n=24), epilepsy (n=32), psychomotor retardation (n=23), meningitis (n=12) and meningism (n=16)(Tables 1 and 2).

•   The most important fact showed in our study is that relatively brief seizures lasting less than 2 minutes did not increase lactate concentration in CSF (Figure A). Increased CSF lactate levels were found in 83% of children with mitochondrial disorders but in only 3% of children with epilepsy and 9% of children with psychomotor retardation (c.f. Figure B). Our results thus suggest that CSF-lactate level may serve as a reliable marker discriminating mitochondrial patients with seizures from patients with epilepsy if the sample of cerebrospinal fluid is not obtained within a short time after an attack of prolonged seizures. This fact is important to consider, because a substantial proportion of patients with mitochondrial disorders may manifest seizures.

•   21% of the children with mitochondrial disorders showed only mildly increased CSF lactate levels (between 2.2 and 3.0 mmol/L). This finding may be of special importance, because, in mild elevation of CSF-lactate may be wrongly considered to be a result of seizures or even overlooked.

•   Two-thirds of children with mitochondrial disorders had higher lactate concentrations in CSF than in blood. This confirmed
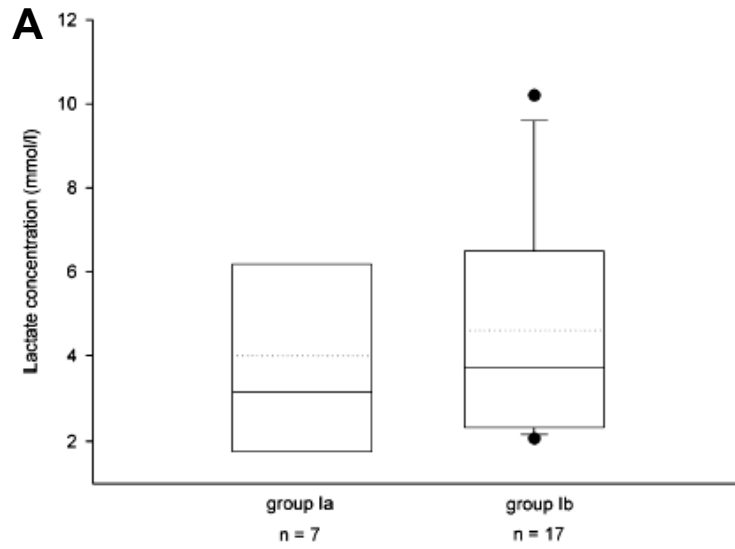
FIGURE A. Levels of lactate in cerebrospinal fluid in 7 mitochondrial patients manifesting seizures (subgroup Ia), in comparison to 17 mitochondrial patients without observed seizures (subgroup Ib). No statistically significant difference between the subgroups was observed (P < 0.621). From Magner et al. 2011.

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B

(y-axis: Lactate concentration (mmol/l), scale 0 to 14)

