Diagnostic Process for Chronic Inflammatory Response Syndrome (CIRS): A Consensus Statement

Report of the Consensus Committee of Surviving Mold

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Abstract:

Clinical management of patients with a complex, multisystem, multi-symptom illness identified as a chronic inflammatory response syndrome (CIRS) has expanded. Often associated with illness due to exposure to low molecular weight biotoxins and inflammagens found (i) inside water-damaged buildings (WDB); (ii) following exposure to blooms of cyanobacteria; (iii) following consumption of ciguatoxic fish; and (iv) following confirmed acute Lyme disease, persistent despite reasonable use of antibiotics, CIRS is increasingly recognized. A need for a formal case definition and case management protocol has arisen. Patients with CIRS will have abnormalities in innate responses, reduced levels of regulatory neuropeptides MSH and VIP, elevated inflammatory markers of C4a, MMP9 and TGF beta-1. Systemic illness, based on abnormal gene activation and suppression, as shown by RNA Seq and transcriptomics, requires a multi-factorial, rigorous diagnostic assessment to assist in both differential diagnosis and monitoring response to therapy. A consensus statement is herein provided to assist practitioners in case identification and management.

Key words: CIRS, water-damaged buildings, transcriptomics, cyanobacteria, Post Lyme syndrome ciguatera, visual contrast sensitivity

Introduction :

As the third decade of CIRS-medicine begins, the key elements of CIRS are reviewed in a consensus statement to enhance accuracy and reliability of the diagnostic process. This consensus statement has been approved by (i) the diagnostic module consensus statement committee of Surviving Mold and (ii) approved by the 65 members of the Surviving Mold list serve.

Section 1. What is CIRS?

Chronic inflammatory response syndromes (CIRS) are multisystem, multi-symptom illnesses acquired following exposure to environmentally produced biotoxins (1, 2, 3, 4, 5, 6, 7, 8, 9).

CIRS has gone through an evolution of names over the years. Initially, in the 1990's, CIRS was called a neurotoxin-mediated illness (10, 11). As more information was added, the term was changed to chronic biotoxin associated illness (CBAI). The third change to CIRS occurred following development of a commercial assay for transforming growth factor beta-1 (TGF-beta-1) in 2008, shortly before the development of a commercial assay for acquired T regulatory cells in 2009. CIRS was confirmed to involve many arms of the immune response systems acting simultaneously and in combination.

CIRS itself is modeled after an acute systemic inflammatory response syndrome (SIRS), an acronym typically used to describe an acute inflammatory illness, most commonly sepsis. In patients with sepsis there is simultaneous activation of Th1, Th2 and Th17 immunity; coagulation factors; and complement in response to an overwhelming stimulus of infection and endotoxin present in the blood stream. In this regard, the illness becomes the host response as eloquently described by Thomas (12).

Survivors of sepsis have been well studied; they have a heightened level of innate immune activation post-sepsis compared to before. Survivors have a significant increase in interleukin 10 and go on to suffer a greater incidence of chronic fatiguing illnesses (13, 14).

In 2008, followed by a publication in 2010 (19), members of the "mold" medical community began using a jargon term, CIRS-WDB to describe illness seen with the same activation of Th1, Th2, Th17, coagulation, complement activation and more. If the illness came from water-damaged buildings, it was called CIRS-WDB. If symptoms came from Post Lyme Syndrome, it was called CIRS-PLS. If symptoms came from ciguatera it was called CIRS-ciguatera. Theoretically, one could call the syndrome most anything; possibly the syndrome would be codified by a regulatory agency, much as the CDC changed the name, "Pfiesteria health illness syndrome (affecting) humans," PHISH, for acute and chronic illness caused by exposure to blooms of toxigenic, fish-killing dinoflagellates, including Pfiesteria, to "Possible estuarine-associated syndrome" (PEAS, 11).

