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Diagnosis and management of patients with mitochondrial disease

Supportive care combined with monitoring and treatment of organ-related complications is key to managing patients with mitochondrial disease.

ABSTRACT: The diagnosis of mitochondrial disease is complex because of its clinical and genetic heterogeneity. Typically, histopathological findings and respiratory chain enzyme results from skeletal muscle are interpreted in the context of clinical findings and results from ancillary laboratory tests. While the investigative process often relies on a muscle biopsy, diagnosis can sometimes be achieved with direct testing of mitochondrial genes and/or nuclear genes, especially when there is a recognizable clinical syndrome. In BC, consultation with the Biochemical Genetics Laboratory at BC Children's Hospital is strongly recommended prior to any muscle biopsy, as sample handling is critical to the diagnostic process. Treatment of patients with mitochondrial diseases currently involves the use of vitamin supplements, arginine, and exercise therapy. While exercise is proven to improve mitochondrial function, the efficacy of other treatments has not yet been established and clinical trials are ongoing. Regardless of the therapies used, management also involves screening for treatable complications of mitochondrial disease, such as diabetes mellitus and cardiac conduction, and providing support for patients and families dealing with chronic illness.

Mitochondrial disease is a clinically heterogeneous, often multisystem disorder that can present from birth to old age. Diagnosis is complex and requires the integration of information obtained by history, laboratory testing, imaging, and muscle biopsy. Patient management focuses mainly on supportive care with monitoring and treatment of organ-related complications of mitochondrial disease as we await the results of ongoing clinical trials looking at the efficacy of various treatments.

Investigation and diagnosis

As many of the investigations required to diagnose mitochondrial disease

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are not widely available and are often difficult to interpret, referral to a specialist to coordinate investigations is warranted when a family physician suspects mitochondrial disease. The clinical features that might arouse suspicion include a broad spectrum of findings that are described more fully in the following article, "Mitochondrial disease clinical manifestations."

Diagnostic criteria

A diagnosis of mitochondrial respiratory chain disorder requires an amalgamation of clinical, biochemical, enzymatic, histopathological, and molecular data. The disparate data may be scored as major or minor abnormalities and are then considered in terms of published classification systems.[1-3] The outcome of this process is a statement of how probable it is that the patient has a primary mitochondrial disease on a scale ranging from "unlikely" to "definite." In between are "possible" and "probable" mitochondrial disease designations. Although these latter categories are unsatisfactory for both patients and care providers, they are necessary given our current lack of understanding of mitochondrial disease. Patients in the possible and probable categories may eventually be diagnosed with an alternate disorder of which mitochondrial dysfunction is only a secondary manifestation.

The published diagnostic criteria are heavily dependent on investigation of skeletal muscle. Muscle biopsy investigations include histopathology, electron microscopy, respiratory chain enzymology, and molecular analysis of mitochondrial DNA. Ancillary investigations, including laboratory tests, imaging, exercise testing, and electromyography (EMG), may be useful to identify patients at highest risk for mitochondrial disease prior to performing a muscle biopsy, and to improve the diagnostic yield of this invasive procedure. Although skeletal muscle biopsy is usually needed for a diagnosis, noninvasive DNA testing on other sample types may be possible in patients with recognizable clinical syndromes.

Table 1. Laboratory tests for investigating mitochondrial disease.

Tests performed before referral	Interpretation
Serum lactate	Useful if elevated but often normal.
Serum CK	May be normal or mildly elevated; patients with very high values (in the thousands) should first be evaluated for other types of myopathies such as polymyositis.
Serum fasting glucose	Diabetes mellitus is a frequent complication of mitochondrial disease.
Serum TSH	Hypothyroidism may mimic some elements of mitochondrial disease.
Other investigations as necessary	The possibility of other more common or treatable disorders considered in the differential diagnosis should be ruled out.
Tests performed after referral to a specialist in mitochondrial disease	
Serum and whole blood acylcarnitine profiles	Can point to a defect in fatty acid oxidation, which can mimic primary dysfunction of the respiratory chain.
Urine organic acids	May show accumulation of metabolites suggesting dysfunction of respiratory chain.

