Chronic Inflammatory Response Syndrome: Diagnosis and Treatment

A Paradigm Change for 21st Century Medicine

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What is Chronic Inflammatory Response Syndrome?

Chronic Inflammatory Response Syndrome (CIRS) is a complex illness caused by immune system dysregulation following exposure to a biotoxin producing organism. The chronic inflammation that results can lead to multiple organ system dysfunction. Patients will often present with numerous symptoms spanning a large number of body systems. [30,35,43] Organ systems impacted by CIRS include gastrointestinal, neuro-endocrine, musculoskeletal and immune.[35] Inflammation is initiated when the body is incapable of removing specific toxins, including biotoxins, neurotoxins and endotoxins.[41] Once this inflammatory cascade begins, patients become chronically ill and may require extensive treatment.

Unfortunately, many patients who experience this syndrome have sought care from a multitude of specialists. This often this leads to excessive health care costs and an extensive laundry list of misdiagnoses. Common misdiagnoses include Fibromyalgia, Chronic Fatigue Syndrome, Multiple Sclerosis, Depression, Stress, Allergies, Irritable Bowel Syndrome, ADD, PTSD or Somatization.[41]

Until recently, a definite diagnosis was not possible due to a lack of formal pathophysiological mechanisms or described biomarkers. Thanks to Ritchie Shoemaker, MD, we now have a foundation for understanding the mechanisms of CIRS and a process of diagnosis and treatment that can be measured and quantified.[41] Dr. Shoemaker originally coined the term "Chronic Inflammatory Response Syndrome" after extensive inquiry into biotoxin illness, beginning in 1997 after billions of fish were plagued with *Pfiesteria* in the Eastern Chesapeake Bay.[25] Residents developed a "mysterious illness", complaining of headaches, secretory diarrhea, rash, cough, muscle ache, fatigue and cognitive impairment. At that time, Dr. Shoemaker made the first connection between toxins and human illness, which led to his eventual discovery of the biotoxin pathway. [25]

This paper will present the mechanism and biomarkers associated with that pathway as well as the 12-step protocol defined by Ritchie Shoemaker, MD, to correct it. [41]

Water-damaged buildings and health effects

According to the United States Government Accountability Office (U.S. GAO), indoor mold is considered a serious health threat that causes immune-mediated and infectious health concerns.[45] The World Health Organization (WHO) has estimated that 50% of all the homes in North America are "sick buildings".[47] Inadequate building and faulty structural materials can lead to leaks, flooding and ground water intrusion.[47] Newer "energy efficient" construction contributes to indoor air pollution by not allowing for proper air ventilation.[23] With the presence of moisture, this creates the perfect environment for mold growth and indoor air contamination.[35,45] Exposure can occur through inhalation, ingestion and contact with the skin. The smaller the particle, the more likely it is to cause adverse health effects. According to the GAO, there are no federal or generally accepted health-based standards for safe levels of mold in the air or on surfaces.[45]

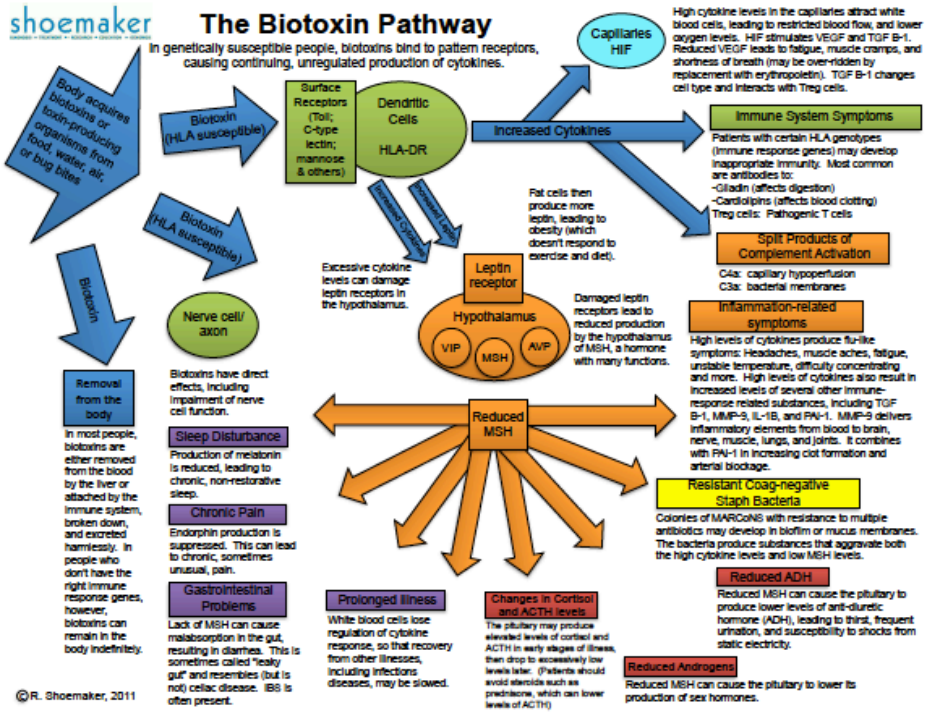
"Water-Damaged Building (WDB) illness is defined as a chronic illness with multiple health symptoms from multiple body systems acquired following exposure of at least 30 days to the interior environment of a building with a history of water intrusion, followed by amplification of growth of resident toxigenic microbes, including but not limited to fungi, bacteria, actinomycetes and mycobacteria; as well as inflammagens and volatile organic compounds." [32] Derived from the above, Table 1 below lists the associated toxins and inflammagens: [23]

Table 1: Types of toxins, inflammagens and microbes associated with WDB[23]

Fungi	*Aspergillus penicilloides, Aspergillus versicolor, Wallemia sebi, Stachybotyrs chartarum, Chaetomium globosum*
Fungal fragments	Mycotoxins, hyphal fragments, conidia, mannans, spirocyclic drimanes, Chitinases
Protozoa	Protozoa and amoebas
Bacteria	*Gram negative bacteria, gram positive bacteria, mycobacteria, chlamydia, mycoplasma*
Bacteria fragments	Endotoxins, Lipopolysaccharides, Actinomycetes, Nocardia, Hemolysins
Inflammagens	Siderophores, beta glucans, cell wall components, cell fragments, proteinases
VOC's	Microbial VOC's, building material VOC's, inorganic xenobiotics
Particulates	Bioaerosols, coarse particulates, fine particulates, ultrafine particulates, nano-sized particulates

The Biotoxin Pathway

When exposed to the microbes, toxins and inflammagens associated with water-damaged buildings or other biotoxin-producing organisms, a series of complex biochemical events begins. The biotoxin pathway is outlined in the following diagram:



It all begins with biotoxins and the immune system

Biotoxins are small, fat-soluble molecules produced by biological microorganisms, capable of causing disease on contact or by absorption by body tissues. Biotoxins are synonymous with ionophores and amphipaths which can move easily from cell to cell due to their small size (as small as 1.4 angstroms) and their ability to share electrons.[36] These toxins store in adipose and nerve tissue, where they interact with cellular receptors including toll 2,4 receptors, mannose, dectin and C-type lectin receptors, turning on innate immune activation. [5,37,40,41]

Listed below are known biotoxin (ionophore)-producing organisms that can cause a chronic inflammatory cascade and severe health effects in humans:

- CIRS-Water Damaged Building (WDB): *Chaetomium globosum, Aspergillus penicilloides, Aspergillus versicolor, Stachybotrys chartarum, Wallemia sebi, Actinomycetes, bacteria and inflammagens*
- CIRS-Post Lyme Syndrome (PLS): *Borrelia species*
- CIRS-Possible estuarine associated syndrome (PEAS): *Pfiesteria (dinoflagellate)*
- CIRS-Ciguatera: *Gambierdiscus (dinoflagellate)*
- CIRS-Arachnids: *Recluse spider*
- CIRS-Apicomplexans: *Babesia spp., Sarcocystis*
- CIRS-Cyanobacteria: *Microcystis, Lyngbya, Cylindrospermopsis, Anabaenopsis* [38]

The body has a complex immune strategy to prevent invasion by foreign pathogens. The skin and the epithelial lining of the lung and gut provide a physical barrier between the inside and the outside world.[1] The epithelial lining secretes mucus to prevent adherence and contains antimicrobial peptides called "defensins" that kill pathogens and prevent their growth.[1] If this layer becomes compromised the innate and adaptive immune system are recruited.

