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# Mitochondrial disease criteria

## Diagnostic applications in children

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**Abstract—Background:** Based on a previous prospective clinical and biochemical study, a consensus mitochondrial disease scoring system was established to facilitate the diagnosis in patients with a suspected mitochondrial disorder. **Objective:** To evaluate the specificity of the diagnostic system, we applied the mitochondrial disease score in 61 children with a multisystem disease and a suspected oxidative phosphorylation disorder who underwent a muscle biopsy and were consecutively diagnosed with a genetic mutation. **Methods:** We evaluated data of 44 children diagnosed with a disorder in oxidative phosphorylation, carrying a mutation in the mitochondrial or nuclear DNA. We compared them with 17 children who, based on the clinical and metabolic features, also had a muscle biopsy but were finally diagnosed with a nonmitochondrial multisystem disorder by further genetic analysis. **Results:** All children with a genetically established diagnosis of a primary oxidative phosphorylation disorder had a mitochondrial disease score above 6 (probable mitochondrial disorder), and 73% of the children had a score above 8 (definite mitochondrial disorder) at evaluation of the muscle biopsy. In the nonmitochondrial multisystem disorder group, the score was significantly lower, and no patients reached a score comparable with a definite respiratory chain disorder. **Conclusions:** The mitochondrial disease criteria system has a high specificity to distinguish between mitochondrial and other multisystem disorders. The method could also be applied in children with a suspected mitochondrial disorder, prior to performing a muscle biopsy.

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Because of the lack of agreement on optimal biochemical assays and cut-off values related to frequent secondary abnormalities in the respiratory chain function and the low diagnostic success rate of mutation analysis, it is difficult to define a primary mitochondrial disorder in children.<sup>1–4</sup> We use the consensus mitochondrial disease criteria (MDC) scoring system (table),<sup>5</sup> which evaluates muscle symptoms (IA; maximal score: 2 points), CNS abnormalities (IB; maximal score: 2 points), and multisystem involvement (IC; maximal score: 3 points), with a maximal clinical score (I) of 4 points, adding metabolic abnormalities and neuroimaging (II; maximum additional score: 4 points). Histologic anomalies (III) could increase the score with 4 points, leading to a maximal score of 12 points (table). A score from 5 to 7 is comparable with a “probable mitochondrial disorder”; score of 8 to 12 confirms the diagnosis (“definite mitochondrial disorder”). This system was originally established to achieve the final diagnosis

irrespective of the presence or absence of a known mutation. Application of the scoring prior to muscle biopsy might facilitate decision making in further diagnostic steps.

In applying the MDC classification for the last 4 years, we used a minimal MDC score of 3 in the workup of patients as the cut-off point for performing a muscle biopsy. To study the specificity of the diagnostic system and distinguish between primary mitochondrial and other multisystem disorders, we evaluated the results of the MDC score in children with different multisystem disorders with a known genetic background who underwent a muscle biopsy at our center.

**Methods.** We evaluated data of 44 children who based on a MDC score of  $\geq 3$  underwent a muscle biopsy for a suspected mitochondrial disease in the period 2001 to 2005, and were consecutively diagnosed with the disorder and a mutation in the mitochondrial or nuclear DNA. We compared this group with 17 children also having a muscle biopsy for a suspected mitochondrial disease (MDC score  $\geq 3$  prior to biopsy), but diagnosed with a nonmitochondrial, nuclear-encoded genetic disorder. To evaluate the specificity of the MDC diagnostic system in these children, we re-evaluated the clinical, biochemical, and histologic data, collected during the standard workup in children with a suspected mitochondrial dysfunction. The data of the children carrying *MTTL1*, *MTATP6*, *MTND1*, *MTTK*, and *SURF1*, *POLG*, *PDHA1*, *NDUSF1*, or *NDUSF7* mutations (table E-1 on the *Neurology* Web site [www.neurology.org]) were compared with those of the pa-

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**Table Mitochondrial disease criteria (simplified version for bedside use)\***