Section 2. Case definition of CIRS

In 2008, the US General Accountability Office (GAO) published an overview of publications from US agencies working on the problem of damp indoor buildings (20). 54 studies were noted, showing no coordination of efforts across agency lines. But for the first time, a Federal case definition for what has become CIRS-WDB was proposed.

1. There must be the potential for exposure to a damp indoor space.

2. There must be a multisystem, multi-symptom illness present with symptoms similar to those seen in peer-reviewed publications.
3. There must be laboratory testing results similar to those seen in peer-reviewed, published studies.
4. There must be documentation of response to therapy

This definition still is used even though there has been an explosion of publications in the CIRS community noting various objective parameters in wide clinical use now (1).

Symptoms are noted to be essentially identical in multiple sources of environmental exposures resulting in CIRS (see Table 1). Of interest is the appearance of clusters of symptoms among the 37 symptoms recorded in CIRS (Table 2). Presence of eight or more of the 13 clusters is virtually diagnostic of an unspecified type of CIRS (US Patent 9,770,170 B2; issued 9/26/2017).

Section 3. Before the first office visit

Seeing a health care provider as a new patient brings a series of expectations of standard medical practice process. Forms will be filled out, insurance data recorded, HIPAA information processed; office policies, billing information sheets and maybe even an overview of the philosophy of the practice will be reviewed. Organ system-related specialists (heart, lung, GI, neurologist and more) will focus on their areas of expertise; maybe the appointment with a primary care provider might center on treating the patient as a whole person, not a heart, or a lung, or a colon in isolation.

CIRS providers are going to approach first office visits differently. Based on the experience of thousands of initial office visits with CIRS providers it is likely that patients will have failed to find improvement from least 10 physicians before, at least one of whom was a psychiatrist. None made any delineation of the abnormalities in physiology, but instead each has often made a diagnosis unsupported by objective biomarkers.

Review of past medical records remains the most important initial duty of the CIRS provider to the patient. The patient, in turn, must show due diligence to provide the provider with every relevant document. When the work involved in providing a thorough copy of records is too onerous, a reasonable suggestion is to postpone the initial visit. Regardless of motivation to get better, many CIRS patients do not have reliable recall of an exposure time-line, symptom time-line and medication time-line (including supplements and all anti-fungals, even topicals). Laboratory testing often involves “cash-only labs” which may be not rigorous. Frequently, the basis for clinical decision making is inadequately supported. Assumptions and frank guesses are rife: these have no role in CIRS case management.

Patients often will insist that their symptom-recall and self-based diagnosis is inherently reliable. (As an example, how do CIRS health care providers analyze chief complaints like, “I get a spasm in my kidneys when my brain inflammation acts up.”) Symptoms of CIRS vary from day to day and are subject to unnoticed environmental exposures. Unfortunately, given the 90% + incidence of cognitive impairment in CIRS, symptoms lasting more than a few months are usually lost in time lines as the illness continues.

An additional problem for the CIRS provider is the demand for a “hurry-up” appointment. There can be no hurry-up when records must be reviewed carefully!

Give the CIRS doctor time to read every page of the medical record. Organize the record, get rid of duplicates, make it chronological but do not leave anything out. Organized summaries make some sense but not at the risk of editing by the patient. In the example of 5000 pages of records usually there will be one or two pages that have the answers persistently ill patients have sought.

One might have become frustrated with impersonal, community-based providers, so-called mainstream physicians, who guessed at what was wrong, but really did not know, so some patients might have "gone alternative." Maybe a CIRS met some caring doctors outside the mainstream, but maybe others met predators who sold snake oil in modern trappings. Once again, guesses, assumptions and lack of support from peer-reviewed literature will dominate the medical record.