The innate (inborn) immune system responds immediately after detecting pathogen-associated molecules. These molecules, also known as pathogen-associated molecular patterns (PAMPS), are recognized by the toll receptors on leukocytes and dendritic cells. Toll receptors stimulate a program of gene expression and activate inflammatory chemicals and phagocytosis. Inflammatory chemicals include histamines, cytokines, complement proteins, interleukins, leukotrienes and prostaglandins. Cytokines act as chemical messengers that communicate between the innate and adaptive immune systems. Fever and flu-like symptoms are the hallmark of innate immune activation. [1,14]

The adaptive immune system, also known as acquired or specific immunity, develops over a person's lifetime. Consisting of T cells and B cells, adaptive immunity assists pathogen removal by developing specific antigen recognition and memory. This allows for rapid recall of original antigen exposure. Adaptive immunity is highly specialized and able to produce enormous immunological diversity. When environmental and genetic factors provoke internal dysregulation, autoimmune conditions can occur.[1, 14]

Normally, when a person comes in contact with a biotoxin-producing organism, the immune system is able to bind, tag and remove it effectively. In CIRS, antigen-antibody recognition becomes impaired causing persistent biotoxin load and continual activation of the cytokine response. [5,36,37] The overactive innate immune system, coupled with a dysfunctional adaptive immune system leads to multifaceted and multi-systemic disease syndrome.[5, 14]

Identifying CIRS in a patient

In order to identify a pattern of illness, a two-tiered case definition was established by Dr. Shoemaker (2005) and revised by the GAO report (2008). A third tier was added for treatment efficacy. [30, 31, 35, 42,45]

CASE DEFINITION: TIER 1 (includes all of the following criteria)

- *Exposure History*
  - There must be a history of exposure with a biotoxin-producing organism. This could be due to water-damaged building with visible microbial growth, identification of fungi using MSQPCR, or presence of musty smells.
  - Other considerations would be history of tic bite, spider bite, ingesting fish that feeds on barrier reef fish, or exposure to blue green algae.[30]
- *Presence of Multiple Symptoms* (in at least 4 of 8 system categories)
  1. General symptoms: fatigue and weakness
  2. Musculoskeletal: muscle aches, cramping (claw-like cramping in hands and feet), joint pain
  3. Respiratory: cough, shortness of breath, chronic sinus issues, asthma
  4. Eyes: redness, blurred vision, tearing, sensitivity to bright lights
  5. Gastrointestinal: abdominal pain or cramping, nausea, diarrhea
  6. Central nervous system: memory loss, difficulty word finding, confusion, disorientation, difficulty concentrating, learning difficulties, mood swings
  7. Neurological: headache, numbness, tingling, lightheaded, vertigo, metallic taste, temperature dysregulation, tremor
  8. Unusual symptoms: increased thirst, frequent urination, appetite swings, frequent electric shock [30]
- *Absence Of Other Explanations For Illness (Differential Diagnosis)* have been considered and ruled out. [30]

CASE DEFINITION: TIER 2 (at least 3 of 6 criteria)

1. Deficit in visual contrast sensitivity (VCS)
2. Genetic predisposition measured by HLA DR PCR
3. Elevation of Matrix Metalloproteinase-9 (MMP9)
4. Deficiency in alpha Melanocyte Stimulating Hormone (MSH)
5. Dysregulation of ACTH/cortisol
6. Dysregulation of ADH/osmolality [30]
   - Antigliadin antibodies (AGA) and Anticardiolipin antibodies (ACLA) are substituted for children < 18 years of age[32] (See Appendix 1)

CASE DEFINITION: TIER 3 (showing improvement in at least 2 of 3 markers)

- Effective response to Cholestyramine/Welchol with resolution of symptoms
- Reduction of leptin, if elevated with treatment
- Reduction in MMP9, if elevated with treatment[30]

Case Definition Tier One:

Evaluation and Exposure History

The initial evaluation is a thorough subjective and objective assessment that includes a comprehensive environmental and exposure history. The subjective assessment is conducted as a dialogue rather than administered as a written checklist of symptoms. The patient must be asked the following questions: has the patient resided or worked in a building with water damage with musty odors or visible mold; lived in an area endemic for Lyme disease; travelled to the Caribbean or South Pacific; ingested or handled fish; or come into contact with blue green algae? Has there been impaired performance in work or school environments? Are there developmental issues if evaluating a child (eg. ADD/ADHD, failure to thrive)? Were there any known exposures to heavy metals, solvents or chemicals? If a woman, do they have a history of Breast implants?

In the objective evaluation, many CIRS patients will show signs of neurological maladies including resting tremors, cold hands or discoloration in the hands or feet. The medical practitioner should check for muscle weakness by testing the upper trapezius muscles and grip strength.[41] Common findings in CIRS patients include fatigue and unilateral weakness in the dominant arm. Some patients exhibit a resting tremor. Hyper-flexibility warrants further evaluation for Ehler's-Danlos.

It may be necessary to perform additional testing to evaluate possible contributing factors such as cardiovascular, respiratory, thyroid, neuromuscular or neurological disease, including:

- EKG to evaluate arrhythmia, palpitations and chest discomfort.
- Stress echocardiogram to measure pulmonary systolic arterial pressure (PASP). PASP rising more than 8 mmHg during near maximal exercise is seen in 90% of patients with shortness of breath and nearly all pediatric patients with postural orthostatic tachycardia syndrome (POTS).
- Pulmonary function tests may reveal a restrictive instead of obstructive pattern.
- VO2 max may show reduction to below 25 ml oxygen/kg/minute causing a "delayed recovery from normal activity".
- Abdominal pain is a common finding in 78% of CIRS-WDB patients.
- A nuclear scan of abdomen will show impaired gastric motility in 5% of CIRS cases.
- Endoscopy is ordered to rule out esophageal reflux in cases of abdominal pain, bloating, belching and acid taste; yet no sign of esophageal irritation will be seen.[4]

Biotoxin Illness symptom clusters

The most common symptoms associated with CIRS have been categorized into 13 clusters. Originally designed for statistical analysis, symptom clusters were found to increase predictive value in CIRS. One should be highly suspicious of CIRS when 6 out of 13 clusters are noted. If a patient reports 8 out of 13 clusters, this is confirmatory for biotoxin-mediated illness.[4]

Table 2: Symptom Cluster Analysis

Persistent fatigue	Unusual skin sensitivity Tingling Tremors Unusual pain	Red Eyes Blurred Vision Night Sweats Mood swings Ice-pick Pain
Muscle weakness Difficulty integrating new knowledge Body aches Headache Light sensitivity	Shortness of breath Sinus congestion	Abdominal pain Diarrhea Numbness
Impaired memory Difficulty finding words	Cough Excessive thirst Confusion	Tearing of the eyes Disorientation Metallic taste
Decreased concentration	Appetite swings Difficulty regulating body temperature Increased urinary frequency	
Joint pain A.M Stiffness Muscle cramps		Static shock Vertigo

Case Definition Tier Two:

Visual Contrast Sensitivity Testing

The visual contrast sensitivity (VCS) test is a measure of neurological function of vision. The ability to discern between visual patterns of grey, white and black is visual contrast. In the 1990's, Ken Hudnell PhD, a neurotoxicologist with the Environmental Protection Agency (EPA), identified VCS as a reliable tool to show adverse changes in neurological function after exposure to *Pfiesteria.* Chemicals, medication and biotoxins have all been implicated in the destruction of the visual nervous system and can selectively target different anatomical structures within the visual pathway.[27]

The visual pathway begins with photoreceptors in the retina, continues along the optic nerve/tract, directly to midbrain nuclei and then extends to the visual cortex. In the presence of biotoxins, the optic nerve and the retina become compromised due to capillary hypoperfusion caused by inflammatory elements. Visual contrast tends to be affected but

visual acuity remains unchanged. Visual acuity is not affected due to the precision of the optic system and its resolution limit for high contrast stimuli. In contrast, VCS assesses visual function from the retina to the cortex and engages neurons sensitive to a wider range of stimulus size. According to Dr. Shoemaker, 92% of CIRS patients will fail VCS testing. The other 8% may falsely pass the test if thy have excellent visual acuity even under severe inflammatory conditions.