I. Clinical signs and symptoms, 1 point/symptom (max. 4 points)			II. Metabolic/imaging studies (max. 4 points)	III. Morphology (max. 4 points)
A. Muscular presentation (max. 2 points)	B. CNS presentation (max. 2 points)	C. Multisystem disease (max. 3 points)		
Ophthalmoplegia†	Developmental delay	Hematology	Elevated lactate†	Ragged red/blue fibers‡
Facies myopathica	Loss of skills	GI tract	Elevated L/P ratio	COX-negative fibers‡
Exercise intolerance	Stroke-like episode	Endocrine/growth	Elevated alanine†	Reduced COX staining‡
Muscle weakness	Migraine	Heart	Elevated CSF lactate†	Reduced SDH staining
Rhabdomyolysis	Seizures	Kidney	Elevated CSF protein	SDH positive blood vessels†
Abnormal EMG	Myoclonus	Vision	Elevated CSF alanine†	Abnormal mitochondria/EM†
	Cortical blindness	Hearing	Urinary TA excretion†	
	Pyramidal signs	Neuropathy	Ethylmalonic aciduria	
	Extrapyramidal signs	Recurrent/familial	Stroke-like picture/MRI	
	Brainstem involvement		Leigh syndrome/MRI†	
			Elevated lactate/MRS	

\* Score 1: mitochondrial disorder unlikely; score 2 to 4: possible mitochondrial disorder; score 5 to 7: probable mitochondrial disorder; score 8 to 12: definite mitochondrial disorder.

† This specific symptom scores 2 points.

‡ This symptom in a higher percentage scores 4 points.

GI = gastrointestinal; L/P = lactate/pyruvate; COX = cytochrome *c* oxidase; SDH = succinate dehydrogenase; EM = electron microscopy; EMG = electromyography; TA = tricarboxylic acid.

tients carrying other nuclear DNA mutations (*SMA1*, *XPD*, *MECP1*, *COG*, *GALC*, *FSHD1A*) or common chromosomal alterations (mosaic Turner syndrome, del22q11.2, del9pter, del22q13, del18q22.1).

As part of the regular workup for a suspected disorder in the oxidative phosphorylation, all children underwent a standard diagnostic protocol of multiple investigations including EKG, chest radiograph, EEG, visual evoked potentials, acoustic evoked potentials, sensory evoked potentials, and cranial MRI prior to the muscle biopsy. Serum lactic acid (multiple measurements), pyruvic acid levels, blood gas, serum acyl-carnitine, amino acid, and urine organic acid profiles were analyzed in all children. CSF investigations were not performed in all patients.

Based on clinical symptoms, metabolic alterations, and abnormal neuroimaging results, we calculated the MDC score,<sup>5</sup> and with a minimal score  $\geq 3$ , a surgical muscle biopsy was performed under general anesthesia in all children. Parallel with routine immunohistologic and electron microscopic analysis, the ATP production from pyruvate oxidation and the activity of PDHc and the respiratory enzyme complexes I to V were measured in the fresh muscle sample according to methods described previously.<sup>6</sup> The MDC score was calculated again 1 week after the muscle biopsy. We compared the clinical section (I) of the score of the patients in the two groups and then compared the MDC score prior to (MDC score section I + II) and after the muscle biopsy including the results of the histology (MDC score section I + II + III).

The MDC score data of the two groups were expressed as means  $\pm$  SEM. Between-group differences were statistically assessed using one-way analysis of variance followed by the Scheffé post hoc test when appropriate. For these statistical analyses, the SPSS package software (version 12.0.1) was used. Double-sided *p* values of  $<0.05$  were considered significant.

**Results.** The mean clinical score (I) in the primary mitochondrial system disorders was 3.9 (range 3 to 4) with a maximum score of 4 (including the multisystem involvement [IC] with a mean score of 1.83 [range 0 to 3]); the metabolic and brain imaging score (II) was 2.9 (range 0 to 4), with a histology score (III) of 0.5 (0 to 4). Prior to biopsy the mean MDC score (I + II) was 7.0 (SEM  $\pm 0.4$ ), and the final score (I + II + III) was 7.6 (SEM  $\pm 0.3$ ). All patients had a total MDC score (I + II + III) above 6, and 73% of

the children had a score higher than 8 (range 6 to 12), comparable with the diagnosis of a definite mitochondrial disorder (table E-1).

The mean clinical score (I) in the nonmitochondrial disorder group was 3.75 (range 3 to 4) with a maximum score of 4 (including the multisystem involvement [IC] with a mean score of 1.9 [range 1 to 3]); the metabolic and brain imaging score (II) was 1.7 (range 0 to 2), with a histology score (III) of 0. The mean MDC score (I + II + III) before and after biopsy was 5 (SEM  $\pm 0.025$ ) with a highest score of 6 (range 4 to 6). This was substantially lower than that of the patients with primary respiratory chain disorder (tables E-1 and E-2).