Group I
n = 24

Group II
n = 32

Group III
n = 23

Group IV
n = 12

Group V
n = 16

* P<0.001 in Group I and Group IV compared to Groups II, III and V
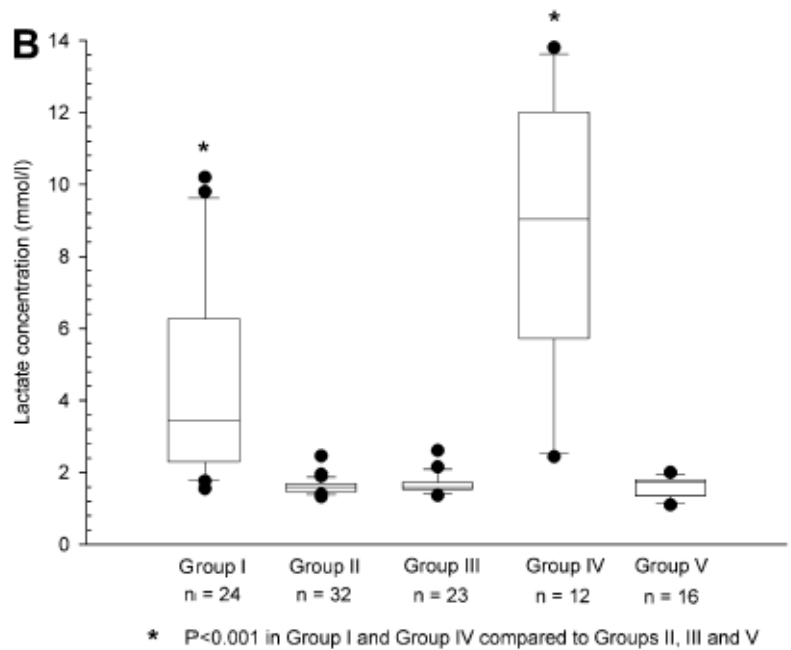
FIGURE B. The levels of lactate in cerebrospinal fluid in 24 children with mitochondrial disorders (group I) in comparison to 12 children with meningitis (group IV), 32 children with epilepsy (group II), 23 children with psychomotor retardation (group III) and 16 children with meningism (group V). From Magner et al. 2011.

Tables 1 and 2. Clinical characteristics of the study polulation. From Magner et al. 2011.

Table 1 – Blood lactate and cerebrospinal fluid lactate and alanine in 24 children with mitochondrial disorders. Seizure duration was a maximum of 3 min.

No.	Patient age (gender)	biochemical diagnosis	molecular diagnosis	seizure description	CSF obtained after seizures	B-lactate (mmol/l; controls ≤2.3 mmol/l)	CSF-lactate (mmol/l; controls ≤2.1 mmol/l)	CSF-alanine (μmol/l; controls ≤35 μmol/l)
1.	2 m (M)	complex I def.	n.k.	clonic	30 h	1.2	1.76	34
2.	4 m (M)	complex I def.	n.k.	clonic	72 h	5	3.17	n.d.
3.	4.5 m (F)	complex I def.	n.k.	clonic	45 min	1.93	1.79	47
4.	8 m (F)	complex I def.	n.k.	n.o.	–	1.8	2.53	33
5.	2.3 y (M)	complex I def.	n.k.	n.o.	–	0.9	2.36	25
6.	3.5 y (M)	complex I def.	n.k.	n.o.	–	4.0	2.19	26
7.	1 m (M)	complex IV def.	n.k.	n.o.	–	10.1	9.85	55
8.	7 m (F)	complex IV def.	n.k.	n.o.	–	5.9	6.59	70
9.	1.3 y (M)	complex IV def.	n.k.	n.o.	–	1.95	2.3	19
10.	6 m (M)	complex IV def.	SCO2	clonic	30 min	2.1	3.7	125
11.	9.5 m (F)	complex IV def.	SCO2	n.o.	–	2.1	3.72	n.d.
12.	7 m (M)	complex IV def.	SURF1	n.o.	–	6.6	6.3	80
13.	1.4 y (F)	complex IV def.	SURF1	n.o.	–	2.56	4.89	25
14.	2 y (F)	complex IV def.	SURF1	n.o.	–	3.2	4.61	30
15.	3.5 m (F)	complex V def.	NARP	n.o.	–	6.7	7.46	n.d.
16.	6 m (M)	complex V def.	NARP	tonic and clonic	1 h	3.13	6.18	57
17.	1.3 y (M)	complex V def.	NARP	n.o.	–	2.1	5.2	84
18.	5 y (M)	complex V def.	NARP	n.o.	–	5.34	3.13	52
19.	3.5 m (M)	combined def.	n.k.	tonic and clonic	1 h	2.23	1.55	29
20.	3.5 m (F)	combined def.	n.k.	n.o.	–	7.5	10.2	137
21.	1.3 y (M)	combined def.	n.k.	n.o.	–	2.8	2.07	11
22.	13 y (M)	combined def.	MELAS	tonic-clonic	48 h	5.1	9.8	n.d.
23.	1 y (M)	combined def.	MERRF	n.o.	–	6.34	3.11	39
24.	1 y (M)	combined def.	POLG	dysrhmsia	–	2.3	2.3	32