The first step in diagnosis of CIRS is to know what has been said about the patient. Ignore the snide innuendo of, "It's all in your head." A NeuroQuant® will show what is wrong cognitively. Ignore the fibromyalgia diagnosis and the gabapentin prescription. The lab results tracking CIRS and transcriptomic findings are the light in the darkness. Once one sees objective, proven, reliable scientific findings that lead to a proven treatment protocol, a CIRS provider will usually show the patient something long-lost: Hope.

Hope is based on data. Hope is based on published work, validated by CIRS physicians over the past 22 years. The Hope in CIRS is supported by favorable Daubert and Frye decisions in courts across the US.

Patients will likely be asked to stop unneeded medications immediately. Throw the amitriptyline bottles into the "worthless psych nostrum" drawer. Eliminate the unneeded bags of supplements and antibiotics possibly sold to patients by those who prescribed them. Patients will be admonished to stop nasal, oral and intravenous anti-fungals, as they are creating resistant mutant MARCoNS that are already threatening desperately ill infectious disease patients with additional antibiotic resistances.

Outside learning will be included; one will probably be instructed to read several hundred pages of documents found (free) on www.survivingmold.com, understanding that few will remember everything. Most CIRS patients have difficulty assimilating new knowledge, but possibly loved ones will read and learn more as well. In time, patients must become an expert in the jargon and pathophysiology of CIRS. Why? Simple: the illness is real. CIRS protocols work to correct the physiology and the underlying transcriptomic abnormalities.

Section 4. Evolution of the paradigm of CIRS

Patients may also be asked to review the history of CIRS. Knowing the past is an ally for expansion of future understanding. The history of the case definition of CIRS is marked by a series of "spike events." A spike occurs when there is a publication or a breakthrough that adds exponentially to prior thought. Initially, CIRS was felt to be simply an inflammatory response to exposure to the environmentally produced neurotoxins. Dinoflagellate illnesses, such as Pfiesteria and ciguatera were the afflictions most relied upon to develop the case definition in the late 1990's. Later additional findings, first from exposure to cyanobacteria (1997) and then to the interior environment of water damaged buildings (WDB, 1998), showed that an uncanny similarity of symptoms was shared by each of these diverse sources of inflammatory illness.

Because the initial sources of illness in some instances could not be identified reliably, the case definition in 2003 simply included: i) the potential for exposure; ii) presence of a multi-system, multi-symptom illness and; iii) absence of confounding exposures or diagnosis.

When objective lab studies could be added, first including visual contrast sensitivity (VCS), HLA DR by PCR and melanocyte stimulating hormone (MSH), a commonality of inflammatory abnormalities together with similarity of symptom groups within subsets of this neurotoxin mediated illness was recognized.

A treatment protocol employed cholestyramine (CSM) after removal from exposure (or treatment of underlying infectious disease) together with clearance of multiply antibiotic resistant coagulase negative staph (MARCoNS), resident in deep nasopharyngeal space.

In 1998, Lyme researchers Donta and Cartwright patented the identification of a biotoxin made by Borrelia burgdorferi (Publication number CA2365424 A1), the causative agent in Lyme disease. Since CSM worked well to treat other biotoxins, its use in Lyme patients followed. The unexpected and precipitous untoward reactions of patients with Post Lyme Syndrome to CSM caused a stunning rethinking of concepts of biotoxin illnesses. The observed group were people with confirmed Lyme disease as shown either by (i) a physician witnessed erythema migrans rash in association with a recent tick bite or (ii) clear evidence of a significant antibody response as shown by Western Blot testing done in reliable laboratories. These patients, when given cholestyramine after a reasonable course of antibiotics, did not improve as dinoflagellate, cyanobacteria and WDB patients did. They got worse.

This never-before-seen adverse reaction, labeled "intensification," was quickly shown to be due to a massive pro-inflammatory cytokine response as manifested by significant elevation of matrix metalloproteinase-9 (MMP-9). This syndrome was recognized initially as showing a fall in VCS scores beginning in row E, followed by a fall in row D. This was not a "Herxheimer" reaction; it had nothing to do with antibiotics. It simply was a pro-inflammatory cytokine event.