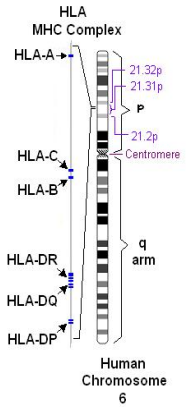
In 1999, Shoemaker and Hudnell demonstrated that VCS testing could be used as a screening tool to show that treatment with cholestyramine or Welchol corrected VCS deficits. This not only makes VCS testing a quick and effective tool in screening for neurotoxicity it also functions as a tool to monitor treatment. Correction was noted by an improvement in the ability to see contrast by 2 rows in any one column or by one row in each of the five columns. A decline was marked by a fall in two rows in any one column. The goal of treatment is to continue to see improvement from the patient's baseline until there is no further improvement in symptoms and VCS.[5, 25]

At present, both a computerized and an in-office test are available. The "in-office test" is considered more accurate as the patient can go back and review failed answers. The computerized test does not allow for this, which may lead to a false negative test. If someone fails the computerized test, they should have an in-office test performed to confirm the result. [41]

Procedure:
Visual acuity must be tested prior to VCS testing and must be better than 20/50 in both eyes to perform the test. The visual contrast sensitivity chart contains five columns of circles containing different shades of vertical and horizontal patterns with various contrast levels using decreased shading from black to grey. Contrast levels go from high to low and columns go from left to right. The patient covers one eye and reports the direction of the bars in each circle through five columns. The ambient lighting of the room needs to be set at 70 ft. lamberts, which can be calibrated by light meter or can be achieved using a desk lamp with 2-15 watt daylight fluorescent bulbs shining directly on the card. [30]

In order to pass the test, a patient must be able to see with both eyes beyond row 6 in column C and beyond row 5 in column D. Column E can be used to assess treatment effectiveness and can also be used to monitor potential intensification reactions seen in patients with Lyme disease.[5]

HLA DR DQ



Human Leukocyte Antigens (HLA DR) are genes we inherit from our parents (one from each) that are necessary for toxin clearance through the adaptive immune system (T and B cells). [5,27] They specifically code for a protein named the major histocompatibility complex II (MHCII) molecule.[15] When there is a defect in the HLA, the MCHII cannot perform its role with antigen presentation

and antibody formation. This allows for biotoxins to persist and leads to increased toxin load and illness. [5,27]

Upon extensive review of the gene registry, Dr. Shoemaker found that approximately 24% of the population has a genetic susceptibility to chronic mold illness and 21% of the population is Lyme susceptible.[5] Lyme susceptible patients have a potential for not responding well to antibiotics and may need assistance in biotoxin clearance as well.[5] The procedure for identifying the different HLA haplotypes is listed in *Surviving Mold: Life in the Era of Dangerous Buildings* under Appendix 2: Rosetta Stone. The chart below is a compilation of the haplotypes discovered by Dr. Shoemaker. [36]

Table 3: Rosetta Stone, Appendix 2: Surviving Mold

	DRB1	DQ	DRB3	DRB4	DRB5
Multisusceptible	4	3		53	
	11/12	3	52B		
	14	5	52B		
	13	3	52A		
Mold Susceptible	7	2/3		53	
	13	6	52A, B, C		
	17	2	52A		
	18*	4	52A		
Borrelia, Post Lyme Syndrome	15	6			51
	16	5			51
Dinoflagellates	4	7/8		53	
Multiple Antibiotic Resistant Coagulase Negative Staph Aureus (MARCoNS)	11	7	52B		
No recognized significance	8	3, 4, 6			
Low-risk Mold	7	9		53	
	12	7	52B		
	9	9		53	

Lab markers associated with CIRS

There are key lab markers that define CIRS. In recent years, the case definition has been expanded to include additional markers of importance in the biotoxin pathway. Patients must present with 4 of 8 markers: MSH, VIP, VEGF, TGF-beta 1, MMP9, C4a, ACTH/cortisol and ADH/osmolality. The following section describes all the serum markers as outlined in the biotoxin pathway and summarizes the importance of each.

Leptin

Reference range: Male: 0.5-13.8 ng/mL; Female 1.1-27.5 ng/mL

Produced in the adipose (fat) cells, leptin helps regulate energy and suppresses hunger. It is the hormone that tells the brain when we are full and satisfied and is the key driver in weight loss. In CIRS, this hormone becomes elevated due to an overactive cytokine response and over time, its receptor in the hypothalamus becomes damaged, which causes leptin resistance. When the leptin receptor is "resistant," we hold on to fatty acids and store them as fat. This leads to rapid weight gain that dieting and exercising doesn't correct. [27]

Leptin play an important role with alpha melanocyte stimulating hormone (MSH) in the hypothalamus. It directly activates pro-opio-melanocortin (POMC) neurons and the subsequent release of MSH. Reduction of MSH occurs when excessive cytokine production interferes with the leptin receptor and communication is diminished through the POMC pathway. [27,30]

Melanocyte Stimulating Hormone (MSH)

Reference range: 35-81 pg/mL

Melanocyte stimulating hormone is a neuro-regulatory hormone that modulates many other endocrine hormones, having effect on melatonin, endorphins, ADH, sex hormones, ACTH and cortisol. It has been studied extensively for its peripheral regulation of mucus membranes of the nasal mucosa, gastrointestinal tract and lungs.[18, 27,36] Low MSH has been implicated in leaky gut, malabsorption and inflammatory bowel disease.[9] Studies aimed at producing MSH as a treatment have demonstrated its anti-inflammatory properties in conditions such as contact dermatitis, cutaneous vasculitis, asthma, inflammatory bowel disease, rheumatoid arthritis and ocular/brain inflammation[9,18] Low MSH means increased susceptibility to mold illness, ongoing fatigue, chronic pain, hormone abnormalities, mood swings, leaky gut, malabsorption, irritable bowel and prolonged illness.[27, 36,42]

MSH and MARCoNS

Multiple Resistant Coagulase Negative Staph (MARCoNS) are bacteria found deep in the nasal passages. CoNS used to be considered benign colonizers of the skin and nose. Unfortunately, with the widespread use of antibiotics, these bacteria have developed resistance to 2 or more antibiotics and have the capability to produce a polysaccharide matrix (biofilm) where they can live undetected in colonies and act like multicellular organisms. The biofilm matrix makes it incredibly difficult for susceptible antibiotics to penetrate.[27]

MSH and MARCoNS have a bidirectional relationship. Under normal conditions, MSH protects the mucus membranes in the nose from colonization. MSH deficiency eliminates the protective barrier and it has been shown that 80% of MSH-deficient patients will be the "victims of colonization", compared to controls with normal MSH being less than 1%. [37,41] Once established, MARCoNS suppress MSH in a two-fold manner. First they release hemolysins, small proteins that destroy red blood cells, so that the bacteria can use iron as a food source. Once hemolysins are released into the blood stream, a secondary cytokine

storm is initiated, further suppressing MSH. They also have the capability to release exotoxins, which split and inactivate MSH.[27]

MARCoNS is a common finding in CIRS patients who have been exposed to water-damaged building (CIRS-WDB). They are also commonly found in the nasopharynx of patients who have been treated with multiple antibiotics for prolonged periods (CIRS-PLS).[27] MARCoNS were first discovered in the 1980's after being cultured on prosthetic surfaces and in artificial heart valves. More recently, MARCoNS have been cultured in osteonecrosis of the jaw.[41] According to Dr. Shoemaker there have been little evidence of MARCoNS in children less than 18 years of age; however, with the increased use of prolonged antibiotics, it is the opinion of this author that there may be evidence that this is no longer the case.

Vasoactive Intestinal Peptide (VIP)

Reference range: 23-63 pg/mL

Vasoactive Intestinal peptide (VIP) is a neuro-regulatory hormone produced in the suprachiasmatic nucleus of the hypothalamus, gut and pancreas. Acting as a potent vasodilator, it regulates inflammation and blood flow. It also plays a major role in intracellular communication and cellular regeneration. When VIP is low, the patient may experience shortness of breath and elevated pulmonary arterial pressure during exercise. Dr. Shoemaker has also reported 98% of CIRS cases with multiple chemical sensitivity will be low in VIP.[36, 39]

VIP has been proposed as an alternative treatment for autoimmune disease, as it down regulates cytokines, inhibits the production of TGF beta 1 and modulates T regulatory cells.[12, 36] In his original protocol, Dr. Shoemaker describes VIP as the "crown therapy" for the treatment of CIRS, as it exerts a positive effect on the entire biotoxin pathway. It should not be misused.[41] Symptom improvement can be seen with the abatement of chronic fatigue, shortness of breath, cognitive impairment, neurological symptoms, depression and chronic joint pain.[12, 22] It can also correct multiple chemical sensitivity and reduce the "sicker quicker" phenomenon by down regulating MASP-2. The "sicker quicker" phenomenon is a term used to describe the amplified immune reaction and symptoms that occur in CIRS-WDB patients in response to future exposure to inflammagens, antigens and toxins even if for a brief period of time.[4] VIP will restore inflammatory regulation, restore circadian rhythms, improve exercise intolerance, normalize genomics, correct nuclear atrophy, down regulate aromatase and release endorphins.[45] The following table demonstrates the normalization of labs seen after the use of VIP:

Table 4: Normalization of Labs measured with VIP[5]

Increased with VIP	***Decreased with VIP***
VIP	Estradiol
MSH	MMP9
VEGF	C4a
Testosterone	TGF beta 1
CD4+CD25++ Treg cells	PASP during exercise
Vitamin D3	

Antidiuretic Hormone and Serum Osmolality (ADH/Osm)

Reference range: ADH: 1.0-13.3 pg/mL; Osmolality: 280- 300 mOsm/L

Antidiuretic hormone (ADH), also known as vasopressin, is a hormone released from the pituitary when osmoreceptors in the hypothalamus detect an increase in osmotic pressure in the blood. This sends a signal to the kidneys to retain water in order to reduce plasma osmolality. When osmolality is low, there is too much water in the blood and the osmoreceptors block the release of ADH, which leads to the loss of free water in the urine.