complex I – NADH-ubiquinone oxidoreductase.
complex IV – cytochrome c oxidase.
complex V – ATP synthase.
SCO2 – SCO cytochrome oxidase-deficient homolog 2 (yeast) gene.
SURF1 – surfeit 1 gene.
POLG – polymerase (DNA directed), gamma gene.
TMEM70 gene – transmembrane protein 70.
NARP – Neurogenic muscle weakness, Ataxia, Retinitis Pigmentosa syndrome.
MELAS – Mitochondrial myopathy, Encephalopathy, Lactic Acidosis and Stroke-like episodes.
MERRF – Myoclonic Epilepsy, Ragged-Red Fibers.
n.k. – not known.
n.d. – not determined.
n.o. – not observed.

Table 2 – Blood and cerebrospinal fluid levels of lactate in children with epilepsy obtained from samples taken between 1 and 8 h after seizures.

No.	patient age (gender)	main epileptic diagnosis	seizure description	seizure duration (min)	CSF obtained after seizures (hours)	B-lactate (mmol/l; controls ≤2.3 mmol/l)	CSF-lactate (mmol/l; controls ≤2.1 mmol/l)
1.	2.5 m (M)	migrating partial infantile seizures	clonic	0.5	1	1.3	1.39
2.	5.5 m (M)	local seizures with complex symptomatology	tonic	0.5	2	2.0	1.65
3.	4 m (M)	hypoxic-ischemic encephalopathy	infantile spasms	1	3	0.8	1.33
4.	4.5 m (M)	hypoxic-ischemic encephalopathy	clonic	0.5	8	2.1	1.46
5.	16 y (F)	complicated febrile seizures	clonic	2	2	2.4	1.58
6.	5 m (M)	West syndrome – unknown etiology	infantile spasms	1	5	2.6	1.56
7.	3 y (M)	first manifestation of partial epilepsy with secondary generalization	tonic-clonic	>30 min	3	1.3	2.46
8.	4 m (M)	epileptic encephalopathy	clonic	2	6	1.7	1.72
9.	14 y (M)	hypoxic-ischemic encephalopathy	tonic	1	1	1.3	1.49
10.	5 m (F)	first manifestation of partial epilepsy with secondary generalization	clonic	1.5	1	3.0	1.78
11.	5 y (F)	secondary epilepsy after ischemic encephalopathy due to cardiorespiratory failure in pneumococcal pneumonia	tonic-clonic	2	1.5	3.2	1.6
12.	6 y (F)	treatment-resistant epilepsy; cerebral congenital defect	tonic-clonic	1	1	0.7	1.46
13.	15 y (M)	first manifestation of partial epilepsy with secondary generalization	clonic	1	5	2.0	1.69
14.	1 m (M)	cortical heterotopia	clonic	1	2	3.0	1.47
15.	5.5 y (M)	Lennox-Gastaut syndrome	tonic-clonic	2	4	0.8	1.4

the previously known fact, that CSF lactate examination is of higher sensitivity than that of blood lactate levels examination.

• The last important result of our study was the confirmation of the role of alanine assessment. Although no correlation was found between lactate and alanine levels in CSF, alanine concentration in blood was increased in 54% of mitochondrial patients but in only 3% of children with epilepsy and 4% of children with psychomotor retardation. Our results were thus in accord with the third diagnostic protocol of mitochondrial disorder assessment published by Wolf and Smeitink (Wolf and Smeitink 2002), in which an absolute elevation in alanine above 450 μmol/l is a factor utilized to determine the likelihood of mitochondrial disease.