While Post Lyme Syndrome patients presented treatment difficulties, these challenges were the source of new therapies for pro-inflammatory cytokine responses added to the simple cholestyramine and MARCoNS protocol.

As the intensification reaction became amenable to diagnosis, treatment and prevention, there were a series of patients who improved beyond what we had seen while others still were ill.

In rapid fire order in the early 2000's physicians were able to see the (i) low levels of vascular endothelial growth factor (VEGF) and (ii) elevated levels of split products of complement activation, especially C4a, which would become additional targets for sequential treatment.

Melanocyte stimulating hormone (MSH) was the first regulatory neuropeptide to be identified as deficient in the early CIRS cases. Because MSH exerts a regulatory role on production of other hormones, especially including gonadotropins, it was not a surprise to find that androgens together with estrogens were affected by MSH deficiency. To this day, physicians are attempting to "balance" androgens and estrogens; this approach remains surprising in that a proximal step, that being correction of MSH deficiency, is mandatory in correcting androgens and estrogens.

Researchers also saw that MSH interacted with antidiuretic hormone (ADH) in the hypothalamus to regulate other hormone activities in addition to salt and water

balance. MSH controls tight junctions in the gut, a fact often overlooked by those who diagnose "leaky gut."

The discovery that vasoactive intestinal polypeptide (VIP), another regulatory neuropeptide, was deficient in > 90% of patients with the syndrome set off another avenue of inquiry in the physiology of the illness. Providers now know that measurements of VIP receptors 1 and 2 are also important to fully understand VIP's efficacy. Transcriptomics shows us how VIP receptors are intimately tied to the activities of a family of nuclear transcription factors, Ikaros.

As objective lab parameters began to be associated loosely with some specific grouping of symptoms, it was the sequential treatment method that enabled providers to approach symptom reduction in a disciplined manner. By correcting one objective parameter at a time, patients could identify symptoms that were improved, implying that there were objective parameters correlating with specific lab abnormalities.

Symptoms reported by patients, by definition, are subjective. However, when symptoms are collected as part of a medical history, the subjective aspect is now funneled through the trained mind of an experienced health care provider to yield an objective picture of symptoms. Symptom recording has shown a lack of reliability when performed by the patients using a check list or (even less reliable) using their memory alone.

Symptoms, however, remain vitally important in understanding the multi-system, multi-symptom elements that are crucial to the differential diagnostic process of this illness. Even though symptoms are subjective, accurate collection of a medical history remains the single most important aspect of the differential diagnostic process of any illness.

As the number of CIRS patients seen and treated has expanded; and the number of physicians and healthcare providers who are competent to treat this complicated illness has grown, there was little variation in symptoms recorded from patients living around the globe. There were also specific and reproducible elements in lab abnormalities that suggested that each of these illnesses must have an underlying basis reflecting differential gene activation or suppression.

The concept of the "final common pathway," began to emerge following the 2010 Physician Consensus Statement (15) in which this inflammatory illness finally was named chronic inflammatory response syndrome (CIRS). In other words, a CIRS caused by chronic exposure to WDB, was nearly indistinguishable from a CIRS caused by Post Lyme Syndrome, ciguatera, Pfiesteria or cyanobacteria. The initial inciting triggers might differ in each case, but the end clinical presentation was similar. Since providers could not rely on VCS deficits, lab abnormalities or symptoms alone to adequately distinguish one illness from another, all physicians had to support a diagnosis was potential for exposure. Even this reliable method of history taking became confounded when say, someone who had a dinoflagellate illness then moved into a moldy apartment creating two sources of biotoxin exposure. Even worse, some individuals would be residing next to fresh water areas, lakes or ponds for example, in which there would be cyanobacteria blooms in addition to moldy buildings and sick fish.

Clearly, physicians needed a diagnostic method that would (i) specify individual illness and provide the (ii) basis for monitoring results of treatment.