A dysregulation of ADH is seen in more than 60% of CIRS patients. [27, 36] Loss of regulation leads to excessive thirst, increased urination, and dehydration. Excess salt on the skin acts as a conduit for electricity and increases the potential for static electric shock. Low ADH and high osmolality can also cause migraine-like headaches. According to Dr. Shoemaker, it is typical to see ADH normalize with biotoxin carriage correction and eradication of MARCoNS. If it does not self-correct, DDAVP is recommended.[41]

Adrenocorticotropin Hormone (ACTH)/Cortisol

Reference Range: ACTH: 8-37 pg/mL; Cortisol: 4.3- 22.4 ug/dL

The hypothalamic-pituitary-adrenal axis is a complex system of hormonal feedback interactions that regulates many body systems in response to stress. Digestion, mood, immune function, sex hormones, and energy production are all regulated to some degree by the HPA axis. When activated, the hypothalamus releases corticotropin-releasing hormone (CRH), which signals the pituitary to cleave POMC into ACTH, MSH and beta-endorphins. ACTH acts on the adrenal glands and stimulates the release of cortisol. Cortisol re-establishes homeostasis by participating in a negative feedback loop and inhibits the release of CRH and ACTH production. When there is a continuous state of stress, this feed back balance becomes disrupted and results in failure of feedback inhibition. It is not uncommon to see a dysregulation of ACTH and cortisol in biotoxin-mediated illness (nearly 85% of cases). [4] Elevated levels of cortisol and ACTH may be seen in early stages of illness, then drop to excessively low levels as illness progresses. [5] Exogenous corticosteroids should be avoided, as they will not correct this problem. Reduction of the biotoxin load remains to be the best solution for adrenal dysregulation caused by CIRS.

Vascular Endothelial Growth Factor

Reference range: 31-86 pg/mL

Vascular endothelial growth factor (VEGF) is a cytokine that stimulates new blood vessel growth and dilation of blood vessels in response to low cellular oxygen levels.
In biotoxin-mediated illness, pro-inflammatory cytokines cause white blood cells to aggregate in capillary beds causing hypoxia. The decrease in available oxygen stimulates the release of hypoxia inducible factor (HIF) which initiates VEGF release.[30] An initial rise in VEGF is seen early in biotoxin exposure followed by a secondary decrease .[5] Once depressed, VEGF leads to cellular oxygen deprivation. This translates to mitochondrial

dysfunction and post-exertional fatigue. In low VEGF patients, the maximum amount of available oxygen to the cell (VO2 max) will be low and anaerobic threshold will be reduced. This causes glycogen stores to stores to become depleted and lean body mass is used for energy instead. [13, 25] Low VEGF has also been implicated in neurodegeneration and permeability of the blood brain barrier. [13,21,43] Elevated VEGF has been associated with cancer. [13]

Transforming Growth Factor Beta-1

Reference Range: <2380 pg/mL

Transforming Growth Factor Beta-1 (TGF beta-1) is a protein with strong immune-regulatory properties.[16] TGF beta-1 is produced by lymphocytes, macrophages and dendritic cells. It controls growth, differentiation, activation and death of immune cells.[16] It plays an important role in tissue repair and fibrosis. It has been linked to autoimmune disorders (SLE, RA, Dermatomyositis, UC), susceptibility to opportunistic infection, fibrotic complications (lung, scleroderma, vocal cord polyps) and gastrointestinal dysfunction.[16, 41] TGF beta-1 increases pulmonary systolic blood pressure and is therefore associated with pulmonary hypertension. There are additional implications that TGF beta-1 may be a factor in learning disabilities consistent with its involvement in "leaky blood brain barrier". [5, 16,41] Blood brain barrier permeability is a result of a combination of TGF beta-1, MMP9 and VEGF.[42]

TGF beta-1, Immune Dysregulation and Autoimmunity

T regulatory cells differentiate into three major types of T helper cells — Th1, Th2 and Th17 respectively. [5,46] TGF beta-1 contributes to the development, differentiation and maintenance of T regulatory cells, which are found in both the peripheral mucosa surfaces (CD4+CD25++) and thymus derived (CD4+CD25++CD127-/lo). CD4+CD25++ regulate Th1 (autoimmunity) and Th2 (allergy). In CIRS, it is typical to see low CD4+CD25++ T regulatory cells and elevated TGF beta-1. Excessive levels of TGF beta-1 will increase the production of Th-17.

T reg and Th17 cell plasticity may be the critical factor for induction of autoimmune disease. Plasticity refers to the ability of the cell to switch from one lineage to another or to a mixed phenotype. Th1 cells are considered the most stable, whereas Th17 are not. Regulation of plasticity between CD4 (+) T-cell lineages is vital for immune homeostasis and prevention of autoimmune disease. Retinoic acid signaling is essential for limiting Th1-cell conversion into Th17 effectors and to prevent pathogenic Th17 responses.[5, 16, 40, 41, 50]

Matrix Metalloproteinase-9 (MMP-9)

Reference range: 85-332 ng/mL

Matrix metalloproteinase-9 is an enzyme involved in the degradation and remodeling of tissue, specifically the structural elements of the extracellular matrix.[27, 48] In CIRS, MMP-9 causes the blood vessels to become "leaky" allowing inflammatory elements to escape out

of the blood vessel wall and into the sub-intimal space. This is its causal relationship with blood brain barrier permeability, inflammatory arthritis, cardiovascular and respiratory disease.[3, 5,27,48] MMP-9 may play a pivotal role in vascular remodeling and development of atherosclerotic lesions and may contribute to the susceptibility of coronary artery disease.[48] It has also been implicated in lung disease, specifically airway remodeling and fibrosis.[49]

Antigliadin Antibodies IgG/IgA

Reference range: < 20 units (Antibody not detected)

Hypersensitivity to gluten is one of many autoimmune responses associated with CIRS. Antigliadin antibodies (AGA) are produced in response to gliadin, a protein found in wheat. In biotoxin illness, this response is due to a dysregulation of T regulatory cells and low MSH causing "leaky gut." According to Dr. Shoemaker more than 58% of children with biotoxin illness will have elevated levels of AGA.[32] AGA is not specific for celiac disease, but if AGA is elevated, tissue transglutaminase (TTG-IGA) should be tested to evaluate for celiac sprue. If TTG-IGA is positive, referral for biopsy should be considered and the patient should abstain from gluten permanently. If TTG-IGA is negative, the patient needs to remove gluten from the diet for 3 months. Retesting for AGA should be performed 2-3 weeks after gluten has been reintroduced. If AGA remains negative, gluten can remain in the diet with careful monitoring for a recurrence of GI symptoms**.** If reintroduction causes a return in symptoms, or AGA becomes positive again, then it is recommended for the patient to stay off gluten indefinitely. [5, 41]

The Complement System

The complement system is part of the innate immune system that functions to elicit inflammatory and immune messengers, to clear microbes and damaged cells. It consists of more than 30 proteins and protein fragments produced in the liver that circulate in an inactive form in the bloodstream. Pathogen exposure causes a cascade of activation of compliment proteins.[10] The complement system has the potential to be extremely damaging and is tightly regulated, with 3 distinct pathways of activation. Over-activation of the complement system can lead to tissue damage and severe anaphylactic type reactions. C4a and C3a are complement proteins of importance in CIRS.