5.2. The lactate level differences in various mitochondrial syndromes

SURF1 missense mutations promote a mild Leigh phenotype
Piekutowska-Abramczuk D, <u>Magner M</u>, Popowska W, Pronicki M, Karczmarewicz E, Sykut-Cegielska J, Krmiec T, Jurkiewicz E, Szymanska-Debinska T, Bielecka L, Krajewska-Walasek M, Vesela K, Zeman J and Pronicka E
Clin Genetics 2009;76:195-204. IF 3.206

SURF1 gene mutations are the most common cause of Leigh syndrome (LS), a rare progressive neurodegenerative disorder of infancy, characterized by symmetric necrotizing lesions and hypervascularity in the brainstem and basal ganglia, leading to death before the age of 4 years. Most of reported mutations create premature termination codons, whereas missense mutations are rare. The aim of this study was to characterize the natural history of Leigh syndrome patients carrying at least one missense mutation in the SURF1 gene. Nineteen such patients (8 own cases and 11 reported in literature were compared with a reference group of 20 own c.845_846delCT homozygous patients, and with other LS SURF- cases described in the literature. Disease onset in the studied group was delayed. Acute failure to thrive and hyperventilation were rare, respiratory failure did not appear before the age of 4 years. Dystonia, motor regression and eye movement dissociation

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developed slowly. The number of patients who survived 7 years of life totaled 9 of 15 (60%) in the ´missense group´ and 1 out of 26 (4%) patients with mutation s leading to truncated proteins.

The study showed that:

•	The presence of a missence mutation in the SURF1 gene may correlate with a milder course of the disease and longer survival of Leigh patients

•	Normal magnetic resonance imaging (MRI) findings, normal blood lactate value, and only mild decrease of cytochrome c oxidase (COX) activity are not sufficient reasons to forego SURF1 mutation analysis in differential diagnosis.

Two patients with clinically distinct manifestation of pyruvate dehydrogenase deficiency due to mutations in PDHA1 gene
Magner M., Vinšová K., Tesařová M., Hájková Z., Hansíková H., Wenchich L., Ješina P., Smolka V., Adam T., Vaněčková M., Zeman J. and Honzík T.
Prague Med Rep. 2011;112:18-28.

In this study we presented first two patients with PDHc deficit due to mutations in PDHA1 gene in the Czech Republic. We documented the broad variability of clinical symptoms of this disease. Although the Leigh syndrome was diagnosed in MRI examination in both patients, the clinical course differed significantly. In patient 1, the initial hypotonia with psychomotor retardation was observed since early infancy. The child gradually showed symptoms of spasticity and arrest of psychomotor development. In patient 2, the disease manifested by seizures and hyporeflexia in toddler age. The diagnosis was confirmed at the age of seven years after attacks of dystonia and clinical manifestation of myopathy with normal mental development.

•	The increased blood and cerebrospinal fluid lactate levels were present in both our patients. In concordance with other studies, biochemical investigations did not help to predict the phenotype of patients, as the correlation between biochemical findings and clinical manifestation is poor and even impossible in the PDH deficiency (Robinson et al. 1987). The patients with various severity of clinical impairment may show mild elevation of lactate and alanine levels

ratios indicate a suspicion on PDHc deficit in both our patients using Barnerias´s criteria.

• Enzymatic analyses revealed PDHc deficiency in isolated lymphocytes in the first but not in the second patient. The direct measurement of PDH E1 subunit revealed deficiency in this individual. We thus proved that normal PDHc activity may not exclude the disease. The literary data on correlation among the PDHc activity, E1αβ protein level and clinical phenotype exists in PDHc deficit were reviewed in the discussion. No clear correlation could be found.

• In patient 1, a novel hemizigous mutation c.857C>T (Pro250Leu) was detected in the X-linked PDHA1 gene. Mutation c.367C>T (Arg88Cys) was found in patient 2.

Mitochondrial disorders of respiratory chain complexes in children
J. Zeman, M. Magner
in Praktische Aspekte bei Diagnostik und Therapie von Mitochondriopathien und ketogener Diät. Eds. W. Sperl, J. Mayr. Heilborn: SPS Verlagsgesellschaft, 2007. ISBN 978-3-936145-38-0

The text reviews current knowledge of prevalence, clinical spectrum, diagnostic approach and possibilities in the field of mitochondrial medicine in children. In association with biochemical diagnosis, it is emphasized that OXPHOS disorders are often accompanied by excessive production of lactic acid and development of metabolic acidosis. According to our experiences, increased lactate and alanine levels in blood and cerebrospinal fluid are present in more than 80% children, usually with increased lactate/pyruvate ratio.

