Abdominal pain and vomiting as first sign of mitochondrial disease

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Abstract

We describe a patient in whom abdominal pain and vomiting were the presenting symptoms of Mitochondrial Myopathy Encephalopathy, Lactic Acidosis with Stroke-like episodes syndrome (MELAS). Mitochondrial disorders usually present with neurological symptoms or with myopathic features at any age. Although many patients develop visceral symptoms at a certain moment during the course of the disease, only in a minority of patients these symptoms are the unique presenting ones. The proband was initially diagnosed as having gastro-oesophageal reflux and it was only after detailed clinical history that an underlying metabolic defect was suspected and the molecular defect identified. (Acta gastroenterol. belg., **2009**, 72, **365-368**).

Introduction

Mitochondrial disorders are variable in presentation, usually presenting with neurological symptoms, myopathic features or with an encephalomyopathy. These degenerative disorders result from the cells' decreased ability to convert a sufficient amount of glucose into adenosine triphosphate (ATP). Organs with high-energy requirements such as brain, heart, liver and skeletal muscle will be especially vulnerable. Mitochondriocytopathies (MCP) are often multisystem disorders affecting peripheral or central nervous system as well as eyes, ears, endocrine organs, the heart, gut, liver, kidneys, blood and dermis (1). In over 30% of paediatric patients the presentation is non-neurological (2). Diagnosing MCPs is a challenge because of their wide clinical and genetic heterogeneity (3).

Vomiting in patients with MCPs is often seen in association with neurological symptoms. We report the first case of an 8-year-old boy presenting initially with abdominal pain and vomiting due to Mitochondrial myopathy Encephalopathy, Lactic Acidosis with Stroke-like episodes syndrome (MELAS). It was the clinical evolution and biochemical findings in blood and skeletal muscle that ultimately led to the diagnosis of an oxidative phosphorylation disorder.

Case

An 8-year-old male patient was referred to the paediatric gastroenterology consultation because of poor appetite and episodes of abdominal pain and vomiting since several years, responding to empirical treatment with ranitidine. His intelligence was normal and he had no learning difficulties. His weight had always been on the third percentile, his height on the tenth. Clinical examination revealed poor muscle mass and a barrel-shaped thorax. Gastroduodenoscopy revealed oesophagitis grade B and 24 hour oesophageal pH-metry confirmed gastro-oesophageal reflux with an acid reflux index of 10% and 65 reflux episodes. Light microscopic examination of a duodenal biopsy specimen was normal. Blood count, iron status, urea, creatinine, liver enzymes and anti-tissue transglutaminase were normal. A sweat test was negative, excluding cystic fibrosis. Treatment with omeprazole resulted in less abdominal pain and weight gain. Following physical exercise, however, he continued to complain of abdominal pain which subsequently improved with rest. Due to nightly snoring, fatigue, concentration difficulties, recurrent upper respiratory tract infections and hearing loss an adeno- and tonsillectomy was performed. Although the hearing loss improved, there was a persistent loss of 30 dB. After initial clinical improvement, complaints of abdominal pain and vomiting, most of the time following physical exercise, reoccurred and exercise intolerance became more pronounced. Additional diagnostic tests were performed. Pulmonary function was within normal limits (forced vital capacity % = 87%, forced expiratory volume in 1 second % = 103%) and remained unchanged following physical exercise and inhalation of β2-mimetics. Electrocardiogram and echocardiography were normal. Ultrasound of the kidneys was normal. Repeat blood tests after exercise showed that blood cells, urea and creatinine were again normal but aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were increased [316 U/L (normal < 50 U/L), 104 U/L (normal < 40 U/L) respectively] as well as creatine kinase (CK) [1210 U/L (normal < 219 U/L)] and lactic acid [57 mg/dL (normal < 16 mg/dL)]. A skeletal muscle biopsy was performed. Microscopic examination revealed the presence of 20 ragged-red-fibres/mm^2. These are muscle fibres with areas of purplish-looking mitochondrial accumulation revealed by the modified