With papers published in 2015, 2016, 2017 and 2018, the importance of transcriptomics in the diagnostic process of CIRS is manifest.

As transcriptomics is added to CIRS case management, based on case-controlled studies and based on prospective intervention trials, there is a molecular basis for the final common pathway suggested by commonality of symptoms in sources of CIRS. While there is an old expression that "All roads lead to a final common path that leads to Rome," what providers are seeing in the approach to CIRS is more like that of a spider web. The greatest movement of the web is in the very center, but the tethers could attach variously to corners around a window or to an object that was stable providing a mechanism for the spider web to survive wind and rain. Whatever came to the center of the web could come from any seemingly unrelated tether.

Genomics can be regarded as the center core of the spider web. Here is the source of all the objective laboratory abnormalities; here is the source of all the confusing complexities of symptoms and their groupings called clusters. Here is the basis for CIRS illness.

To quote a current Primer in Transcriptomics (16):

"Transcriptomics has now crossed from research to application. It not only serves as a diagnostic aid but it also provides precision in monitoring the complexities found in many immune-based illnesses, such as chronic inflammatory responses acquired following environmental exposures. We see transcriptomics as an ideal mechanism to fine tune therapies aimed at correction of inflammatory abnormalities.

"The initial finding by Ryan, et al (5) showed that intranasal use of VIP in CIRS patients, part way through their treatment protocols, modulated expression of both nuclear encoded mitochondrial genes and ribosomal genes. We later confirmed that patients naïve to treatment had profound suppression of these same genes. These genes usually recover with the first ten steps of the Shoemaker Protocol; they often become higher than normal controls. A tantalizing possibility for the overshoot lies with genes important in glycolysis (breakdown of glucose into two 3-carbon fragments). One of these genes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), also functions to regulate inflammatory gene suppression after initial activation in the presence of interferon gamma.

"What we think is happening is an attack on the basic cellular metabolic elements. It is well documented that microbial toxins (i) attack protein production at the level of the ribosome and (ii) energy production in the mitochondria. Patients with CIRS will not recover unless exposure to these microbes and their cellular toxins ceases. Further, it is well documented that in the face of infectious diseases, especially from viruses, that cells become "hypometabolic" to prevent viral "takeover" of cellular function.

"This idea is relevant for the abnormal immune responses we see in CIRS. Hypometabolic immune cells activate primordial mechanisms: innate immunity, complement, defensins. These protective inflammatory elements do not require participation of cells. When cells begin to recover from the illness-induced hypometabolic state, the protective mechanisms of innate immunity begin to wane. Cells then upregulate protein production, energy production and begin to use sugar properly. Call it a "synchronized reboot," of cellular systems. Re-exposure simply means a recall of innate immunity and all its protective mechanisms.

"The sequence of hypometabolism and recovery are shown by changes in transcriptomics. Certainly, the CIRS protocol could prime ribosomal and

mitochondrial gene expression. Then VIP could upregulate GAPDH gene expression. Could increased pyruvate from glycolysis fire up the mitochondrial furnace for energy production and then protein expression?" Or will it stay suppressed as to not increase lactate production?"

With a genomics basis established for CIRS, physicians began to see application of the principles of CIRS to other illnesses that involve inflammation but did not have all the elements of CIRS. For example, in a small number of patients with impending heart transplant for dilated cardiomyopathy, gene expression of adrenoceptors and "contractility receptors" found in peripheral blood cells respond to CIRS protocols. If it is confirmed that cardiac myocytes also improve like white blood cells do, perhaps providers will see dramatic changes in quality of life if further myocardial injury from genomic abnormalities can be prevented.