5.3. The characterisation of clinically and laboratory data of neonates with confirmed mitochondrial disorders. The proposal of new diagnostic algorhytms.

Mitochondrial encephalocardio-myopathy with early neonatal onset due to TMEM70 mutation

Tomáš Honzík, Markéta Tesařová, Johannes A Mayr, Hana Hansíková, Pavel Ješina, Olaf Bodamer, Johannes Koch, Martin Magner, Peter Freisinger, Martina Huemer, Olga Kostková, Rudy van Coster, Stanislav Kmoch, Josef Houštěk, Wolfgang Sperl, Jiří Zeman

The aim of this multi-site survey was to characterise the natural course of a novel mitochondrial disease with ATP synthase deficiency and mutation in the TMEM70 gene. Retrospective clinical data and metabolic profiles were collected and evaluated in 25 patients (14 boys, 11 girls) from seven European countries with a c.317-2A>G mutation in the TMEM70 gene.

In the majority of patients (92%), muscular hypotonia, apnoic spells and acute metabolic distress characterised by lactic acidosis and hyperammonaemia (86%) were present from birth. Artificial ventilation was necessary in 19 neonates. Only in 2 children was the onset of disease delayed until 1–3 months of age. Ten patients died during the first episode of metabolic disturbance within the first 6 weeks of life. Six others died later between 14 months and 4.5 years of age following metabolic deterioration as a result of acute respiratory infection or gastroenteritis. Interestingly, one of a pair of monochorionic–monoamniotic twins died during the neonatal period whereas the other twin was alive at the age of 8 years. Nine patients were alive, the oldest one being 13 years old. Failure to thrive and growth retardation (below the third percentile) were present in all patients surviving the neonatal period. Microcephaly was documented in 59% of all patients. Mild cranio-facial dysmorphy with low set ears, a prominent nasal bridge and retrognathia were apparent in 16/24 patients. In P23 (compound heterozygote), apart from the early neonatal onset similar to the other patients, the course of the disease was much milder allowing almost normal psychomotor development with attendance at a regular school.

Clinical and laboratory data in 75 children with neonatal manifestation of mitochondrial disease: proposal for diagnostic algorhytms

Honzík T., Tesařová M., Hansíková H., Wenchich L., Veselá K., Ješina P., Magner M., Zeman J.

The diagnosis of mitochondrial diseases on biochemical and/or molecular level has been confirmed in more than 350 patients during last 18 years at our department. 75 of them manifested symptoms at the neonatal age. Aim of our work was to characterise the clinical and laboratory presentations of mitochondrial diseases at the neonatal age and to propose the algorythms for the diagnosis of mitochondrial diseases at this age.

•   The complex I deficiency was found in 5 children, complex IV deficiency in 40 children, ATP-synthase deficiency in 26 children, the PDHc deficiency in 2 children and 2 children had a combined respiratory chain deficiency.

•   ATP-synthase deficiency due to mutations in TMEM70 gene was found in 18 children, the 8993A>G mutation (NARP syndrome) was confirmed in 7 children. The mutation analysis confirmed the diagnosis of Barth syndrome in one child. We found the mutations in SCO2 and SCO1 genes, which encode the assembly proteins for complex IV, in 5 patients with complex IV deficiency. Pearson and Alpers-Huttenlocher syndrome were found in 2 children with the combined respiratory chain deficiency. The mutations in E3 subunit of PDHc were found in 2 children.