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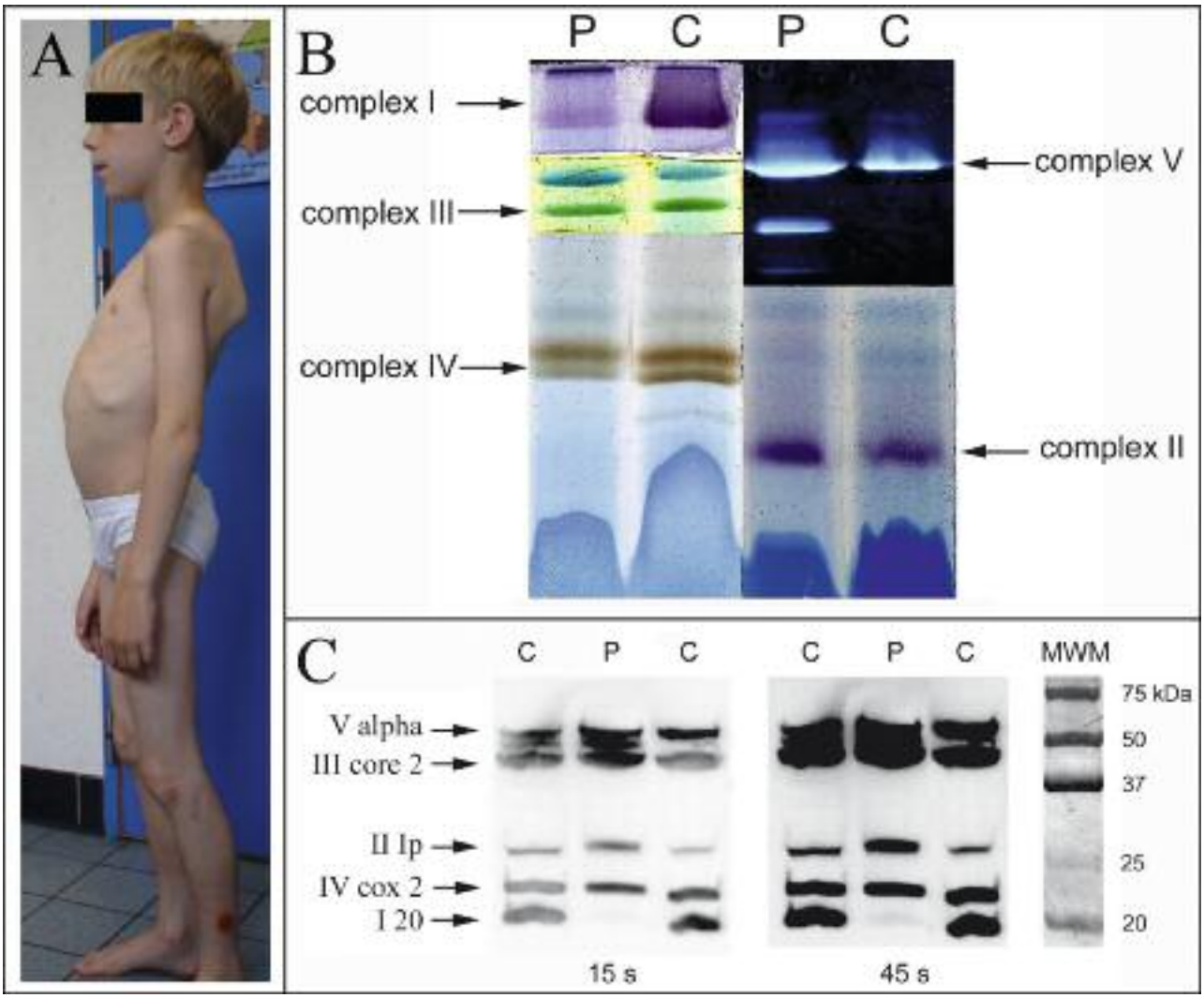

Fig. 1. — Panel A : Clinical picture of the patient in profile.
Panel B : In-gel activity staining following blue native–polyacrylamide gel electrophoresis analysis BN PAGE and in-gel activity staining were performed as described previously (9). Solubilised muscle mitochondria from patient and control were loaded in duplicate. The first set of lanes was used for activity staining of complex I, III (10) and IV, the second set for activity staining of complex II and V. BN-PAGE of the patient's (P) muscle tissue showed severely decreased complex I activity, and low normal complex IV activity, while the activities of complexes III and II were normal compared with control (C) specimens. Note the presence of complex V subcomplexes which are a sign of a disturbed intramitochondrial protein synthesis.

Panel C : Immunostaining of skeletal muscle proteins from patient (P) and controls (C) using specific antibodies against subunits of the five OXPHOS complexes. MWM : molecular weight marker. Respectively 15 and 45 seconds of exposure time. The signal from the complex I subunit (NDUFB8-20 kDa) is strongly decreased in the patient compared to the controls. The signals of the other subunits : complex V alpha, complex IV cox 2, complex III core 2 and complex II Ip are comparable with the controls.

Gomori trichrome stain. These are the pathological hallmark of a mitochondrial disease. A significantly decreased activity of complex I activity was detected by biochemical analysis (Fig. 1, Table 1). Mitochondrial DNA (mtDNA) analysis revealed a classical MELAS mutation [3243 A → G, 44.6 % in white blood cells (WBC) and 89.6% in skeletal muscle]. The mutation load in the mother was 3.6% in WBC and the maternal grandmother had 1% mutation rate in WBC. The faecal elastase-1 test was normal [452 µg/g faeces (normal > 200 µg/g faeces)]. Cerebral MRI revealed no brain abnormalities.