Specific neuroimaging findings were detected in a small percentage of patients, in particular in the children with *MTATP6*, *MTTL1*, and *SURF1* mutations. No characteristic histologic alterations were found in the nonmitochondrial patient group. On the other hand, histologic alterations were found in only one-third (14/44) of the patients with oxidative phosphorylation disorders (*MTTL1*, *MTTK*, *POLG*, and *SURF1* mutations).

No significant difference was observed between the clinical score of the two patient groups, including the score for multisystem involvement. Prior to biopsy, thus without being able to include the histology score in the classification, the total MDC score was above 6 (probable respiratory chain disorder) in all of the primary mitochondrial cases and above 8 (definite respiratory chain disorder) in 8 of 13 children. Owing to metabolic alterations (chronic lactic acidemia, increased serum alanine levels, increased urinary organic acid excretion) and abnormalities found by neuroimaging, there was a higher total MDC score prior to biopsy ( $p < 0.001$ ) in this group. With the inclusion of the specific histologic alterations, the final total MDC score was higher ( $p < 0.0001$ ) in children with a primary respiratory chain disorder.

**Discussion.** Muscle biopsy, especially when performed under general anesthesia, is an invasive procedure, with an additional risk for patients with a dysfunction in oxidative phosphorylation, severe muscle hypotonia, cardiac symptoms, and CNS abnormalities. In this high-risk patient group, it is important to evaluate the chance for successful diagnosis prior to deciding on a muscle biopsy.

Using the data of the 61 patients studied with a genetic multisystem disorder, we analyzed the specificity of the MDC scoring and assessed whether the use of the classification is also adequate in the decision making prior to performing a muscle biopsy. Comparing the clinical classification section of the MDC score in primary mitochondrial and nonmitochondrial disorders, we did not find a significant difference between the two patient groups. We detected a significant difference in the total score of the patients after the addition of the results of the brain imaging and metabolic studies.

The use of the biochemical markers is important in the diagnosis of patients with a suspected respiratory chain disorder. Metabolic alterations, including recurrent/chronic lactic acidemia, high serum alanine levels, and increased excretion of the metabolites of the citric acid cycle are characteristic in patients with mitochondrial dysfunction.<sup>2</sup> Lactic acidemia and a marked increase in the serum alanine concentrations have been described in other conditions as well: in association with sepsis, in different conditions with tetraparesis, hyperinsulinism, chronic thiamine deficiency, or as a side effect of certain medications like valproic acid or nucleoside analogues.<sup>7-9</sup> Seizures due to hypoxic brain injury might result in recurrent elevation of lactic acid, alanine, and glutamate, especially in myoclonic epilepsy.<sup>10,11</sup> One should carefully evaluate the possibility of secondary mitochondrial dysfunction in this patient group.<sup>12</sup>

Regarding the use of (functional) brain imaging studies in the disease classification, the finding of Leigh(-like) syndrome or abnormal signal intensity or metabolic alterations detected in the basal ganglia are very indicative of primary mitochondrial dysfunction. This picture occurs only in a few other disorders (methylmalonic acidemia, glutaric acidemia, Wilson disease, and some other neurodegenerative disorders) with a characteristically different clinical presentation.<sup>13-15</sup> Performing brain MRI/MRS in the patient group with a suspected respiratory chain disorder is sometimes technically difficult owing to the need for a strong sedation or narcosis, but could be itself diagnostic. In the original MDC scoring system, we included a list of unique features, scoring double (2 points) according to the criteria, including Leigh disease, progressive external ophthalmoplegia, chronic lactic acidemia, elevated serum alanine levels, elevated CSF lactate, and abnormal muscle MRS, emphasizing the importance of these features in the differential diagnostics. The presence of these features in patients with a clinically suspected mito-

chondrial disorder might warrant further invasive investigations, even in the absence of a very high MDC score. In our own patient population with mitochondrial disorders, we have found only a few children with a known genetic alteration and a relatively low MDC score.