•   A substantial lactic acidosis was present in 93 % neonates immediately after delivery. Hyperammonaemia was documented in 22% of children, mostly in children with Tmem70 protein deficit, in some children with isolated complex IV deficiency and in one child with the NARP syndrome. The increased creatin kinase activity was found in 28 % of the neonates. The selective metabolic screening showed the increased alanine concentration in blood and increased excretion of citric cycle intermediates (malate, fumarate, citrate,

succinate, akonitate, 2-oxoglutarate). All neonates with Tmem70 protein deficit had the increased elevation of 3-methylglutaconic acid.
•   The diagnostic algorhytms (Fig. 1) for the diagnostics of neonates with suspicion of mitochondrial age were proposed.
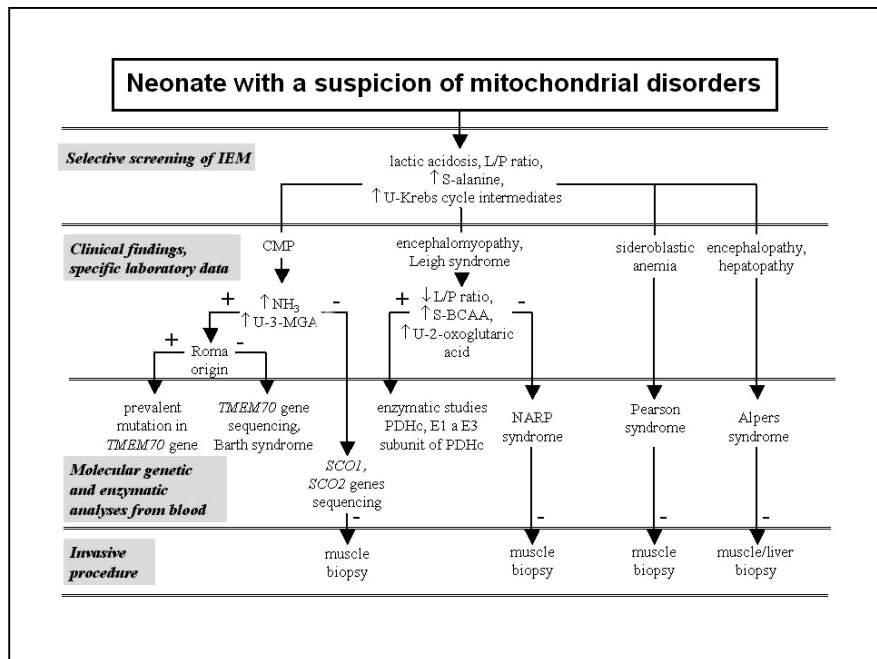

Fig.1. Diagnostic algorhytm for a neonate with a suspicion on mitochondrial disorder. According to Honzik et al. 2010. IEM – inborn errors of metabolism, CMP – cardiomyopathy, U-3-MGA – 3-methylglutaconic acid in urine, S-BCAA – branched-chain amino acids in serum

6. DISCUSSION

The role of lactate analysis in the differential diagnosis between children with mitochondrial disorders and children with other disease

We showed that elevated cerebrospinal fluid lactate is a more reliable marker of mitochondrial etiology of disease in children than increased blood lactate. Also the discriminative value of the CSF-lactate to other diseases is high – this is valid even after short-lasting seizures. The main reason for this conclusion is that brief seizures lasting less than 2 minutes did not increase lactate concentration in the CSF in our study. This fact is of great importance in the differential diagnosis, as many of children with mitochondrial disorders manifest seizures and are mistreated under the diagnosis of epilepsy.

We also showed, that some children with mitochondrial disorders manifest only mild increase in lactate levels, and that even small increases in lactate require some explanation. Repeated lactate analyses are sometimes necessary, because lactate may be elevated only intermittently in children with mitochondrial disorders.¨

The lactate level differences in various mitochondrial syndromes

There are some mitochondrial diseases, for which the characteristic level of lactate can be found. The good example is the LHON syndrome, in which the increased lactate level is very uncommon. Robinson (Robinson 2006) even classified several mitochondrial disorders according to the severity of lactic acidosis (s.Table 10). However, as the clinical spectrum is very variable in many mitochondrial diseases, we believe in contradiction with Robinson, that the severity of particular phenotype is more deciding for the severity of the lactic acidosis than the particular syndrome.