Atherosclerosis is increasingly shown to be an inflammatory illness. Oxidized LDL is a potent nuclear transcription factor. Insulin and insulin resistance are the inflammatory bases of diabetes and obesity. Alzheimer's of particular types, specifically Type III, is shown to be an inflammatory illness. Inflammation is now recognized as the underlying mechanism, not just a source of the symptoms and not just the cause of lab abnormalities. For further information, see the Transcriptomic Primer (16).

As the next decade of CIRS approaches, the expansion of application of the principles of CIRS to other illnesses, possibly including pediatric acute-onset neuropsychiatric syndrome (PANS), is becoming clear. An important field is the TH17/Treg imbalance. T-regulatory cell abnormalities also are related to abnormalities of genomics and abnormalities in some of the elements of CIRS but not all.

CIRS is now one end of the spectrum of inflammation, with each new element as it is being described contributing to understanding of each other element previously described. By adding one inflammatory feature of an illness on top of another, much as blocks laid on a foundation wall, we begin to recognize that our attempts to define diseases by organ system will fail. Physicians recognize, however that infectious diseases often set off systemic inflammation that will persist beyond clearance of infectious agent. In others, not so much. For the illnesses that do effect changes in genomics, physicians and patients alike are in the middle of a revolution of understanding and a revolution in treatment.

Section 5. Specific symptoms

From a list of 37 different symptoms, there are eight main categories (general, musculoskeletal, eye, respiratory, gastrointestinal, cognitive, hypothalamic and neurologic) formed in an effort to classify symptoms. Cluster analysis provides a mechanism to take subjective symptoms, pass them through a skilled medical history and then convert them to objective elements using abstruse statistical methods, including cluster analysis. Clusters can take the seemingly endless roster of symptoms to make their use amenable to a scoring system.

Table 1 - Percent incidence of symptoms in various CIRS conditions

Symptoms	Controls	Cyano	WDB-1	WDB-2	WDB-3	PEAS	Ciguatera	Lyme
N=	239	10	156	288	21	42	100	352
Fatigue	6	100	89	83	100	70	91	94
Weak	<5	80	75	70	84	-	83	89
Ache	8	90	77	68	95	43	77	81
Cramp	<5	80	66	56	63	14	68	77
Unusual Pains	<5	50	62	51	42	-	82	86
Ice Pick Pain	<5	40	49	41	-	-	45	82
Headache	9	90	78	66	84	73	78	88
Light Sensitivity	<5	90	71	66	89	68	67	85
Red Eyes	<5	50	52	48	63	68	48	61
Blurred Vision	<5	40	61	56	63	-	53	66
Tearing	<5	30	41	48	63	-	28	55
SOB	11	60	78	63	74	57	63	77
Cough	7	50	72	53	53	43	62	71
Sinus Congestion	8	60	79	65	74	41	70	68
Abdominal Pain	<5	60	61	39	37	41	79	42
Diarrhea	<5	50	48	39	21	57	72	51
Joint Pain	11	70	75	53	84	-	62	88
Morning Stiffness	6	70	72	44	-	-	59	80
Memory Impairment	<5	80	83	66	68	84	81	80
Difficulty Concentrating	<5	70	81	62	53	35	83	82
Confusion	<5	40	75	57	26	24	66	72
Decreased Word Finding	<5	80	81	66	11	-	80	84
Decreased Assimilation	<5	80	72	65	37	-	78	88
Disorientation	<5	30	51	40	11	-	28	33

Mood Swings	<5	20	69	65	-	-	42	65
Appetite Swings	<5	50	58	58	-	-	61	77
Sweats (Night)	<5	50	61	54	-	-	42	68
Difficulty Reg. Body Temp	<5	50	63	60	-	-	67	72
Excessive Thirst	<5	60	69	54	-	-	59	71
Increased Urinary Frequency	<5	60	66	58	-	-	66	75
Increased Susceptibility to Static Shocks	<5	40	41	44	-	-	38	32
Numbness	<5	40	48	44	37	-	74	66
Tingling	<5	40	61	51	47	-	78	71
Vertigo	<5	40	39	48	42	16	29	37
Metallic Taste	<5	40	45	36	47	-	46	38

When physicians use persistent health symptoms as a group of 37, recorded by a trained health care provider in a medical history (never rely solely on patient-completed checklists), individual symptoms can collate individual symptoms into groups, called clusters. Statistically, these clusters of symptoms, 13 in number, yield a diagnostic capability to separate out CIRS from essentially all diseases. If an adult patient has 8 or more clusters of symptoms, the likelihood of CIRS exceeds 95%.