Some patients with the T8993C/G, A8993C/G, or A3243G point mutation might present with isolated MRI findings or muscle weakness with normal blood lactate levels; however, the diagnosis is seldom made in this phase, except for familial cases. In the case of lower levels of tissue heteroplasmy, the MDC score is also low, making the diagnosis difficult. It is hard to assess the diagnostic value of neurophysiologic studies in children. In our experience, hearing loss or visual disturbances occur frequently late in the course of the disease, and the absence of symptoms therefore has no "ruling-out" value. Sensory evoked potentials are seldom informative, except for in somewhat older patients with T8993C/G or A8344G mutations.

One should note the importance of the muscle histology. In our original MDC system, we included a score up to 4 points for the presence of cytochrome *c* oxidase (COX)-negative fibers, reduced COX staining, and ragged red fibers, which leads to a substantial MDC score even in the presence of one clinical feature.

There is some bias in our study regarding the sensitivity and specificity assessment in the pediatric patient group. One might miss the diagnosis in a few cases with low MDC score due to an organ-specific presentation. Evaluating the data of all children in our patient population who underwent a muscle biopsy, we have found completely normal biochemical and histologic results in 19% during the same diagnostic period. On the other hand, we did confirm classic mitochondrial point mutations in two of these children with Leber hereditary optic neuropathy (LHON) and mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome and normal muscle biochemistry. Furthermore, we found some additional patients with LHON, myoclonic epilepsy with ragged red fibers (MERRF), and MELAS syndrome (with low heteroplasmy), where no muscle biopsy was indicated, and the diagnosis was confirmed by direct mutation analysis.

## References

1. Thorburn DR. Mitochondrial disorders: prevalence, myths and advances. *J Inherit Metab Dis* 2004;27:349-362.
2. Rubio-Gozalbo ME, Sengers RC, Trijbels JE, et al. A prognostic index as diagnostic strategy in children suspected of mitochondriocytopathy. *Neuropediatrics* 2000;31:114-121.
3. Smeitink JA. Mitochondrial disorders: clinical presentation and diagnostic dilemmas. *J Inherit Metab Dis* 2003;26:199-207.
4. Smeitink JA, Zeviani M, Turnbull DM, Jacobs HT. Mitochondrial medicine: a metabolic perspective on the pathology of oxidative phosphorylation disorders. *Cell Metab* 2006;3:9-13.
5. Wolf NI, Smeitink JA. Mitochondrial disorders: a proposal for consensus diagnostic criteria in infants and children. *Neurology* 2002;59:1402-1405.
6. Janssen AJ, Trijbels FJ, Sengers R, et al. Determination of the mitochondrial energy generating capacity in human muscle mitochondria: diagnostic procedure and application to human pathology. *Clin Chem* 2006;52:860-871.

7. Thabet H, Brahmi N, Amamou M, Ben Salah N, Hedhili A. Hyperlactatemia and hyperammonemia as secondary effects of valproic acid poisoning. *Am J Emerg Med* 2000;18:508.
8. Thauvin-Robinet C, Faivre L, Barbier ML, et al. Severe lactic acidosis and acute thiamin deficiency: a report of 11 neonates with unsupplemented total parenteral nutrition. *J Inher Metab Dis* 2004;27:700-704.
9. Noguera A, Fortuny C, Munoz-Almagro C, et al. Hyperlactatemia in human immunodeficiency virus-uninfected infants who are exposed to antiretrovirals. *Pediatrics* 2004;114:598-603.
10. Howse DC. Metabolic responses to status epilepticus in the rat, cat, and mouse. *Can J Physiol Pharmacol* 1979;57:205-212.
11. Yager JY, Armstrong EA, Miyashita H, Wirrell EC. Prolonged neonatal seizures exacerbate hypoxic-ischemic brain damage: correlation with cerebral energy metabolism and excitatory amino acid release. *Dev Neurosci* 2002;24:367-381.
12. Hui J, Kirby DM, Thorburn DR, Boneh A. Decreased activities of mitochondrial respiratory chain complexes in non-mitochondrial respiratory chain diseases. *Dev Med Child Neurol* 2006;48:132-136.
13. Billard C, Dulac O, Bouloche J, et al. Encephalopathy with calcifications of the basal ganglia in children. A reappraisal of Fahr's syndrome with respect to 14 new cases. *Neuropediatrics* 1989;20:12-19.
14. Lowry RB. Early onset of Cockayne syndrome. *Am J Med Genet* 1982;13:209-210.
15. Straussberg R, Shorer Z, Weitz R, et al. Familial infantile bilateral striatal necrosis: clinical features and response to biotin treatment. *Neurology* 2002;59:983-989.

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