Complement 4a (C4a)

Reference range: <2830 ng/mL

C4a is the marker of greatest significance in CIRS-WDB patients. It is specifically activated in response to cell wall components of all pathogenic fungus. It will also elevate in the presence of dinoflagellates, cyanobacteria and persistent Lyme disease.[34, 44] Activation occurs in the classical or lectin pathway. Continual activation of mannan binding lectin associated serine protease enzymes (MASP-1 and MASP-2) leads to persistent elevation of C4a.[32, 34,36] Acute exposure to toxigenic fungi and inflammagens associated with water-damaged buildings will cause a rise in C4a within 10 minutes of exposure which makes it a great screening tool for both current exposure and re-exposure trials.[5, 44]

C4a elevation recruits other immune cells and causes histamine release, resulting in smooth muscle contraction, capillary hypoperfusion and increased vascular permeability. Cellular hypoxia and mitochondrial dysfunction are the outcome. Symptoms that follow include fatigue, respiratory concerns and cognitive decline.[14, 34]

Capillary hypoperfusion induced by C4a, can cause changes in six cognitive executive functions: memory decline, inability to concentrate, difficulty finding words, decreased assimilation of new knowledge, confusion and disorientation. These changes can be measured in the brain through magnetic resonance (MR) spectroscopy with findings of elevated lactate and a low ratio of glutamate to glutamine (G/G) in the frontal lobes and hippocampus.[33] Complement activation may also play a role in Alzheimer's dementia and may be present in beta amyloid plaques. [6] Fortunately, correction of C4a results in improvement of the six executive functions and central nervous system defects.[5, 33, 42]

Finally, C4a can interact with von Willebrand's Factor (vWF) polymerization, leading to bleeding from mucosal surfaces. If a patient reports frequent nosebleeds, easy bruising or bleeding tendencies, acquired von Willebrand disease should be investigated as the blood coagulation system is another example of an enzyme-triggered cascade.[14]

Complement 3a (C3a)

Reference range: < 940 mg/ml

C3a is an anaphylatoxin that also stimulates histamine release causing smooth muscle contraction, increased vascular permeability and capillary hypoperfusion. It differs from C4a as it can stimulate the adaptive immune system and can induce and moderate both T cell and B cell proliferation and production.[10] Many C3a receptors are found in smooth muscle, adipose tissue and endothelial cells of the lung, brain, liver and kidney. C3a elevates in response to the presence of bacterial membranes.[10]

von Willebrand's Profile

von Willebrand's disease is a common genetic clotting disorder with increased bleeding tendencies. In cases where there is a "hemostatic challenge" such as autoimmune, connective tissue and myeloproliferative disorders, an acquired von Willebrand's disease may manifest causing unexpected bleeding that will not stop.[24] Shetty et al (2011) proposed several mechanisms for acquisition which include "development of autoantibodies, selective absorption of high molecular weight von Willebrand factor (VWF) multimers, non-selective absorption of VWF, mechanical destruction of VWF under high shear stress, and increased proteolysis."[24] It is not uncommon to see a disturbance in coagulation with an increase in bleeding tendencies, specifically the nose or lung, in patients with CIRS-WDB; however the mechanism has not been clearly identified. If a patient presents with epistaxis or hemoptysis, acquired von Willebrand's disease must be considered. Serum analysis will show low levels of Factor VIII, ristocetin associated cofactor and multimers of von Willebrand's antigen.[32] Utilization of DDVAP can produce a rapid reduction in hemorrhage as it enhances polymerization of von Willebrand's

multimers and allows platelets to become stickier.[37] In most cases, the symptoms are reversible with a return to normal hemostasis.[24]

Plasminogen activator inhibitor-1 (PAI-1)

Reference range: 5-40 mg/l

Plasminogen activator inhibitor-1 (PAI-1) is a serine protease inhibitor secreted by the endothelial layer of blood vessels and adipose tissue. It inhibits fibrinolysis, increases clotting and causes increased risk for thrombosis. When MMP-9 combines with PAI-1, it can facilitate oxidized LDL to move through blood vessel walls. This brings inflammatory elements into the sub-intimal space, which can cause fibrosis of connective tissue and atherosclerosis.[5, 48]

Anticardiolipin Antibodies (IgA, IgG, IgM)

Reference range (Negative) = ACA IgA < 11 APL; ACA IgG < 14 GPL; ACA IgM < 12 MPL

- *APL = IgA phospholipid units; GPL = IgG phospholipid units; MPL = IgM phospholipid units*

Anticardiolipin antibodies are directed against the cardiolipin component of the cell membrane and can cause increased risk for blood clotting and abnormal bleeding tendencies. Anticardiolipin antibodies are found in collagen vascular diseases such as lupus and scleroderma. Complications include miscarriage, blood clots of the veins or arteries (thrombosis), low platelet counts (autoimmune thrombocytopenia), stroke, transient ischemic attacks (stroke warnings), Libman-Sacks endocarditis (formation of a clot on a specific heart valve), pulmonary emboli and pulmonary hypertension. Livedo reticularis is a common presenting sign of elevated anticardiolipin antibodies.[8]

Additional Imaging

MRI with NeuroQuant

MRI with NeuroQuant is an FDA-cleared computerized program developed by CorTechs Labs that quantifies absolute and relative volumes of 11 different brain areas and compares an individual's brain structure volume measurements to those of a healthy population. Several peer review studies have demonstrated its reliability in evaluating Alzheimer's, epilepsy, traumatic brain injury and PTSD.[2, 7,11,21] Due to the correlation of neurocognitive deficits seen in CIRS patients and the potential for blood brain barrier permeability, several studies have demonstrated brain changes associated with biotoxin exposure using NeuroQuant. [37,42]

The following neurotoxic effects can be seen when inflammatory elements pass the blood-brain barrier:

- CIRS-WDB: Microscopic interstitial edema in the forebrain parenchyma (white matter), cortical grey matter and pallidum; as well as caudate nuclear atrophy.
- CIRS-PLS: Right thalamic hypertrophy and atrophy of the putamen.

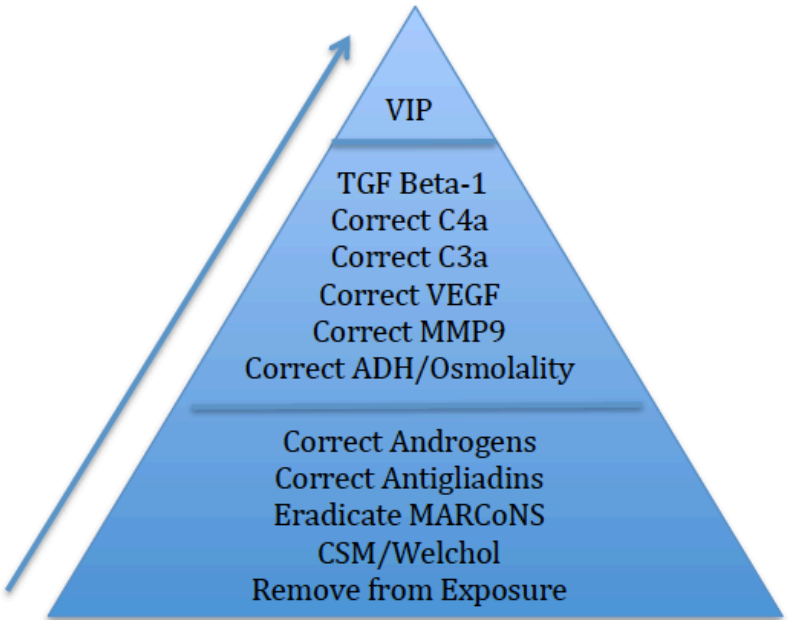
- Hypermobility: Enlargement of the cerebellum.
- PTSD: Atrophy of the thalamus, hippocampus and amygdala.
- Multinuclear Atrophy: Four or more central nuclei of the basal ganglia. This may cause a resting tremor that resembles Parkinson's disease.

These neurotoxic effects impact all areas of brain function, including executive function, problem solving and learning, mental flexibility, attention, short and long-term memory and fluency. Caudate and thalamic nuclei are implicated in motor, cognitive and sensory dysfunction.[42] Specifically, atrophy of the caudate nuclei has been a finding in obsessive-compulsive disorder and Tourette's syndrome, as inflammatory elements passing through the blood brain barrier cause cortico-striatal-thalamo-cortical circuits to become disrupted.[6, 7, 42] This could imply that inflammatory elements associated with CIRS-WDB could be a causative factor in many childhood developmental issues on the rise today.

