

# Simplified Evaluation and Treatment of Autism Using Biomarker Directed Algorithms

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For parents and clinicians interested in caring for children with autism, the *Defeat Autism Now!* protocols constructed by Jon Pangborn and Sid Baker have served as an excellent understanding of the complex chemistry, toxicology, and immunology of these disorders. And we use the plural form because autism should more properly be thought of as *autisms*.

While autisms present a complex clinical problem for physicians, it seems to us that the approach can now be streamlined for many children. This is possible as a result of advances in the research on biomarkers. This has always been the goal of the DAN! movement. And given the most recent data from the U.S. and the U.K. which reflect that 1-2 % of males under 12 may have autism<sup>1,2</sup>, a simplified approach is needed for broad application to make it reasonable for mainstream pediatricians and family practitioners.

ICDRC has records on about 4,000 autistic children which we have evaluated and treated over the past decade. This provides an extensive clinical experience, and it is very much similar to other practitioners, e.g.: Baker, Bock, Boris, Goldblatt, Freedenfeld, Levinson, Mumper, Neubrandner and many others. In various DAN! Think Tanks we have all shared data and findings with one another and a central theme has emerged from these exchanges. This is our take on it and the way we generally evaluate and treat children on the spectrum. Obviously this process does not replace a good history and physical exam and a physician's clinical judgment – it is not medical advice – but a general framework of how we look at this disorder and decide on treatment.

Autisms generally have abnormalities in these related and overlapping areas:

- 1) oxidative stress<sup>3</sup>
- 2) decreased methylation capacity and limited transsulfation<sup>4</sup>
- 3) increased toxic burden – primarily of heavy metals and especially of mercury<sup>5</sup>
- 4) immunological dysregulation with a unique inflammatory bowel disease and immune activation of glial cells in the brain<sup>6</sup>
- 5) central nervous system hypoperfusion or abnormal regulation of blood supply to the brain<sup>7</sup>.

Most children we evaluate have ALL of these happening at the same time. All of these have broad negative influences on development and will impact all of the secondary features of autism including sensory abnormalities, coordination, cognition, mood, general health, sleep, and gastrointestinal function. They all connect like gears in the child's system and for that reason we need all of them turning in the right direction and properly connected.

By necessity of brevity, this discussion will only provide an overview of the evaluations and treatments. It is our general belief, that unless all of the underlying major biological disruptions are dealt with simultaneously, the cyclical negative impact of these problems on each other will perpetuate the autism symptoms and delay recovery.

**BIOMARKERS:** Note, some of these tests may be considered by Medicare, Medicaid and other carriers as investigational and, therefore, may not be payable as a covered benefit for patients. This is not an exhaustive list – it is intended to target core issues in autism.

**Immune Blood Markers:** It is very difficult to get direct measurement of brain inflammation and even cerebral spinal fluid has offered inconsistent findings. Stool findings for things like fecal calprotectin are also inconsistent predictors of disease. For this reason I use other markers:

- 1) Autoantibodies to endovasculature<sup>8</sup> performed at Washington University in St. Louis
  - Predicts immune involvement in speech delay and need to deal with immune vascular issues
  - Forms can be downloaded from <http://www.neuro.wustl.edu/neuromuscular/lab/serumreqc.htm> and check the box labeled Autism/Landau-Kleffner Syndrome variant.
  - If you are new to this test procedure, download the instructions for shipping and handling at <http://www.neuro.wustl.edu/neuromuscular/lab/seruminst.htm>
- 2) Neopterin/Biopterin<sup>9</sup>: these are two sides of the same coin.
  - Neopterin predicts the degree of cell mediated immune activation
  - Biopterin is a measure of attempts to down-regulate immune activation
- 3) ASO & Anti-DNase B
  - These are strep bacterial markers and if significantly elevated indicate recent strep infection
  - When associated with a change in obsessive compulsive or tic behavior these predict response to immune therapy and possible treatments. These are PANDAS like issues which are common in autism<sup>10</sup>
- 4) Immunoglobulin subsets: IgG (1-4), IgM, IgA and IgE
  - All helpful to know prior to starting treatment.
  - They are most important in sickly or autoimmune children where they can predict response to IVIG therapy.
- 5) Brain antibodies
  - Are rarely ordered by us anymore because antibodies to a variety of brain proteins can be detected in autism
  - A more careful analysis indicates MBP is not the primary antigen. Furthermore, the anti-brain antibodies are not available for commercial testing<sup>11</sup>.
- 6) Vaccine titers
  - Can be used to show immunocompetency and may reflect a response to IVIG.
  - They are also helpful to know if further vaccines are not going to be given since the immunization success of past vaccinations can be measured.
  - High levels can only be interpreted as immunity to that agent and not likely a reflection of problems, e.g., a high measles titer after vaccination means immunity to measles and cannot be interpreted as viral persistence.