Blood and urine testing

The investigation of a patient for mitochondrial disease includes blood and urine testing as shown in **Table 1**. Lactic acidemia is an important but inconsistent indicator of mitochondrial disease. This finding is often present in more severely affected patients with childhood onset of disease, but may be missing in patients with less severe involvement. Thus, the absence of an elevation in lactate cannot be used to exclude a diagnosis of mitochondrial disease.

Radiological investigations

Because the central nervous system is involved in 30% to 60% of patients with mitochondrial disease, brain magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) are important diagnostic tools. The brain MRI results may display both relatively specific abnormalities (such as basal ganglia calcification) and nonspecific abnormalities (such as white matter changes). Brain MRS, available in Vancouver, may be used as an alternative to lumbar puncture for CSF lactate testing to document intracerebral lactate elevations.

Exercise testing

Exercise testing is an alternative technique for the evaluation of possible mitochondrial myopathy. The key features of a mitochondrial myopathy are a low anaerobic threshold (indicating impaired or inefficient oxygen utilization) and an increased respiratory exchange ratio indicating an inefficient utilization of fatty acids as an energy source. However, the sensitivity and specificity of exercise testing have not been clearly defined and interpretation remains difficult.

Electromyography

The majority of patients with mitochondrial disease have skeletal muscle

complaints and sometimes symptoms of a peripheral neuropathy. Electromyography (EMG) is a useful diagnostic test to screen for myopathic changes and to delineate the type of peripheral neuropathy that may also be subclinical.

Many patients with mitochondrial myopathy have normal or nonspecific changes on EMG studies. However, normal EMG findings can still be helpful. A metabolic myopathy may still be present since patients with most other forms of clinical myopathy (such as inflammatory myopathies) usually have diagnostic abnormalities on EMG testing.

Muscle biopsy

A biopsy of skeletal muscle permits histopathology, electron microscopy, respiratory chain enzymology, and mtDNA testing, provided a specialized sample handling protocol is followed. The logistics of sample handling must be worked out clearly prior to the procedure. Physicians considering mitochondrial investigations should contact the Biochemical Genetics Laboratory for guidance and special sample handling procedures (phone 604 875-2307 or e-mail lab_bdl_office @cw.bc.ca).

Muscle histopathology and electron microscopy. Certain histopathological features are specific indicators of mitochondrial dysfunction. For example, the presence of multiple ragged-red fibres with modified Gomori trichrome stain indicates abnormalities of mitochondrial function with compensatory mitochondrial proliferation. Electron microscopy may show abnormal mitochondria with increased size and abnormal cristae (see histopathological findings in Figures 5 and 6 of "Primer on mitochondrial disease" in this issue). Further, a histologically and ultrastructurally normal muscle biopsy does not exclude a mitochondrial disease since the biopsied muscle may not be involved in the disease process.

Muscle respiratory chain enzymology. In BC respiratory chain enzymology is performed on frozen skeletal muscle tissue. The activities of the enzyme complexes involved in making adenosine triphosphate (ATP) are measured and expressed relative to citrate synthase, a marker enzyme; see Figure 2 of the article "Primer on mitochondrial disease" in this issue. However, because not all respiratory chain defects are expressed in skeletal muscle, these tests may be normal in some patients with mitochondrial disease. When abnormal, the pattern of enzyme results often helps elucidate the underlying genetic cause and thus directs further investigations.

Molecular analysis

Depending on the clinical and pathological features, testing of nuclear DNA (nDNA) or mitochondrial DNA

Resources for patients, their families, and their physicians

The Internet is full of information on mitochondrial disease, but the credibility varies widely and the content changes frequently. The following are recommended.

Mitoaction (www.mitoaction.org)

An excellent, comprehensive, patient-friendly website with information for physicians as well. Includes:

- A primary care physician's guide (www.mitoaction.org/guide/-contents).
- A list of medications that are mito-toxic.
- Nutrition recommendations.
- Practical resources for children and adults living with mitochondrial disease.

United Mitochondrial Disease Foundation (www.umdf.org)

A great resource for patients and professionals. Includes:

- A guide on patient evaluation for professionals (e.g., deciding when to refer patients, diagnostic indicators).
- Mito 101: A primer for physicians and patients.
- Treatment and therapy recommendations.

Muscular Dystrophy Association (www.mda.org)

Provides access to good explanations of mitochondrial disease and the systems affected:

- Mitochondrial myopathy (www.mda.org/publications/Quest/q64mito2.html).
- Mitochondrial disease in perspective (www.mda.org/publications/Quest/q65mito.html).

