

Simplified Evaluation and Treatment of Autism Using Biomarker Directed Algorithms©

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While autism presents complex medical problems 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 biomedical therapy advocates. 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^{1,2}, a simplified approach is needed for broad application to make it reasonable for mainstream pediatricians and family practitioners. Simultaneously, abundant evidence shows us that Applied Behavioral Analysis (ABA, previously know as Behavior Modification) can recover children as well. So how can this be? Simply the brain is bidirectional in its functioning and requires input as much as it gives output. So the programming of a child with curriculum to master skill deficits with ABA is vital to their recover as well and needs to be address along with issues of inflammatory bowel disease and other medical problems. And all of us need to avoid reductionistic thinking about the human being as a biological machine as it is clearly far more complicated than that. To that end I wanted to include the abstract of the UCLA follow-up study demonstrating long-term success with ABA.

Long-term outcome for children with autism who received early intensive behavioral treatment.

McEachin JJ, Smith T, Lovaas OI.
Am J Ment Retard. 1993 Jan;97(4):359-72
UCLA, Department of Psychology 90024-1563.

After a very intensive behavioral intervention, an experimental group of 19 preschool-age children with autism achieved less restrictive school placements and higher IQs than did a control group of 19 similar children by age (Lovaas, 1987). The present study followed-up this finding by assessing subjects at a mean age of 11.5 years. Results showed that the experimental group preserved its gains over the control group. **The 9 experimental subjects who had achieved the best outcomes at age 7 received particularly extensive evaluations indicating that 8 of them were indistinguishable from average children on tests of intelligence and adaptive behavior.** Thus, behavioral treatment may produce long-lasting and significant gains for many young children with autism.

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 with the medical aspects of this disorder, and it is very much similar to other practitioners, e.g.: Baker, Bock, Boris, Goldblatt, Freedendfeld, Levinson, Mumper, Neubrandner and many others. In various Defeat Autism Now! 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 stress³
- 2) decreased methylation capacity and limited transsulfation⁴
- 3) increased toxic burden – primarily of heavy metals and especially of mercury⁵, and bacteria generated neurotoxins (particularly Clostridia species bacteria) .⁶
- 4) immunological dysregulation with a unique inflammatory bowel disease and immune activation of glial cells in the brain⁷
- 5) central nervous system hypoperfusion or abnormal regulation of blood supply to the brain⁸.

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 endovasculature⁹ 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/Biopterin¹⁰: 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 and D8/17 Lymphocytes¹¹
 - 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 may predict response to immune therapy and possible treatments. These are PANDAS like issues which are common in autism¹². D8/17 is available through Immunoscience Laboratories.
- 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 may predict response to IVIG therapy (Oleske).
- 5) Brain antibodies
- Are rarely ordered by us anymore because antibodies to a variety of wide brain proteins can be detected in autism
 - A more careful analysis indicates MBP is not the primary antigen. Furthermore, the other anti-brain antibodies are not available for commercial testing¹³.
- 6) Vaccine titers
- Can be used to show immunocompetency and obviate the need for booster vaccination, while immune deficiency may reflect a more likely response to IVIG.¹⁴
 - 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 disease¹⁵
 - Is also elevated in asthma
 - Mayo Laboratory and Specialty Laboratory can perform this test. (ref 9)

Immune Fecal Markers:

- 1) Lactoferrin
- 2) Calprotectin
- 3) Eosinophil X
- 4) S100A12
- 5) Culture
- 6) Analysis

Oxidative Stress Blood Markers:

- 1) Reduced Glutathione¹⁶ (**not easily available commercially at this time**).
 - Is the opposite of oxidative 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 siblings¹⁷.
 - 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 stress¹⁸.

- 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 stress¹⁹.
- 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 stress²⁰.

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 the 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 lead²¹.
- Ideally the lab should provide the entire chromatograph from the HPLC printout. If not, it would best to report precoproporphyrin 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 tract²².