**Immune Urine Markers:**

- 1) Neopterin and Biopterin. We prefer urine over blood.
- 2) Urinary N-Methylhistamine: May be useful in some cases of autism
  - Is a useful marker of significant inflammatory bowel disease
  - Is also elevated in asthma
  - Mayo Laboratory and Specialty Laboratory can perform this test. (ref 9)

### **Oxidative Stress Blood Markers:**

- 1) Reduced Glutathione (**not easily available commercially at this time**).
  - Is the opposite of oxidized glutathione, so it is inversely related
  - The higher the number the better.
- 2) GSSG, which is oxidized glutathione (**not easily available commercially at this time**).
- 3) Levels of major antioxidant proteins in the serum (standard blood tests)
  - Transferrin (iron-binding protein)
  - Ceruloplasmin (copper-binding protein)
  - Both are significantly reduced in autistic children as compared to their developmentally normal non-autistic siblings<sup>12</sup>.
  - A striking correlation was observed between reduced levels of these proteins and loss of previously acquired language skills in children with autism.
- 4) Blood ammonia and lactate
  - Reflect mitochondrial function and as such reflect the state of mitochondrial function in the presence of likely oxidative stress<sup>13</sup>.
  - These are standard tests done at all hospital, but we are amazed how many places have difficulty properly performing these tests.
  - Blood for ammonia and lactate require immediate icing of the blood tubes. If at all possible, lactate and ammonia levels should be drawn without the tourniquet released after the venopuncture or IV is started. Ideally, the child should not be fighting during the process. These may require sedation to get accurate results.
  - Special tubes are required: Typically Lactate = 3 ml Grey Stopper; K Oxalate and NaF, and Ammonia = 3 ml Green Stopper Heparin Tube or Green Microtainer with Lithium Heparin.

### **Oxidative Stress Urine Markers:**

- 1) 8 hydroxyguanine (8-OHG)
  - Is a marker for RNA oxidation in the mitochondria and cytoplasm of cells.
  - It is an easy marker to obtain for intracellular oxidative stress<sup>14</sup>.
  - The DNA marker of oxidative stress (8-dOHG) is not elevated in most cases.
- 2) Isoprostane looks at fatty acid oxidation and reflects cell membrane stress<sup>15</sup>.

### **Heavy Metal Markers Blood:**

Packed erythrocyte levels of toxic metals (mercury, lead, and arsenic in particular)

- Reflect ongoing exposure or rapid turnover from tissue.
- Lead in particular is trapped in bone and can be released during growth spurts without renewed exposure.
- These tend to tell us about the safety or lack of it in the child's current environment.
- We also will not chelate more than once if zinc and selenium are low until they can be adequately replaced. So a full panel of packed erythrocytes is helpful since it looks at nutritional minerals as well as toxic metals.
- Special tubes are required and vary by laboratory method.

### **Heavy Metal Markers Urine:**

- 1) Urinary fractionated porphyrins
  - Are an ideal way to assess the metals that are left behind. It can tell if mercury is present but is a little hard to distinguish how much is mercury versus lead.

- Ideally the lab should provide the entire chromatograph from the HPLC printout. If not, it would best to report precoprophyrin levels as this is the more sensitive mercury test.
- 2) Heavy metal challenge
- Ideally this means a post provocation urinary metal collected for either 6 to 24 hours. We typically choose 6 hours as most studies show the majority of chelation takes place in the first 6 hours.
  - A first morning urine after a bedtime dose of chelator is an excellent reflection of chelator efficacy.
  - Generally, we do not get pre/post urines – only post. This is both due to the added expense of a pre and the challenges of having two collections from most children.

### **Decreased methylation capacity and limited transsulfation - blood:**

Both of the following are frequently deficient in autism. Decreases in either or both of these may help to determine Methyl B12 responder status.

- 1) Fasting Plasma Cysteine or Cystine (the double form of cysteine)
- Cysteine is the sulfur containing amino acids that will act as the rate limiting step in production of glutathione – the key intracellular defense against oxidative stress.
  - Cysteine and glutathione also are involved in defending against heavy metals.
- 2) Fasting Plasma Methionine. Methionine is the main donor of methyl via an intermediate S-adenosylmethione (SAM or SAMe).

**Intestinal Permeability:** Abnormal absorption of lactulose and mannitol can be used to determine altered permeability in the gastrointestinal tract<sup>16</sup>.

**Urinary Organic Acids:** Reveal many factors useful in clinical management – unfortunately these levels have not been published and are generally not covered expenses by insurers. Yeast and anaerobic bacterial biomarkers in the urine do seem to correlate with clinical responses to antifungal and/or antibacterial intervention.

**Oxalates:** Can be measured in the urine and if high, support reduction in oral oxalates and perhaps reduce vitamin C intake, although this is controversial.