12-Step Shoemaker Protocol

Once the case criteria have been met and labs/physical findings are consistent with CIRS, the following protocol is followed in sequential order. First and foremost, the patient must understand that these recommendations are based on peer reviewed evidence-based medicine. The steps to treatment need to be adhered to and the sequence must be followed in order. This allows for consistency and measurability.

Educating the patient about the length of treatment and the necessity of consistent follow up is essential. Follow up appointments are recommended every 4-6 weeks. Regular follow up visits are mandatory as they help to monitor symptoms and maintain treatment efficacy.



STEP ONE: *Remove from exposure*

It is necessary to identify the source of exposure. Once identified, the source must be eradicated. This could mean moving or remediation, if exposure source is due to water-damaged building (WDB). This is the most important and yet the hardest step, as it brings up many issues, including where the patient will live and the potential for changes in work and school environments. It can be very difficult to address these concerns when meeting resistance by other family members. Sometimes the patient may be in complete denial that their home, work, or school could be the cause of deleterious health effects. Then there is the reality of addressing the cost of remediation vs. the cost of replacement. It takes compassion to help patients make individualized and appropriate decisions. Dealing with personal belongings that hold sentimental and financial value and "letting go" are all things to consider. This can be overwhelming for anyone, let alone someone who is already having a difficult time functioning and thinking clearly.

In the case of water damage, the first thing that must happen is to have the home tested properly with an Environmental Relative Mold Index (ERMI) or the Health Effects Roster of Type Specific (formers) of Mycotoxins and Inflammagens (HERTSMI-2) test, which can be obtained from http://www.mycometrics.com. Both have been found to be the best predictors as to whether or not a building is safe enough to make clinical progress. Surface samples capture a history over a period of time that is a better predictor of exposure than air sampling. Air sampling only gives a current snapshot of approximately 5-10 minutes. It is helpful to go over instructions on how to collect dust samples correctly to ensure accurate results with the patient.

The following guidelines should be followed if there is mold susceptibility:

- If the ERMI score <2 and the MSH score is < 35 with a C4a < 20,000, then the patient will most likely be able to tolerate the environment. However, if the MSH score is <35 and the C4a is > 20,000, then an ERMI of <-1 is required. [41]

In comparison, the HERTSMI-2 can be obtained either individually or can be calculated from the ERMI scorecard. It looks at the five toxigenic mold species associated with indoor air including *Aspergillus penicilloides, Aspergillus versicolor, Chaetomium globosum, Stachybotyrs chartarum and Wallemia sebi.*

- A HERTSMI-2 ≤10 is considered safe for CIRS patients; however, very sensitive patients may need a score of ≤ 8 to see improvement in overall health. [41]

A quality Indoor environmental professional (IEP) could be recommended by the physician if the patient does not feel they can adequately determine if the house is contaminated or not, or if they are not clear as to whether they should remediate or move.

Some may try to suppress mold growth by treating the air with air purifiers. This will not address the adverse health effect caused by the other microbial components, such as

endotoxins, exotoxins, beta glucans, mannans and actinomycetes. Air purification such as the IQ air that removes particles as small as 0.3 microns can be used in conjunction with other measures but never as a stand-alone treatment.

If remediation is chosen, it must be performed in a cleanroom environment. All furniture needs to be removed from areas where demolition may need to be done. Negative air pressure differential containment is constructed to prevent cross-contamination into other areas of the home. All contaminated building materials must be removed under strict guidelines. All surfaces are cleaned using specific physical dust removal cleaning methods and HEPA scrubbers. All porous materials must be sealed and removed. Floor coverings and carpet must be removed in a contained environment. Dust that collects in carpet will be a reservoir for fungal fragments and bioaerosols. As soon as the carpet is lifted or disturbed, dust containing bioaerosols will become airborne again. Beta 1,3- glucans are commonly found in dust collected from floor coverings and play a role in increased risk of inflammatory reactions. Ventilation, heaters and HVAC systems are also checked and cleaned. Unplanned moisture intrusion needs to be corrected and post remediation testing must be performed.[41]

Another sensitive topic is how to handle personal belongings. Personal belongings can hold both monetary and sentimental value making it difficult to decide on what to keep and what to discard. The basic guidelines for managing this task is to determine what in the home could cause a potential for re-exposure. Items that are non-porous such as leather furniture can be wiped clean. When cleaning, bleach should never be used. Not only can bleach give off toxic fumes, the chlorine will evaporate leaving behind water that feeds mold growth. Clothing can be washed. Photographs and personal documents can be scanned into a computer then put into storage. Cloth furniture and mattresses should be discarded. These porous materials hold on to bioaerosols that are impossible to remove. Books are typically discarded. If books must be kept, a HEPA- Vacuum can be used while the book is closed to remove contaminants. Future use of books should be done near a HEPA filtration unit to prevent recontamination.[41]

The bottom line is that materials exposed to inflammagens, antigens and fungal fragments can re-contaminate a remediated home or a new home. If items are brought and not cleaned appropriately, this will cause re-exposure. Re-exposure, even if small and brief, can lead to profound worsening of symptoms that can last for many days.

STEP TWO: *Reducing Biotoxin Carriage with Cholestyramine/Welchol*

In 1997, during a *Pfiesteria* outbreak in rural Maryland, Dr. Shoemaker discovered that cholestyramine could be used to mitigate the symptoms associated the biotoxin-mediated illness.[4,25] Cholestyramine is a non-absorbable FDA-approved medication safely used to treat high cholesterol until 1973 and has been used extensively off label for the treatment of secretory diarrhea associated with *Clostridium difficile.*[5]

Cholestryamine is made of a strong non-absorbable anion exchange resin with positively charged nitrogen side groups.[4, 37] It works to reduce negatively charged anions such as bile

acids by intraluminal binding of bile salt, preventing reabsorption at the terminal ileum and excretion in the stool. It is effective at reducing biotoxin carriage because anion dominant ionophores have a similar size measuring 1.4 angstroms and a central anion dipole in aqueous solution.[37]

Ionophores are typically removed from the blood by the liver through the creation of bile acid conjugates and secreted against a gradient through the organic anion transport system (OATS). This transport system is responsible for removal and reabsorption of a variety of toxic substances including ionophores, xenobiotics and some medications. OAT transporters are found in numerous body tissues, including the brain, liver, lung, kidneys and skeletal muscle and may play an important role in the transport of biotoxins through these body systems.[5, 20]

The use of cholestyramine for the reduction of biotoxin carriage is considered an "off label" use; however, the FDA ruled in 1999 that there was no reason to expect increased risk to the health of a person in its use to treat biotoxin illness such as Ciguatera, mold, Pfiesteria, or post Lyme syndrome. It is therefore exempt from repeating FDA trials to show safety.[5, 41] Cholestyramine has relatively few side effects, but may cause gastrointestinal symptoms including nausea, GERD, belching, bloating and constipation.

In cases where CSM is not tolerated, Welchol can be used instead, but it will take longer to see labs normalize since Welchol only has 25% the binding sites as cholestyramine. Another strategy that is helpful is to use the two in tandem, with cholestyramine taken in the morning before breakfast and at bedtime and Welchol with lunch and dinner meals. [5,41] Cholestyramine can also be titrated up slowly for those who are unable to manage it at the higher doses, keeping in mind that it is not a therapeutic dose until at least reaching 3 scoops per day. Cholestyramine is prescribed through traditional pharmacies by trade name of Questran and Questran Light. Questran Light contains aspartame, which should not be used in patients with anxiety or depression. In this author's opinion, if the patient has extreme sensitivities to dyes, fillers and sucrose, it may be better to use the compounded sugar free version, or stevia blend available through compounding pharmacies.[5, 41]

Keep in mind that an "intensification reaction" similar to a Jarisch-Herxheimer reaction can occur in patients that have post Lyme syndrome. If intensification occurs, there will be a worsening of visual contrast sensitivity and elevation of MMP-9. Preloading with high-dose omega 3 fatty acids (2.4 g EPA and 1.8 g DHA) for 5-7 days and adhering to a low amylose diet can help to prevent intensification. The original protocol recommended that 45 mg of Actos be used; however, due to its black box warning of bladder cancer, it is preferred to use omega 3 fatty acids in place of Actos, and the no amylose diet.[5, 41]

Cholestyramine can inhibit the absorption of other medications and should be taken at least two hours away from Coumadin, thyroid hormones and thiazide diuretics. It can also interfere with fat-soluble vitamins A, D, E and K, so they should also be taken at least two hours away as well.[5, 41]

Cholestyramine dosing

ADULTS: > 120 lbs or > 18 yrs old

Questran: 9 grams (1 scoop) mixed in 6 oz water up to four times daily, 30 minutes before food, followed by extra 4-6 oz water

Compounded cholestyramine (CSM): 4 grams mixed with 6 oz. of water up to four times daily, 30 minutes before food and before bedtime, followed by extra 4-6 oz. water

PEDIATRICS: <120 lbs or < 18 yrs old

Questran or compounded CSM: 60-mg/kg/dose mix with 6 oz. of water, up to three times daily 30 minutes before food, followed by extra 4-6 oz. water

Welchol Dosing

ADULTS: Welchol 625mg: Take 2 tablets up to 3 times daily with meals.