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. **Microarray Testing:** The proper and harmonious expression of a large number of genes is a critical component of normal growth and development and the maintenance of proper health. Disruptions or changes in gene expression are responsible for many diseases. Microarrays are miniaturized biological devices consisting in molecules, for example DNA or protein, identified by the probes, that are orderly arranged at a microscopic scale onto a solid support such as a membrane or a glass microscope slide. The array elements bind specifically to labelled molecules, the "targets", present in complex molecular mixtures, generating signals that reveal the identity and the concentration of the interacting labelled species. Microarray analysis has a broad range of applications that involve different types of probes and/or targets. Microarrays have the potential to look at the true consequences of toxic or immune exposures on DNA transcription (i.e., functional analysis). It allows for evaluation of gene activation, protein production, immune activation and toxicological interference. Eventually it will be feasible for routine case management.

ICDRC Preferred Clinical 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. In the future, quantitative analysis of bacterial DNA in the gut will be routine. The nature of the good vs. bad bacteria in the gut is a major determinant of intestinal immune function.²³

OXIDATIVE STRESS:

- **Urine:** 8-OHG (Oxidized RNA byproduct) and when available Isoprostane

- **Blood:** Transferrin, Ceruloplasmin, Ammonia and Lactate. Autoantibodies to cholesterol are reasonable biomarkers of oxidation in adults²⁴ and may be in autism as well, but this has not been evaluated to our knowledge.

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 TRANS-SULFATION:

- **Plasma:** Fasting Cysteine and Methionine, and if available the SAM/SAH ratio (not readily available apart from research applications).

METABOLIC PROFILE:

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

OTHER:

- **Urinary** Organic Acid Test and **Urinary** Oxalates.
- William Shaw of Great Plains Lab believes a tyrosine derivative, which is very similar to but is not identical to 3,4-dihydroxyphenylpropionic acid is a good biomarker for Clostridia and it appears to be clinically helpful to us as well.

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 Defeat Autism Now! 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 is in early research development, but appears promising²⁵. If the intestinal permeability study is abnormal, we try to use both immune agents and secretin via nasal spray twice a day. However, restoring normal gut flora may be the ideal way to regulate the immune system in the gut. We prefer cultured foods over probiotic supplements²⁶. Experience has taught us that in the ASD population it is difficult to get lasting results without using cultured foods. Some children may require frequent rounds of vancomycin as an oral antibiotic to eliminate Clostridia²⁷ until full function of the new restored gut flora is accomplished. Consider lactobacillus as the critical good flora and clostridia as the most likely bad one. Resources: www.culturedvegetables.com or www.net and www.bodyecologydiet.com. We do not use cultured cows milk, but some children can consume cultured goat milk without adverse reactions. Saccharomyces (nutritional yeast) are in some cultured foods and many children with the ASD group do not do well with this at least initially. We would start with simply *Lactobacillus rhamnosus* GG cultured foods like coconut or vegetable water. The following study is important in its ability to help us understand the variations in probiotic responses.

Probiotics for treatment of acute diarrhoea in children: randomised clinical trial of five different preparations.

BMJ. 2007 Aug 18;335(7615):340. Epub 2007 Aug 9.

Canani RB, Cirillo P, Terrin G, Cesarano L, Spagnuolo MI, De Vincenzo A, Albano F, Passariello A, De Marco G, Manguso F, Guarino A.
 Department of Paediatrics, University of Naples Federico II, Naples, Italy.