In many mitochondrial diseases both of the severe forms with early manifestation and significant lactic acidosis, intermediate and mild forms with far milder course were described. As a good example may serve the example of SURF1 deficit. Robinson classified this disorder as the one with typical lactatel level over 5 mmol/l

(Robinson 2006). We demonstrated the group of the patients with milder clinical course and corresponding much lower levels of lactate (Piekutowska-Abramczuk et al. 2009). A PDHc deficit may be also taken as an example. We described two boys with severe and intermediate phenotypes (Magner et al. 2011B), but the clinical variability is much wider (Barnerias et al. 2010).

However, many exceptions may be found in this correlation. This is documented by case reports of two boys with PDHc deficit with quite different course of the disease, but similar levels of lactic acidosis (Magner et al. 2011B). The next very important exception from this rule are mitochondrial syndromes manifesting usually at higher age, in which the typical episodes of metabolic disturbance with severe lactic acidosis are observed, as MELAS (Connolly et al. 2010).

The characterisation of clinically and laboratory data of neonates with confirmed mitochondrial disorders. The proposal of new diagnostic algorhytms.

The early presentation at the neonatal age (and thus very serious manifestation in the most of cases) is in concordance with severe lactic acidosis. This is documented by our works describing patients with mitochondrial disorders, which manifested at the neonatal age (Honzík 2010; Honzik, Tesarova et al. 2010). On contrary, in the milder forms of the diseases with presentation at higher age, the incidence of lactic acidosis was far lower. Our work with SURF1 patients with missensse mutations illustrate this possibility (Piekutowska-Abramczuk et al. 2009). We may roughly conclude, that the severity of lactic acidosis correlates with the age of the disease onset and thus also with the severity of its clinical course.

We described the group of patients with mostly severe form of TMEM70 protein deficit in patients with manifestation in neonatal age (Honzik et al. 2010). The milder course of the disease has been described, recently (Shchelochkov et al. 2010). The severity of single mutation can be wide spread on the scale of clinical manifestations of the disease. In our study with TMEM70 protein deficit patients with the same mutations had a variable clinical course of the disease. Interestingly, one of a pair of monochorionic-monoamniotic twins died during the neonatal period whereas the other twin was alive at the age of 8 years. General outcome is

with no doubts affected also by allelic configuration of th genome and/or many epigenetic factors.

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The practical impact of the work is the proposal of the diagnostic algorythms for the newborns with suspicion on mitochondrial disorder.

7. CONCLUSION AND PRACTICAL IMPACT OF PHD THESIS

Lactate is an important biochemical marker of children with mitochondrial disorder. The main aim of this PhD Thesis was to evaluate some aspects of the role of lactate in mitochondrial disorders in order to help to better indicate and interprete its values. The most important results could be summarized as follows:

•   Elevated cerebrospinal fluid lactate is a more reliable marker of mitochondrial etiology of disease in children than increased blood lactate. Also the discriminative value of the CSF-lactate to other diseases except neuroinfection is high – this is valid even after short-lasting seizures. The main reason for this conclusion is that brief seizures lasting less than 2 minutes did not increase lactate concentration in the CSF in our study. This fact is of great importance in the differential diagnosis, as many of children with mitochondrial disorders manifest seizures and are wrongly treated / mistreated under the diagnosis of epilepsy.

•   There are some mitochondrial diseases, for which the characteristic level of lactate can be found. However, as the clinical spectrum is very variable in many mitochondrial diseases, the severity of particular phenotype is more important for the severity of the lactic acidosis than the particular syndrome.

•   The early presentation of mitochondrial disease at the neonatal age was usually connected with severe lactic acidosis. On contrary, in the milder forms of the diseases with later onset, the incidence of lactic acidaemia was lower.

•   The valuable clinical and laboratory data of 75 neonates diagnosed with mitochondrial disorders were evaluated. The special focus was given to the group of children with novel disease - TMEM70 protein deficit. Based on our experiences the diagnostic algorhytms for the newborns with suspicion on mitochondrial disorder were proposed.