PEDIATRICS: Welchol 625 mg: Take 1 tablet up to 3 times daily with meals.

Important guidelines to follow once treatment begins:

- Visual contrast sensitivity should be checked every 30 days after starting cholestyramine.
- Labs are obtained at baseline and then with each step, including TGF beta-1, MMP-9, VEGF, MSH, ADH/osm and ACTH/cortisol.
- Lipase is tested monthly once the patient has met the criteria for VIP.
- Once visual contrast has normalized and the home has been cleared as "safe," the patient can be switched to a maintenance dose of Welchol (625 mg 1 tablet twice daily).
- It can be very difficult to abstain from "mold hits." If re-exposed, patients should use Welchol or Cholestyramine for 3 days minimum to decrease biotoxin load and inflammatory elements.
- Treatment failure is usually due to new exposure, poor compliance, or failure to eradicate MARCoNS.

STEP THREE: Eradicate MARCoNS

A MARCoNS test is obtained on the first visit as part of baseline labs, using an API- STAPH nasal culture. The culture must be obtained from the nasopharynx. The sample will be taken from one side only and the swab is inserted all the way back to the nasopharynx and turned 3 times (3 seconds). Walking the patient through the procedure can reduce anxiety and ensure a successful culture. When you insert the swab at a slight angle and then straighten out the swab gently, you can easily go all the way back the full 3"- 4"necessary to reach the nasopharynx without causing too much discomfort. [41]

A positive result is when there is presence of coagulase negative staph that is resistant to 2 or more antibiotics. If positive, treatment should begin 30 days after beginning cholestyramine using BEG spray. A muco-adhesive polymer gel can be added to the BEG spray to keep it in the nasal passages longer if needed. Treatment of MARCoNS typically takes 30-50 days. Instruct the patient to complete on full bottle, wait 2 weeks and retest. If results remain positive, a discussion about loved ones and dogs as potential carriers should be addressed and another month of BEG spray is recommended. An addition of oral Rifampin 300 mg twice daily for 30 days can also be recommended. If symptoms worsen after one month, then it may be necessary to go back to step one and discuss the possibility of re-exposure; recheck VCS and MMP-9 as well. MARCoNS must be cleared to move on to the next step.

MARCoNS Treatment:

ADULTS: BEG (Bactroban (mupirocin) 0.2%, EDTA 0.1%, gentamicin 0.02%): 2 sprays in each nostril three times daily. Blow nose first, then spray.

PEDIATRICS: BEG (Bactroban (mupirocin 0.2%, EDTA 0.1%, gentamicin 0.02%): 1 spray twice-daily, alternating nares.

STEP FOUR: Correction of Antigliadin Antibodies

In patients that test positive for antigliadin antibodies (AGA) but negative to transglutaminase antibodies (TTG), a gluten free diet must be maintained for at least 3 months. After 3 months, retest antigliadin antibodies. If AGA is negative, then gluten can be introduced. Recheck for elevation of AGA after one month. If AGA is positive again, then remaining gluten free may be necessary. Regardless of test results, patients report that they feel better without gluten in the diet.

STEP FIVE: Correct low Androgen levels due to aromatase up-regulation

Abnormal androgens are caused by unregulated aromatase enzyme, which causes low levels of testosterone and high levels of estrogens. Men often present with low testosterone that is not correctable with androgen replacement. Women may present with dysmenorrhea, menorrhagia, clotting, ovarian cysts, chronic vulvar pain and interstitial cystitis.

Treatment can include one of the following options, while monitoring DHEA, testosterone and estradiol levels during the course of treatment. Treatment should not involve testosterone replacement, as this can further suppress natural production of testosterone.

- DHEA 25 mg: three times daily for 30 days
- HCG injections or sublingual drops: 125 mg per week for 5 weeks
- VIP nasal spray: Four sprays intranasally, alternating nostrils, up to four times per day for one month.
  VIP will stabilize aromatase, thereby rebalancing androgen production

STEP SIX: Correct Antidiuretic hormone and osmolality imbalance

ADULT: DDAVP 0.2 mg tab every other night for 5 doses, or DDVAP nasal spray with one spray intranasally twice daily for 5 days.

- The day after the last dose, check electrolytes, ADH and osmolality.
- If both are normal but symptoms of polyuria, polydipsia, orthostatic hypotension, recurrent headaches and increased static shock are still present, increase DDVAP to 0.2 mg every day for another 10 days, with close monitoring of weight gain and edema.
- After 10 days measure electrolytes, ADH and osmolality again.

PEDIATRICS: 1 spray of DDAVP spray based on child's weight and age

This step may also help to reduce MMP9 and correct acquired von Willebrand's Syndrome.

STEP SEVEN: Correction of elevated matrix metalloproteinase 9 (MMP-9)

As stated previously, correction of elevated MMP-9 is achieved using high-dose omega 3 essential fatty acids at **2.4 g EPA and 1.8 g DHA** total daily dose along with a **low amylose diet**. This is often implemented 5-7 days prior to beginning cholestyramine and has provided a solution to minimize "intensification" reactions. In cases where high-dose fish oils and the low amylose diet are not effective at lowering MMP-9, **Actos 45 mg** once daily for 30 days can be used. Actos up-regulates PPAR-gamma production by increasing the sensitivity of the body to insulin, and reduces MMP-9. As stated previously, Actos is not used as often in current treatment protocols, due to its black box warning for bladder cancer, so it should be used with caution. Kidney function should be monitored as well as symptoms of hypoglycemia.

Note: *Actos is contraindicated in patients where leptin < 7 or in children younger than 18 years.*

STEP EIGHT: CORRECTION OF LOW VEGF

As outlined in treatment step number seven, low VEGF is corrected with a low amylose diet and high-dose omega 3 essential fatty acids and activation of PPAR-gamma.

Graded exercises can also be used to correct low VEGF, low V02 max, capillary hypoperfusion, and post-exertional malaise.

GRADED EXERCISES
Measure anaerobic threshold with cardiopulmonary stress test (measuring VO2 max) before starting graded exercises.

1. Start with <u>CARDIO EXERCISES:</u> bike or walk
   - 5 min/day → working up to 15 min/day.

2. Next add FLOOR EXERCISES: abdominal (sit ups/crunches) and leg lifts
   - 5 min/day → working up to 15 min/day
3. Then add FREE WEIGHTS: upper extremities (biceps, triceps), shoulders and chest (pectoral fly)
   - 5 min/day → work up to 15 min/day
4. After 1 month, go back to step 1 and INCREASE INTENSITY following the same sequence

STEP NINE: Correction of C3a

Start with **150 mg CoQ10** daily for 10 days, to minimize the potential for side effects, then begin 80 mg statin daily until reduction of C3a is achieved. This competes with other medications/supplements that are processed through CYP450, so watch for interactions.

STEP TEN: Correction of C4a

Treatment: VIP 50 mcg/ml: 1 spray QID

Original Protocol: Low-dose Procrit (erythropoietin) SQ 8,000 Units Monday and Thursday for 5 doses, along with baby aspirin.

- PROCRIT WAS NEVER RECOMMENDED FOR USE IN CHILDREN.
- Caution should be used, as Procrit has a black box warning of increased risk of blood clotting.
- If Procrit is used, an informed consent must be obtained for "off-label" use and only should be used after assessment for the 6 executive functions has been performed, followed by MR spectroscopy. A low glutamate/glutamine ratio meets eligibility for Procrit in ADULTS ONLY.
- Due to the black box warning of thrombosis, D-dimer needs to be checked as well as hemoglobin and blood pressure.
- At the end of treatment, MR spectroscopy should show normal glutamate/ glutamine, normal lactate and improvement of all 6 executive functions.

STEP ELEVEN: Correction of TGF-B1

ADULTS: Cozaar (Losartan): 25 mg twice daily for 30 days
PEDIATRICS: Cozaar (Losartan): 0.6-0.7 mg/kg/day divided twice daily

In patients with low blood pressure or those who cannot tolerate Losartan, use 4 sprays daily of VIP 50 mcg/dose intranasal spray.