OBJECTIVE: To compare the efficacy of five probiotic preparations recommended to parents in the treatment of acute diarrhoea in children. Design Randomised controlled clinical trial in collaboration with family paediatricians over 12 months. **SETTING:** Primary care. **PARTICIPANTS:** Children aged 3-36 months visiting a family paediatrician for acute diarrhoea. **INTERVENTION:** Children's parents were randomly assigned to receive written instructions to purchase a specific probiotic product: oral rehydration solution (control group); Lactobacillus rhamnosus strain GG; Saccharomyces boulardii; Bacillus clausii; mix of L delbrueckii var bulgaricus, Streptococcus thermophilus, L acidophilus, and Bifidobacterium bifidum; or Enterococcus faecium SF68. **MAIN OUTCOME RESULTS:** 571 children were allocated to intervention. **Median duration of diarrhoea was significantly shorter (P<0.001) in children who received L rhamnosus strain GG (78.5 hours) and the mix of four bacterial strains (70.0 hours) than in children who received oral rehydration solution alone (115.0 hours).** One day after the first probiotic administration, the daily number of stools was significantly lower (P<0.001) in children who received L rhamnosus strain GG and in those who received the probiotic mix than in the other groups. The remaining preparations did not affect primary outcomes. Secondary outcomes were similar in all groups. **CONCLUSIONS: Not all commercially available probiotic preparations are effective in children with acute diarrhoea.**

Oxidative Stress: Vitamin C must be sufficiently supplemented. 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 anti-oxidants. We have followed urinary 8OHG levels and find normalization with Acai at a dose of 1-2 scoops of the freeze-dried powder daily. Grape seed extract is also very good. See the ORAC power of fruits below (per gram of whole food). Freeze dried concentrates are much higher per gram.

Muscadine Grape Seed**	559
Acai*	387
Goji Berry*	253
Noni*	151
Pomegranates*	105
Raspberries*	82
Blueberries*	77
Red Grapes*	74
Prunes*	57
Cherries*	67
Strawberries*	36

*[Antioxidant Capacities of Foods](#). J. Agric. Food Chem., Vol. 52, No. 12, 2004 4027

*Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL, J Agric Food Chem. "Lipophilic and hydrophilic antioxidant capacities of common foods in the United States." 2004 Jun 16;52(12):4026-37

Normalizing cysteine levels are important to this, so supporting the folate-methylation-transsulfation pathway is important. Various cofactors are required: B6, Riboflavin, DMG/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. Commonly used chelators are:

- **DMSA:** (Succimer) FDA approved for children with lead intoxication. Only 20% absorbed orally. No IV form. Suppositories well tolerated. Fairly significant oral intolerance with regression in some children. May be related to poor absorption and increased dysbiosis.

- **DMPS:** (Dimaval) Not licensed in the US, but available legally via compounding pharmacies. More reactive than DMSA. IV form available – so with this technique assured delivery to the blood stream. About 50% is absorbed orally. Suppositories well tolerated and seem effective. DMPS is considered better Hg chelator than DMSA and may also reduce brain levels of mercury based on neurocognitive testing.²⁸

- **CaNa2 EDTA:** (Calcium Disodium Edetate) Licensed for Lead. Poorly absorbed orally. IV appears well tolerated. Suppository may be more effective than oral where only < 5% absorbed.

- **D-Penicillamine:** 5-15 mg/kg per day has been used safely in lead intoxication²⁹ – issues with safety at higher doses. Check CBC and LFTs frequently – rashes – allergy. It is also used in autoimmune disease, but at higher doses which may increase side-effects in ASD.

We prefer CaNa2-EDTA and DMPS via either IV or Rectal Suppositories. Although not as well utilized, D-penicillamine is a well established chelator that is a good mercury chelator as well.³⁰ In this study they showed that in a case of potentially fatal mercury exposure in a child, 9 months of treatment with D-penicillamine, demonstrated the patient's clinical condition, biochemical laboratory parameters, and mercury concentrations all returned to normal. The T2-weighted MRI images of the patient's brain initially showed multiple hyperintense lesions in cerebral white matter, left globus pallidus, and putamen, which also improved with D-penicillamine. Oral D-penicillamine is fairly well tolerated in ASD. Penicillin allergy may be a contraindication to its use. These chelators 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 CaNa2-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 CaNa2-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 test 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 has 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 or DMG may be an alternative method of helping.

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