8. LIST OF PUBLICATIONS

8.1. Key publications for the PhD Thesis

1. **Magner M**, Szentiványi K, Svandová I, Ješina P, Tesařová M, Honzík T, Zeman J.Elevated CSF-lactate is a reliable marker of mitochondrial disorders in children even after brief seizures. Eur J Paediatr Neurol. 2011;15:101-8. IF 2.007

2. Piekutowska-Abramczuk D, **Magner M**, Popowska E, Pronicki M, Karczmarewicz E, Sykut-Cegielska J, Kmiec T, Jurkiewicz E, Szymanska-Debinska T, Bielecka L, Krajewska-Walasek M, Vesela K, Zeman J, Pronicka E. SURF1 missense mutations promote a mild Leigh phenotype. Clin Genet. 2009;76:195-204. IF 3.206

3. **Magner M.,** Vinšová K., Tesařová M., Hájková Z., Hansíková H., Wenchich L., Ješina P., Smolka V., Adam T., Vaněčková M., Zeman J. and Honzík T. Two patients with clinically distinct manifestation of pyruvate dehydrogenase deficiency due to mutations in PDHA1 gene. Prague Med Rep. 2011;112:18-28.

4. Zeman J and **Magner M**. Mitochondrial disorders of respiratory chain complexes in children. In Praktische Aspekte bei Diagnostik und Therapie von Mitochondriopathien und ketogener Diät. Eds. W. Sperl, J. Mayr. Heilborn: SPS Verlagsgesellschaft, 2007. ISBN 978-3-936145-38-0
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5. Honzík T, Tesarová M, Mayr JA, Hansíková H, Jesina P, Bodamer O, Koch J, **Magner M**, Freisinger P, Huemer M, Kostková O, van Coster R, Kmoch S, Houstêk J, Sperl W, Zeman J.
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6. Honzík T., Tesařová M., Hansíková H., Wenchich L., Veselá K., Ješina P., **Magner M.,** Zeman J. Clinical and laboratory data in 75 children with neonatal manifestation of mitochondrial disease: proposal for diagnostic algorhytms. Čes.-slov. Pediat. 2010;65:7-8:422-431

8.2. Related publications

1. **Magner M**, Krupková L, Honzík T, Zeman J, Hyánek J, Kožich V. Vascular presentation of cystathionine beta-synthase deficiency in adulthood. J Inherit Metab Dis. 2010 Jun 22. [Epub ahead of print] IF 3.598

2. Pejznochova M, Tesarova M, Hansikova H, **Magner M**, Honzik T, Vinsova K, Hajkova Z, Havlickova V, Zeman J. Mitochondrial DNA content and expression of genes involved in mtDNA transcription, regulation and maintenance during human fetal development. Mitochondrion. 2010;10:321-9. IF 4.262

3. M.Pejznochova, M.Tesarova, T.Honzik, H.Hansikova, **M. Magner** and J.Zeman. The developmental changes in mitochondrial DNA content per cell in human cord blood leukocytes during gestation. Physiol Res. 2008;57:947-55. IF 1.653

4. Alena Čížková, Viktor Stránecký, Johannes Mayr, Markéta Tesařová, Vendula Havlíčková, Jan Paul, Robert Ivánek, Hana Hansíková, Vilma Kaplanová, Marek Vrbacký, Hanna Hartmannová, Lenka Nosková, Tomáš Honzík, **Martin Magner**, Zdeněk Drahota, Kateřina Hejzlarová, Wolfgang Sperl, Jiří Zeman, Josef Houštěk, Stanislav Kmoch. Mutations in TMEM70 cause isolated deficiency of F1F0 APT synthase and neonatal mitochondrial encephalo-cardiomyopathy. Nat Genet. 2008;40:1288-90. IF 30.259

5. Katerina Vesela, Hana Hansikova, **Martin Magner**, Jiri Zeman. Cytochrome c oxidace deficienty in childhood. Paediatr Croat 2009;53:122-126.

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