The proband was treated with riboflavine, thiamine, idebenone (= co-enzyme Q10) and L-arginine. Parents are able to measure lactic acid at home and sustained exercise is avoided. He has been stable since diagnosis 1.5 year ago but has increased lactic acid concentrations during illness or physical exercise. He develops some learning difficulties and is very tired at the end of the school day.

Table 1. — **OXPHOS enzyme activities in muscle homogenate following spectrophotometric analysis, values in nmol/min/mg protein**

Complex	*Enzyme*	*Patient*	*controls*			
			mean	median	P5	P95
I	NADH-CoQ reductase	2	26	24	13	44
II	Succinate CoQ oxireductase	66	47	44	23	86
II + III	Succinate cytochrome C reductase	68	43	39	20	75
III	Ubiquinol cytochrome C reductase	135	57	53	21	118
IV	Cytochrome C oxidase	92	126	123	75	205
V	ATP-ase	ND	–	–	–	–
CS	Citrate synthase	215	214	204	140	334

Discussion

The term ''mitochondrial diseases'' refers specifically to defects of the mitochondrial oxidative phosphorylation, the bioenergetic pathway in all cells, except the red blood cell. The diseases affect mitochondrial ATP production resulting in impaired energy-requiring cellular functions. MCPs can be caused by nuclear mutations since most oxidative phosphorylation subunits are nucleus-encoded, and also because correct folding and functioning of the oxidative phosphorylation complexes require many steps, all of which are under control of nuclear DNA (nDNA). On the other hand 13 of the approximately 80 proteins that make up the oxidative phosphorylation are encoded by mitochondrial DNA (mtDNA). Complexes I, III, IV, and V contain several subunits encoded by mtDNA.

The diagnosis of a MCP is not easy because of the wide clinical heterogeneity of these disorders. Literature states that mitochondrial diseases should be considered whenever a patient displays two or more seemingly unrelated symptoms in more than one organ system especially when symptoms are progressive (4).

In most patients signs of encephalopathy and myopathy are present, possibly in association with symptoms in visceral organs (pancreas, heart, liver, gut and kidneys) and blood. In case a patients has a recognisable phenotype (Fig. 2), a known mtDNA or nDNA mutation should be searched for (4).

Some patients, like ours, present with predominantly non-neurological symptoms. In those patients, diagnosis is difficult and often delayed. The proband displayed initially the classical symptoms of gastro-oesophageal reflux, a far more common disorder than MCP. Treatment with proton pump inhibitors only partially resolved symptoms despite normalisation of the pH-metry parameters.

It was only after a detailed clinical history that the suspicion of exercise induced metabolic decompensation was raised. Transaminases were only disturbed after physical exercise, with a AST higher than ALT, and associated with increased CK. This pointed towards a muscular problem ; therefore further investigations were pointed towards the skeletal muscle and not to liver or intestine. The skeletal muscle biopsy revealed the presence of ragged-red-fibres and complex I deficiency leading to the diagnosis of MELAS. Since the diagnosis was confirmed by the presence of the classical mutation in the mtDNA there was no need for further investigation of liver or intestine.

When the clinical picture is suggestive of a MCP (Fig. 2), extra arguments can be found in plasma lactic acid, ketone bodies and urinary organic acids (4). If these studies are abnormal, a muscle biopsy with measurement of the oxidative phosphorylation complex activities should be performed, as was the case in the proband (4).

The classical symptoms of MELAS are included in its name : mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes. The patient started with gastrointestinal symptoms that progressively became associated with physical exercise and he developed signs of a myopathy. The lactic acidosis became only evident by blood sampling following exercise. The other classical MELAS symptoms were not yet present in this patient, although he developed some learning difficulties.

Visceral symptoms usually accompanying MELAS are vomiting (as was the case in this proband), short stature, diabetes mellitus and dilated cardiomyopathy (2). Paralytic ileus can also be seen in case of severe metabolic decompensation (5).

