

Summary – Immune Function & Mitochondrial Disease

Presented by



Dr. Katherine Sims is a Pediatric Neurologist at the Massachusetts General Hospital. Her clinical work over the last 30 years has focused on the broad scope of neurogenetic disorders including those of the lysosome, particularly neuronal ceroid lipofuscinosis [Batten disease, NCL disorders], Fabry disease, Norrie disease, general neurometabolic disorders and, most recently, the primary mitochondrial energy metabolism disorders. Over the last 15 years, Dr. Sims, working as clinician scientist, has directed the design and development of Patient Registries and BioRepositories for Mitochondrial, NCL and Norrie diseases. She is an expert diagnostician and works with great facility in identifying clinical cases and facilitating entry into appropriate clinical translational studies.



Jolan E. Walter, MD, PhD is the Director of Pediatric Immunodeficiency Program at Massachusetts General Hospital *for Children*. Dr. Walter's clinical care focuses on patients with immune deficiency. She jointly follows patients with mitochondrial disease and immune dysfunction with the Neurogenetics Program (Dr Kathy Sims, Dr Amel Karaa and Dr Melissa Walker). She also conducts translational research on autoimmune manifestation of primary immunodeficiencies. Dr. Walter has graduated with a MD and PhD from University of Pecs, Hungary. Dr. Walter is has trained in Pediatrics at Children's Hospital of the King's Daughters, Eastern Virginia Medical School and in Allergy/Immunology at Boston Children's Hospital. During her training, she conducted research both in the field of Virology and Immunology.



Melissa A. Walker, MD, PhD is a fourth-year trainee in the Massachusetts General Hospital Child Neurology Residency Program. Dr. Walker's clinical and scientific interests focus on improving the understanding and treatment of primary mitochondrial disorders. Dr. Walker received her MD and PhD degrees from Columbia University College of Physicians and Surgeons in New York City, New York. She trained in Pediatrics at the Massachusetts General Hospital for Children in Boston, Massachusetts.

The focus of the discussion centers on eight years of collaborative experience with mitochondrial patients at Massachusetts General Hospital (MGH) titled: "Predisposition to Infection and SIRS in Mitochondrial Disorders: Eight Years' Experience in an Academic Setting," published in the *Journal of Allergy and Clinical Immunology in Practice* in 2014.

Primary Mitochondrial Disorders - To fully grasp the impact of mitochondrial disease on the immune system, an understanding of basic mitochondrial function is necessary (slide 3). Primary Mitochondrial Disorders are diverse group of multi-system diseases, caused by dysfunction of the mitochondria. These "powerhouse" organelles of the cell produce the energy that both the cells and the entire body need to function properly.

Functions of the mitochondria include:

- oxidative-phosphorylation - energy production
- fatty acid oxidation - energy metabolism or energy turnover
- apoptosis - controlled or programmed cell death
- calcium regulation, as well as many other functions

Primary mitochondrial disorders can disrupt one or more of these vital processes in the cell, causing a broad range of medical problems. Incidence is estimated to be 1 out of 4,000 individuals, although difficult to know exactly how many individuals are affected.

The diagnosis of primary Mito diseases (slide 4) is a difficult and complicated process as:

- Multiple steps needed to ascertain the likelihood of mitochondrial disorder as well as a potential cause
- Can have symptoms in multiple organs systems, including the immune system
- No definitive biomarkers (blood test to measure Mito function, for example)
- All genes that may cause mito are not currently known
- Phenotypic variations - the same genetic mutation can produce different symptoms or presentations in different individuals

- Heteroplasmy - an unequal distribution of mitochondria and their DNA in cells
- Maternal inheritance - All mitochondria and mitochondrial DNA is passed down through the egg cell given by the mother. Each mitochondrion within that egg cell can have different mitochondrial DNA mutations. With each cell division, there can be a different number of mutations passed to the new cells (heteroplasmy).
- Secondary mitochondrial dysfunction can mimic primary mitochondrial disorders, rendering it difficult to discern between the two.

Diagnosis of primary mitochondrial dysfunction has multiple steps (slide 5). Clinical Criteria is examined first. Bernier, Morava, and others have created different sets of criteria based on signs and symptoms a patient may have and, at times, laboratory testing, which try to predict the probability that a person is affected by primary mitochondrial disease. For the purpose of this research study, the Bernier criteria (<http://www.ncbi.nlm.nih.gov/pubmed/12427892>) and Morava criteria (<http://www.ncbi.nlm.nih.gov/pubmed/17130416>) were utilized.

The second step that may be taken is genetic analysis, closely examining two types of DNA where mutations for primary mitochondrial disorders can be found. This step is most useful when a patient has the signs and symptom to support a known mitochondrial syndrome or disorder.