When combined with VCS deficits, symptom clusters can yield an accuracy in diagnosis of 98.5% (that means the sum of false positives and false negatives is less than 2%). In the formal case definition of CIRS, however, cluster analysis is performed as an additional analysis to total symptoms reported.

Cluster Analysis of Symptoms

Individual clusters (one or more symptom in a group counts as a cluster)

1. Fatigue
2. Weakness, assimilation, aching, headache, light sensitivity
3. Memory, word finding
4. Concentration
5. Joint, morning stiffness, cramps
6. Unusual skin sensations, tingling
7. Shortness of breath, sinus congestion
8. Cough, thirst, confusion
9. Appetite swings, body temperature regulation, urinary frequency
10. Red eyes, blurred vision, sweats, mood swings, icepick pains
11. Abdominal pain, diarrhea, numbness
12. Tearing, disorientation, metallic taste
13. Static shocks, vertigo

Table 2 - Cluster analysis

A positive cluster analysis for biotoxin illness is presence of 8 or more of 13 clusters. For pediatric patients, 6 clusters are adequate to support a CIRS diagnosis.

CIRS providers have looked at symptoms described by character, including fatigue, weakness and executive cognitive dysfunction. The results are underwhelming, except for pain.

In a medical history, the physician is not using a check list. Symptoms questioning is perhaps better understood after observing how a skilled attorney will question a witness. Essentially, he will likely be asking for the same information from multiple different angles trying to have a clear idea of what the witness says. Similarly, a physician will follow the line of thought of a patient in discussing symptoms but will circle back to pin down any possible vagaries that might be present. In this manner, a physician will learn to a reasonable degree of medical certainty, more likely than not, on a day to day basis, does a patient have muscle aching? Does a patient have muscle cramps? Are there unusual pains, sharp stabbing pains that seemingly come unexpectedly and lancinate in one area of the body only to disappear and reappear elsewhere the next day? This type of pain description is typical of what CIRS patients experience-and is not confabulated-but will sound odd to the unaware physician.

During the pain interview with a CIRS patient, ask about unusual posturing of fingers or toes, sometimes called "clawing." These involuntary spasms in small muscles of fingers and toes can be painful; and are certainly unusual elements of history. Some patients will have their long and fourth finger split apart making a sign of a V as we saw so often from Mr. Spock in Star Trek. Sometimes there will be arching of the MCP and MTP joints as well. If you do not ask about clawing, you will not be told. Patients will recognize a physician as an insightful historian when he knows to ask the "odd" questions about odd symptoms, describing clawing in particular.

Muscle cramping is a common problem in athletes (and CIRS patients!). How many times have we seen a star basketball player clutching at his calves in the middle of the 3rd quarter of a heated basketball game? Cramps are disabling!

The calf cramps CIRS patients often have are not related to heat or to exertion. They are far more commonly brought on by lying down in bed or upon arising from sleep. The cramp is a muscle spasm; CIRS patients quickly learn that the spasm experienced in the middle of the night can be severe, especially if they have been sleeping with their ankles extended. Simple dorsiflexion of the ankles, stretching, for example, is mandatory but sometimes the spasm is so severe the gastrocnemius muscle essentially twists into a knot, occasionally tearing. In the middle of the night, that pain is agonizing. If you do not ask about cramps like these, you will not be told. Physicians just are not taught to take CIRS spasm histories. The spasms are real.