**EMERGING TESTS:** Functional imaging studies with SPECT, fMRI and PET are not routine although they can be expected to be abnormal in virtually all cases.

### **ICDRC Preferred Biomarkers and Lab Tests:**

#### IMMUNOLOGICAL:

- **Urinary:** Neopterin and Biopterin
- **Blood:** Anti-endothelial Antibodies at WUSTL, ASO and Anti-DNase B, IgG subclasses, IgM, IgA and IgE, Complete Blood Count.
- **Special:** if Neopterin elevated and/or GI symptoms are present, check intestinal permeability to lactulose and mannitol.
- **Urine** tested after standard dose at timed interval.

#### OXIDATIVE STRESS:

- **Urine:** 8-OHG and when available Isoprostane
- **Blood:** Transferrin, Ceruloplasmin, Ammonia and Lactate.

#### HEAVY METALS:

- **Blood:** Packed Erythrocyte Minerals and Toxic Metals
- **Urinary:** Fractionated Porphyrins and if elevated get a post chelation challenge 6 hour urine toxic metal assay.

#### METHYLATION AND TRANSSULFATION:

- **Plasma:** Fasting Cysteine and Methionine

#### METABOLIC PROFILE:

- **Blood:** Electrolytes, Liver and Renal Chemistries.

#### OPTIONAL:

- **Urinary** Organic Acid Test and **Urinary** Oxalates.

Note: If you are experienced with DAN! treatments you might notice several things are missing: e.g., Stool cultures (important if you suspect parasites), and food allergy tests (these may still be helpful but are not primary biomarkers).

### BIOMARKER DIRECTED TREATMENT

Working with your local physician and/or DAN! Consultant, you can use the biomarkers to guide treatment. Our plan would be to normalize all of the abnormal areas detected via these biomarkers as rapidly as possible.

**Immune:** Elevated neopterin would likely respond to immune modifying drugs: steroids, IVIG, pioglitazone, montelukast, spironolactone and others. Biopterin can be supplemented as well and this may emerge in the future as a useful therapy. Nasal administration of secretin and oxytocin are in early research development, but appear promising. If the intestinal permeability study is abnormal, we try to use both immune agents and secretin via nasal spray twice a day.

**Oxidative Stress:** Vitamin C in dose up to 4 grams a day. It is important to follow urinary oxalates if high dose Vitamin C is being used. Bioflavonoids of a variety of types are excellent antioxidants. Grape seed extract and French maritime pinebark have excellent antioxidant properties. Euterpe oleracea Mart, (Acai) fruit and Garcinia mangostana (Mangosteen) fruit are also excellent antioxidants. Normalizing cysteine levels are important to this, so supporting the folate-methylation-transsulfation pathway is important. Various cofactors are required: B6, Riboflavin, TMG, Folate, and MethylB12 will likely be helpful. Equally important is the removal of heavy metals via chelation. Finally, mitochondria must be supported. Here we use: acetyl L-carnitine, ubiquinone or liposomal CoQ 10, and D-ribose. Melatonin also aids oxidative stress.

**Heavy Metals:** The goal is to reduce the abnormal porphyrins to normal via chelation without depleting vital trace minerals. We prefer CaNa<sub>2</sub>-EDTA and DMPS via either IV or Rectal Suppositories. These approaches seem to cause the least number of side-effects. You must consult a knowledgeable clinician if the porphyrins are elevated. NaEDTA used incorrectly appears to be responsible for at least one death of a child with autism. NaEDTA can remove vital calcium from muscle tissue causing failed muscle contraction – especially in the heart. This is NOT a problem for CaNa<sub>2</sub>-EDTA which has an excellent safety record. Remember chelators are always chelated to something. They will reduce healthy minerals, so replacing zinc, selenium, copper and magnesium are critical during chelation. One child also developed iron deficiency during CaNa<sub>2</sub>-EDTA chelation. These are all manageable issues if you are informed and follow the blood mineral levels. We use the example of a well or cistern when we describe the differences of porphyrins and urine metal post - challenge tests. A challenge test tells you how much is in the bucket while porphyrins

tell you how much is in the cistern. So the challenge tests demonstrates the efficacy of the chelation process, while the porphyrin test tells you what is left in the child.

**Methylation – Transsulfation:** Methyl B12 via injection and/or nasal spray – possibly transdermal but uptake not determined. Cysteine may be directly supplemented with NAC but this is tricky due to dysbiosis issues with oral supplements. Methionine can be supplemented at meal times but doses should not be too high or other amino acids may be artificially depleted. Oral folate, leucovorin, or methylfolate can help. Supplementing B6, and other B vitamins, magnesium, and alleviating oxidative stress all helps this pathway to function. TMG may be an alternative method of helping.

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