- 1) Mitochondrial DNA-encoded genes - inherited solely through maternal inheritance (mother) and due to cell division, display heteroplasmy, or the uneven passing of genes
- 2) Nuclear DNA-encoded genes - half passed from the mother and half from the father

Finally, biochemical studies, including blood tests (peripheral biochemical screening), tissue tests, (muscle or other tissue biopsies), and tissue test function (physiology), may give some idea of how well mitochondria are functioning in the body. Tissue test function includes physiology, imaging studies, and other testing. By looking at a combination of these different steps, physicians will be able to determine the likelihood of a primary mitochondrial disorder.

The Immune System (slide 6) Simply stated, the immune system is the body's major defense against infection, providing protection by fighting the pathogens throughout the entire body. Pathogens are microorganisms (bacteria, fungi, and viruses) capable of causing disease. Immune cells are found in multiple tissues (blood, skin, gut, lungs, muscles, etc.). Immune cells are heavily dependent on energy for both migrating to a site of infection or invasion, as well as combating the invading organism. Immune dysfunction can lead to multiple problems, including localized infections (infection in one part of the body), systemic immune response syndrome or SIRS (entire body is affected). SIRS is overwhelming and dangerous, requiring critical care in a hospital setting. Immune deficiency is now linked to autoimmunity, malignancy (cancer) and severe allergies. Specialized immune system cells include:

Mast cells - important to allergic reactions

Basophils - fight off some bacterial infections

Eosinophils - component in inflammatory responses, especially allergy, and also fight against parasites

B-cell and T-cells - educated cells, responding specifically to very specific organisms
Subsets of the primary immune system (slide 7) include:

Innate Immune System is the inborn first line of defense. Individual are born with this system which does not become “educated” or change with exposure to pathogens. The innate system is nonspecific, meaning it can work against multiple different pathogens, not requiring previous exposure to the pathogen. A whole complement of immune cells including phagocytes, macrophages, eosinophils, basophils, and innate T cells work together. This system carries the advantage of being ready to work at the time of an infection, yet may not be enough to fight pathogens.

Adaptive Immune System contains T and B-cells which are pathogen specific. The cells diversify with age, responding to previous exposures. The cells become “educated” over time, learning to recognize a specific pathogen and creating a reserve army to fight that exact pathogen with very specific weapons. With the aid of B-cells and T-cells, pathogen specific antibodies are produced to fight specific pathogens, which can then eliminate or seclude that pathogen more effectively.

The immune system is closely linked to mitochondrial function because all immune responses, both innate as well as adaptive, depend on energy metabolism to function (slide 8). Current research supports the role of mitochondrial function in viral and bacterial immunity as well as T-cell memory and function.

Mitochondrial gene mutations/changes are known to impact immune system dysfunction in these specific diseases: Barth Syndrome, Omenn Syndrome, and Cartilage Hair Hypoplasia (slide 9).

The Collaborative Study At MGH - “Predisposition to Infection and SIRS in Mitochondrial Disorders: Eight Years’ Experience in an Academic Setting,” was published in the *Journal of Allergy and Clinical Immunological in Practice* in 2014. The report reviews 8 years of clinical experience with patients with primary mitochondrial disease in light of immune system health (slides 10 & 11).

The cohort of patients registered at MGH with possible/probable Mito was about 250 patients, with 106 patients having met the criteria for probable or definite Mito using Bernier and Morava Criteria and either supporting genetics or biomedical studies. Nine patients had incomplete follow up, so were excluded from the study, leaving 97 patients - 60 females, 37 males, ranging in age from newborn to 68 years. On average, the study group was followed for 8.5 years at MGH (slide 12). Approximately 32 patients had some form of genetic study which supported Mito Disease (slide 13), most commonly: POLG mutations (7 individuals), MELAS (7 individuals), MERRF (5 individuals). The larger subgroup (75 patients) had a Mito diagnosis supported by biochemical studies, with 7 overlapping patients with both the genetic and biochemical studies (slide 14). The majority identified through biochemical studies showed a defect in oxidative phosphorylation - a deficiency in the electron transport chain. Dr. Walker explains biochemical studies as testing done to muscle tissue obtained via biopsy to test the function of electron transport chain.

Study question:

Does this diverse group of study patients show an increased likelihood of infection, either recurrent infections or serious infection, which includes systemic immune response syndrome (SIRS)? (slide 15)

No standard number or severity of infections has been established for “serious or recurrent” infections, although most physicians seem to know which patients fall into this category. For the purpose of this research, the inclusion and exclusion criteria were (slide 16):

Inclusion Criteria

- Infection requiring hospitalization
- Infection requiring surgical intervention (i.e. tympanoplasty (ear drainage tubes), incision & drainage)
- Infection meeting well defined, published SIRS criteria to quantify infection severity by vital sign changes and clinical and lab findings. Step wise progression of increasing severity is: infection, sepsis, severe sepsis, refractory septic shock, and multi-organ dysfunction syndrome.