Anesthetic guidelines

(Available from the Adult Metabolic Diseases Clinic, 604 875-5965.)
A research-based guide to the safe use of anesthetic agents in people with mitochondrial disease. Includes a list of contraindicated anesthetic agents, recommendations for major and minor surgery, and metabolic stressors that can lead to decompensation of patients with mitochondrial disease.

Table 2. Mitochondrial cocktail.

Supplement	Recommended adult dose (p.o.)	Comments	Possible side effects
Coenzyme Q10	30 mg (gel caps or liquid) t.i.d.	Take with food and vitamin C (500 mg b.i.d.)	Nausea, diarrhea, upset stomach, or appetite loss.
L-Carnitine	5 ml (500 mg) b.i.d.	Increases transport of long chain fatty acids into the mitochondria for conversion into energy.	
Creatine monohydrate	2.5 g (powder) b.i.d.	• Creatine supplementation may improve muscle strength by increasing energy stores and prevent accumulation of lactic acid. • Add powder to sugar-free drinks, including hot drinks, but do not boil. Boiling or adding sugar to creatine will increase its conversion to creatinine. • Avoid purchasing creatine manufactured in China.	Occasional mild stomach upset.
Thiamine and riboflavin	100 mg o.d.	• Useful to reduce incidence and severity of migraine headaches in patients with mitochondrial diseases. • Take with large glass of water to increase absorption.	Riboflavin causes urine to turn bright yellow, a harmless side effect.
Alpha lipoic acid	200 mg t.i.d.		• Occasional mild stomach upset. • Very rarely can trigger an allergic skin reaction.
Arginine	500 mg b.i.d.	• Has been shown to reduce stroke-like episodes in MELAS patients. • Check blood levels of arginine 1 month post initiation of oral supplementation. Dose should be titrated to achieve plasma arginine levels of > 80 umol/L.	

(mtDNA) (or both) may be indicated to confirm the presence of a specific inherited mitochondrial disease.[4]

In the case of a suspected maternally inherited syndrome, mtDNA is analyzed. For this analysis, skeletal muscle mtDNA is preferred because of its relative abundance and its retention of mtDNA mutations over time. Urine and blood testing may also be adequately sensitive depending on the mutation type. In general, if one of several relatively common known pathogenic mtDNA mutations is identified in a symptomatic patient, a diagnosis of mitochondrial disease is definite. However, even patients who have a known pathogenic mtDNA mutation may be asymptomatic depending on the percentage load of mutant mtDNA (degree of heteroplasmy). For this reason, once an index case has been identified with a mtDNA defect, family members should be offered pretest genetic counseling to work through the implications, limitations, and potential benefits of mtDNA testing.

Unlike mtDNA testing, nDNA testing can be performed in any nucleated cell type or tissue, with peripheral blood being the most commonly used. Finding a nuclear gene defect permits accurate prenatal testing in a subsequent pregnancy. The success of finding a nuclear gene defect is variable, and such testing is only pursued in the context of a specific clinical or pathologic phenotype. Advances in molecular technology and the identification of novel nuclear genes are expected to improve diagnostic yield of mitochondrial disease in the future.

Therapy and management

The management of patients with mitochondrial disease involves a three-part approach:

- Prescribing medications and lifestyle measures designed to improve mitochondrial function.
- Screening for potentially treatable complications.
- Providing support for the patient and family who have been affected by this chronic disease.

Improving mitochondrial function

The vitamins and other supplements listed in **Table 2** are referred to as the "mitochondrial cocktail"—a combination of agents known to be involved in mitochondrial function. The use of the cocktail is largely based on the assumption that higher doses of these agents may improve mitochondrial energy generation. With the exception of coenzyme Q10,[5] this assumption remains largely unproven, although randomized trials are ongoing.

Since the supplements in the cocktail are expensive and are not covered in BC by Pharmacare, we often introduce the agents sequentially, having the patient chart symptoms to see which is of benefit. However, for patients with very severe manifestations (such

as liver disease and cardiac failure), the time needed to try the supplements sequentially may be a concern and the entire cocktail can be started at once.