STEP TWELVE: VIP

VIP is the final step in the 12-step protocol and is considered the "crown" treatment and should not be misused. Often patients will have normalized by the time they have gotten to this step.

There are four required criteria to begin VIP:

- No exposure as demonstrated by an ERMI of <2 or HERTSMI-2 <10
- Negative API-STAPH nasal swab for MARCoNS
- Normalized VCS test
- Normal Lipase

It is also recommended to order a stress echocardiogram prior to beginning VIP, to evaluate pulmonary artery systolic pressure (tricuspid regurgitation). Normally pulmonary artery systolic pressure should not rise more than 8mm Hg during exercise. Elevated PASP, which is often seen in CIRS, can be a source of palpitations and of dyspnea that does not respond to beta 2 antagonists.[41]

Treatment:

ADULTS: VIP 50 mcg/ml: 1 spray alternating nostrils four times daily x 30 days

PEDIATRICS: No defined VIP dosing has been established as most children resolve before reaching this step.

The first dose should be administered in the office to monitor blood pressure, abdominal pain and rash. This also allows for TGF beta-1 to be redrawn within 15 minutes after the first dose. A rapid rise in TGF beta-1 is an indication that the patient is still in exposure.

- Fasting lipase is measured prior to beginning VIP and is measured every 30 days along with C4a, TGF beta-1 and any other abnormal labs.
- Anytime during treatment if patient experiences NEW ONSET ABDOMINAL PAIN, RASH, OR CHANGE IN BLOOD PRESSURE, OR AN ELEVATION IN LIPASE, VIP SHOULD BE DISCONTINUED.
- If TGF beta-1 is not improving or symptoms are not improving, check for re-exposure.
- Can titrate up on VIP dose if no improvement every 30 days
- Once TGF beta-1 is stable, lipase is normal and symptoms are improving then continue VIP for 30 more days, then start to taper down to twice daily dosing for 30 days, following by daily dosing for 30 days then discontinue.

End of treatment and follow-up care

A follow up should be performed 6 months post VIP to ensure the patient is stable and there has been no return of symptoms or abnormal labs.

Unfortunately, staying safe can become a lifelong ordeal for mold sensitive patients which requires ways to determine whether a building is “safe” or not for a patient. It may not always be possible to run a HERTSMI-2; therefore a re-exposure trial can be used instead.

Re- exposure instructions (Sequential Activation of Innate Immune Elements-SAIIE)

1. Get patient back to control lab values by treating with cholestyramine or Welchol.
2. Stop all biotoxin medications and have patient stay in safe environment.
3. After 3 days, measure VCS and labs (C4a, leptin, MMP-9, TGF beta-1, VEGF, Factor VIII).
3. Patient continues to stay off all medication.
4. Re-expose patient to the building in question for 8 hours.
5. Retest labs the morning after.
6. Patient is exposed again for another 8 hours after labs are drawn.
7. Retest labs the morning after.
8. Have patient stay in the building for another 8 hours after labs are drawn.
9. Retest for the third time the morning after.
10. After the last blood test, restart cholestyramine if the patient became ill.[5]

Proteogenomics: Welcome to 21st Century Medicine!

Proteogenomics is a newly emerging field that involves examining RNA sequencing as a method for dissecting complex medical illness. It was once thought that DNA was the blueprint that living organisms must follow to remain functional and that the purpose of RNA was to help carry out these guidelines. However, ongoing research is now proposing that RNA may also play a regulatory role in DNA transcription as well. RNA also may regulate two layers that Dr. Shoemaker calls "regulation of regulation of gene activation." This new data provides insight into the role of ribosomal and mitochondrial transcriptional response.[22]

Both ribosomes and mitochondria have critical roles in immune response, as one involves protein production and the other energy production. Both are needed for an immune cell to proliferate and differentiate in response to an infectious threat.[22]

Testing RNA is very different than DNA testing such as 23andMe and methylation profiles, as it measures "dynamic gene changes." SNP testing can only measure stationary genetic structures and "susceptibility" to certain traits but cannot answer the question of whether these genes are active. DNA mutations cannot define the cause of biotoxin illness nor offer treatment solutions to biotoxin illness.

It has been well documented that microbes associated with WDB can secrete a wide variety of toxins and chemical compounds; one such toxin is the "ribotoxin." Ribotoxins cleave 28S ribosomal RNA of the sarcin-ricin loop in the ribosome and the 16S ribosomal RNA in the mitochondrion, leading to dysregulation of both ribosomes and mitochondria, which causes an up-regulation of gene expression.[22]

Dr. Shoemaker and James Ryan. PhD, (2016) published a recent study examining RNA sequencing in patients with CIRS treated with VIP. They discovered that VIP caused a down-regulation of gene expression with a concomitant resolution of symptoms.[22]

RNA sequencing is providing a new pathway for the evaluation, diagnosis and treatment outcomes in biotoxin-mediated illness. This new genomics kit is available from http://www.survivingmold.com or http://www.progenedx.com.

Appendix 1: Acceptable Interventions for Children

With the rise of Autistic Spectrum Disorder (ASD), PANS/PANDAS, ADD/ADHD and increased treatment for pediatric Lyme disease, assessment for CIRS may be necessary in many of these young patients. Exposure to water-damaged buildings is as detrimental for our youth and not as clearly recognized or considered. Dr. Shoemaker and Margaret Maizel (2009) examined the IACFS Pediatric case definition for Chronic Fatigue Syndrome and emphasized how exposure to water-damaged buildings was not addressed in these cases. It was recommended that exposure to the interior of a water-damaged building be included in the case criteria, along with laboratory testing and assessment of CIRS in all pediatric CFS patients. Listed below is a summary of things to consider in the diagnosis and treatment for patients age 18 and younger.

Pediatric patients may not be able to report symptoms in the same manner as adults. When evaluating young children, the symptoms reported may only manifest as 1-2 concerns, the most common being chronic headache, recurring abdominal pains and chronic fatigue. Sleep disturbance, widespread pain, neurocognitive issues and immune manifestations may also be reported.

When considering lab testing, the case definition excludes pituitary hormone abnormalities. Autoimmune parameters have greater diagnostic significance, including antigliadin antibodies and anticardiolipin antibodies. The most important labs in assessment of children should include HLA DR, MSH, TGF beta-1, C4a, acquired von Willebrand's profile, antigliadin antibodies and anticardiolipin antibodies. MMP-9 should be collected and used more for assessment of treatment rather than as a diagnostic tool.

Children under the age of 9 years old may not be able to perform VCS testing accurately, as maturation of neurological function may not be fully developed.

The main objective in pediatric cases is to remove them from exposure, normalize high TGF beta-1 and bring up low CD4+CD25.

In cases where intervention is needed, the following treatments are acceptable:

1. CSM/Welchol for biotoxin carriage
2. BEG spray for MARCoNS based on a positive API-STAPH nasal culture
3. DDAVP to correct ADH/osmolality issues and acquired von Willebrand's Syndrome
4. High dose Omega-3 fatty acids (2.4 g of EPA and 1.8 g DHA) for elevated MMP-9 and low VEGF
5. Gluten-free diet
6. Pediatric dosing of Cozaar for high TGF beta-1 in children older than 6 years of age

Medications that should not be used in children include: Procrit, oral steroids and Trental

Appendix 2: No Amylose Diet

Objectives:

1. *To avoid foods that contain amylose and glucose, which cause a rapid rise in blood sugar.*
2. *To lower elevate MMP9 levels.*

Directions: Follow the "00-2-3 rule." Zero amylose, zero sugar, 2 servings of at least 6-8 ounces of protein each day and 3 servings of above ground vegetables and fruit every day.[26]

Forbidden Foods

- Roots and tubers including white and sweet potatoes, beets, peanuts, carrots and other vegetables that grow underground, with the exception of onions and garlic.
- Bananas
- Wheat and wheat based products- bread, pasta, cakes, cookies
- Rice
- Oats
- Barley
- Rye
- Foods with added sugar, sucrose, corn syrup, or maltodextrin

Allowed Foods

- Anything not on the list of forbidden foods
- Corn
- Onions
- Garlic
- All vegetables that grow above the ground, including lettuce, tomatoes, beans of all types, peas, cucumbers and celery
- All fruits except bananas
- Meat, fish and poultry
- Condiments (avoid low-fat varieties as they usually contain added corn syrup).
- Spices
- Eggs
- Dairy (avoid sugar-laden products)
- Nuts
- Sunflower, pumpkin and squash seeds

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