Almost all MCPs develop at some point visceral manifestations. A gastrointestinal presentation is typically the case in myoneurogastrointestinal encephalomyopathy (MNGIE). This disorder is usually diagnosed only in the second or third decade and is associated with polyneuropathy, ophthalmoplegia, leucencephalopathy, gastrointestinal dysmotility ranging from chronic diarrhoea to pseudo-obstruction episodes and severe malnutrition (6). In MNGIE, nDNA mutations in the gene encoding for thymidine phosphorylase lead to impaired mitochondrial DNA replication due to accumulation of thymidine and deoxyuridine. Pseudo-obstruction episodes can also be seen in patients with myoclonic epilepsy and ragged-red-fibres (MERRF) and in patients with Leigh syndrome. MERRF patients classically presents with tonic-clonic seizures, myopathy, ptosis, ophthalmoparesis, cerebellar ataxia, dementia, and deafness. MERRF is caused by a point mutation in the mitochorndrial $tRNA^{Lys}$ gene. Nuclear Leigh syndrome phenotype

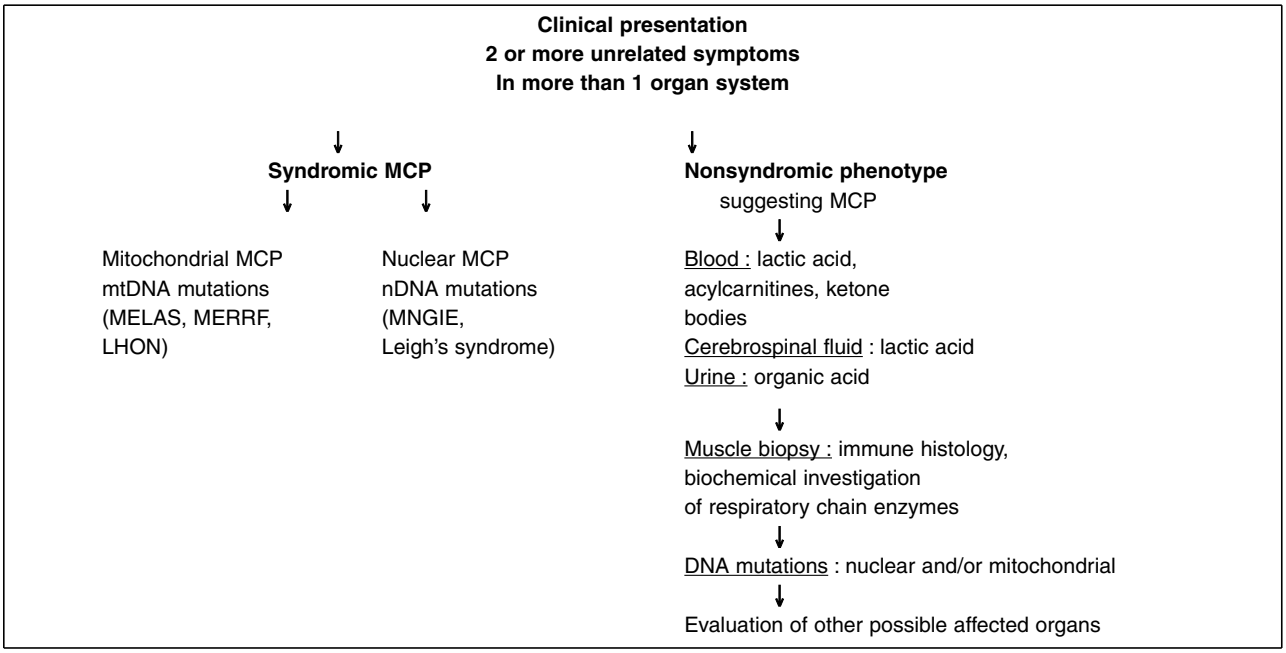

Fig. 2. — Stepwise procedure for the diagnostic work-up of mitochondriopathies (MCP)
MELAS : Mitochondrial myopathy Encephalopathy, Lactic Acidosis with Stroke-like episodes syndrome ; MERRF : Myoclonic Epilepsy and Ragged-Red-Fibres ; LHON : Leber's Hereditary Optic Neuropathy ; MNGIE : Myoneurogastrointestinal Encephalomyopathy.

consists of vomiting, hepatopathy, cardiomyopathy, encephalopathy, and generalised weakness. It is caused by mutations in nDNA genes encoding for subunits of the pyruvate-dehydrogenase complex or for oxidative phosphorylation components, or genes coding for proteins involved in maintenance of oxidative phosphorylation complex functioning.

MCP should be considered in every patient with multisystem disorder and progressive course involving seemingly unrelated organs (4,7). So far, therapeutic options for MCP are limited.

For MELAS several medications are used in an effort to improve the oxidative phosphorylation function or to reduce the levels of reactive oxygen species (L-arginine, coenzyme Q_{10}, thiamine, riboflavine,...) (8). The patient is stable with this treatment. Sustained exercises should be avoided to prevent high blood lactate concentrations. Parents are able to follow blood lactate concentrations at home and try to keep it as low as possible. Correct molecular diagnosis is important for genetic counselling.

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