Another cocktail component, the amino acid arginine, has been studied with some degree of rigor in the treatment of patients with mitochondrial encephalopathy with lactic acidosis and stroke-like episodes (MELAS). In these patients, there is endothelial dysfunction that impairs the delivery of substrate to the brain to make energy. Thus, although this differs from stroke due to atherosclerosis, where there is an anatomic lesion (a plaque) preventing the delivery of substrate, the net result is the same in that the brain has an energy deficit. Arginine improves endothelial function through the generation of nitric oxide. Researchers have found that intravenous arginine[6] improved stroke-like symptoms when given during the episode and oral arginine reduced the number of stroke-like events in a Japanese cohort of patients with MELAS.[7] The use of arginine in patients with disorders other than MELAS has not been systematically investigated. A protocol for the use of arginine can be obtained by contacting the Adult Metabolic Diseases Clinic at Vancouver General Hospital.

Exercise training is one of the few treatment modalities that has shown a benefit in terms of mitochondrial function in randomized trials.[8] The effect of exercise training is to improve the efficiency of the mitochondria in generating ATP. In one study, an 8-week aerobic training program (three to four times/week for 20 to 30 minutes each time with a defined target heart rate) resulted in an improvement in aerobic capacity of 30% and even greater improvements in other parameters designed to look at mitochondrial function.[8] Many patients with mitochondrial disease complain of exertional myalgias and are therefore reluctant to exercise. However, the resultant deconditioning effect will only worsen their mitochondrial function. Thus, one of the most important recommendations that can be made to patients is that they do some low-intensity exercise training. Consultation with a rehabilitation therapist to guide exercise training may be helpful, particularly in patients with disabilities that limit exercise options.

A biopsy of skeletal muscle permits histopathology, electron microscopy, respiratory chain enzymology, and mtDNA testing, provided a specialized sample handling protocol is followed.

Monitoring patients

The monitoring protocols for patients with confirmed or suspected mitochondrial disease are intended to look for complications that arise more frequently in patients with disorders of energy metabolism. These protocols are not meant to be inclusive and would need to be modified based on the particular symptoms demonstrated by the patients and on the underlying genetic cause of their disease. A suggested monitoring schedule used by the Adult Metabolic Disease Clinic is shown in **Table 3**. Pediatric patients who have more severe disease may need to be assessed more often.

Table 3. Monitoring protocol for adult patients with mitochondrial disease.

Assessment	Complication to be identified	Suggested frequency in adults
History and physical exam	Symptoms of myopathy and diabetes; other general presenting symptoms	Annually, although less frequent review may be appropriate for some stable patients
ECG	Conduction defects	Annually
Echocardiogram	Cardiomyopathy	At baseline and then every 3–5 years if baseline is normal
Fasting glucose	Diabetes mellitus	Annually or more frequently if symptoms present
Calcium	Hypocalcemia secondary to hypoparathyroidism	Every 1–2 years or sooner if symptoms present

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Family A: Diagnosis and management

The family introduced in the previous article, "Primer on mitochondrial disease," provides an example of the diagnostic and management process. The index patient (IV-4) presented with clinical features of subacute necrotizing encephalopathy (Leigh syndrome). After a second person in the family (patient V-2) developed severe symptoms of mitochondrial disease, a defect in the mtDNA was found by molecular analysis. Once the specific familial mutation was identified, direct testing of other family members became relatively simple. Several therapies were tried in the most severely affected family members, including a mitochondrial cocktail and ketogenic diet, but these were ineffective and did not prevent progressive neurological deterioration. Symptomatic and supportive therapies then became the mainstays of care for members of Family A with manifestations of mitochondrial disease.

Conclusions

Despite the complexity of the investigations required, a definitive diagnosis of mitochondrial disease can be achieved in many cases. Such a diagnosis may provide prognostic information for the patient and other family members, as seen in the example of Family A. Given the heterogeniety of respiratory chain defects and the variable patterns of inheritance (sporadic, autosomal dominant, autosomal recessive, or maternal), a specific diagnosis is also invaluable for family counseling and possibly for providing the option of prenatal testing in a subsequent pregnancy. Evidence-based therapeutic options to improve the course of disease are limited. However, there remains a need for patient counseling, supportive care, and symptomatic therapy to minimize secondary complications associated with mitochondrial disease.

Competing interests

None declared.

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