Exclusion Criteria to exclude infections that may be related to the mitochondrial disorder

- UTI (increased rate of neurogenic bladder with Mito)
- Pneumonia with respiratory insufficiency (frequently due to myopathy causing respiratory muscle weakness in Mito patients)
- Blood stream infections because of central venous lines - unless occurring at higher rate than in all patients with CVLs

Findings: Infection type and percentage, occurring during the study period, were tabulated and graphed (slide 17). Forty patients, or 40%, experienced serious or recurrent infections (bacterial, viral, fungal). Twelve patients, or about 10%, had one or more episodes of SIRS. The CDC reports a very small percentage of SIRS infection in the general public with just 0.0017% or less than 2/1000 SIRS infections, demonstrating a 200-fold increase in the mitochondrial patient study group as compared to the general population. Specific infection producing pathogens most commonly found included: Staph aureus, including MERSA (15 patients), Candida Albicans (8 patients), and Clostridium difficile (6 patients) (slide 18). Since the common pathogens included bacteria, viruses and fungi both the innate and adaptive arms of the immune system were likely affected negatively by mitochondrial disease. Some of these infections may be acquired during a hospital stay, so families are reminded to be extra careful with hygiene when a Mito patient is hospitalized.

Nine study patients had a clearly defined immunodeficiency (slide 19) which was an important finding because treatment of these patients was tailored to support their immune systems.

For example, 5 patients were treated with immunoglobulin replacement therapy and had:

- decreased frequency and severity of infections
- prevention of developmental regression

- improved quality of life

In mito patients, weekly subcutaneous immunoglobulin treatments were better tolerated than monthly intravenous treatment because subcutaneous version had less side effects (headaches and chills), and tended to be more compatible with autonomic dysfunction (blood pressure changes, flushing, unusual heart rates).

This study had limitations (slide 21). As a retrospective study (looking at the cases after they happened by relying on medical records), the potential for selection bias, information bias, and incomplete or inaccurate records exists. Though the group of patients was diverse, most patients had oxidative phosphorylation defects which may also create a bias. Testing for immune deficiencies was done only for a small subset of patients, creating an incomplete data set. Ideally, all patients should have been tested.

Clinical implications (slide 22):

- Patients with primary Mito may benefit from baseline screening for immune deficiency and autoimmune disease.
- Patients with otherwise unexplained multi-system disorders, including immune deficiencies, warrant screening for primary Mito disorders.
- Providers caring for patients with primary Mito disorders should maintain increased clinical suspicion for infections and SIRS.
- Patients with primary Mito disorders and recurrent infections may benefit from aggressive hydration during illness, prophylactic antibiotics and/or immunoglobulin therapy in conjunction with appropriate specialist's recommendations.

Future Directions: (slide 23) Clinical immunologic screening has been extended to all primary Mito patients at MGH, but additional work includes:

- Further prospective clinical studies and laboratory investigations to better understand the connection between primary Mito disorders and immune dysfunction.
- Additional studies and improved assays for auto immune dysfunction (including atopy, autonomic dysfunction, & small fiber neuropathy)
- Guidelines for infection prophylaxis and treatment in primary Mito disorder
- Complex multidisciplinary teams for optimal management of the immune and autoimmune features of primary Mito disorders.

For example, combination therapy (CoQ10 treatment and SC immunoglobulin) in a 2-year-old boy improved symptoms (slide 24). With additional research, more insight into treatment options will result in improved care.

Additional Recommended Reading

Deficits in Bioenergetics and Impaired Immune Response in Granulocytes From Children With Autism. Napoli E1, Wong S, Hertz-Picciotto I, Giulivi

C. <http://www.mitoaction.org/autism/deficits-bioenergetics-and-impaired-immune-response-granulocytes-children-autism>

MitoAction Clinician's Guide - Infections: <http://www.mitoaction.org/guide/when-patient-sick>

Immunodeficiency Disorders and Mitochondrial Disease - Dr. Susan Pacheco. See more at: <http://www.mitoaction.org/blog/immunodeficiency-disorders-and-mitochondrial-disease#sthash.dLWGvzJt.dpuf><http://www.mitoaction.org/blog/immunodeficiency-disorders-and-mitochondrial-disease>

Sick Protocols and Illness Management Suggestions -

<http://www.mitoaction.org/autism/sick-protocol-and-illness-management-suggestions-kids-mito-autism>