Joint stiffness is what is seen often in CIRS as well as in day to day musculoskeletal medical practice. Stretching upon arising is a common way to start the day for both types of patients. Shoulders, elbows, knees, low back, all will require some sort of attention. The rate of stiffening, however, with *cessation* of activity, called "gelling," is far faster in CIRS than it is for patients with wear and tear degenerative arthritis. If a patient says that he would prefer to stay standing after activity rather than sit down and rest that may be an indication of his awareness of his own rate of gelling.

Aching is perhaps the most common CIRS pain. Not from over-doing the work in the yard or trying to work out excessively, muscle aching can be vague until the aching occurs almost every day regardless of antecedent activity, but not in the same area and not with the same intensity. CIRS aching will not respond to most meds,

including NSAID. The aching often comes from areas of muscle insertion on tendons, raising the concern re: enthesopathies. The enthesium has reduced blood supply and reduced capillary perfusion to begin with; inflammatory responses in CIRS make capillary hypoperfusion worse. Pain will be enhanced in the face of hypoperfusion. If one sees an enthesopathy (extensor epicondylitis, patellar tendinitis, Achilles tendinitis, and more) but does not find a convincing history of overuse, spend a few minutes exploring the rest of the CIRS symptom roster with the patient.

As will be discussed in the section on transcriptomics, down-regulation of nuclear encoded mitochondrial genes can be profound. Instead of postulating *intrinsic* mitochondrial disorders leading to abnormal mitochondrial metabolism of glucose, leading to lactic acid accumulation, a better approach is to recognize that nuclear transcription factors exert direct effect on mitochondrial function. By recording nuclear encoded mitochondrial gene activation, using transcriptomics, the physician can understand reduced energy delivery is abnormal due to aberrant genomic control. Still, whatever causes lactic acid accumulation, excessive lactic acid in capillary beds is a source of muscle pain, including aching.

Understanding that mitochondrial research is ongoing (17) looking at mitochondrial stress responses CIRS providers feel that a delineation of mitochondrial gene expression must first be examined as an upstream regulator of stress responses.

Headaches are not necessarily a musculoskeletal problem but can overlap with any pain syndrome. With CIRS, look for intravascular volume depletion and reduced antidiuretic hormone (ADH) levels for a given osmolality. If patients are troubled with headaches, especially when told that they have "migraine that lasts for more than 24 hours," think of ADH/osmolality and not an actual migraine.

A special case in CIRS patients is the unexplained, sudden weight gain just after the onset of CIRS illness. Ask the question, "was there a time that you gained 20 or more pounds unexpectedly that you just cannot lose?" The physician is asking about leptin resistance, a feared complication of inflammatory changes that abnormally alters responsiveness of the primordial gp-130 cytokine receptor that responds to leptin. A great month of weight loss for a leptin-resistant patient is usually 0.5 pounds per month. When the patient is a young female, and this is often the case, societal values of thinness adds to her CIRS pain.

The overwhelming majority of CIRS patients know all about the symptom of "push/crash." Years ago, this phenomenon was felt to be diagnostic for CFS. Now that CIRS providers are confident that CFS is just a subset of CIRS, the experience of having a better day than the usual bad ones and trying to do a little extra to catch up, is invariably met with two or more days of significant worsening. Once thought to be simply due to capillary hypoperfusion and anaerobic metabolism, it is known that the final common pathway of CIRS involves abnormalities in glycolysis, and ribosomal and nuclear encoded mitochondrial genes that reduce the availability of energy.

Be sure to ask about abdominal pain. Bile acid reflux is found in over 66% of CIRS patients. The reflux sounds typical of acid reflux but never responds to acid blockers. Even worse, after a gastroenterologist is called in to perform a scoping (done fasting when bile acid reflux will not be a problem), the chances are high that the abdominal pain after meals will be called "functional." Functional, by definition, means there is no

discernible pathology: the problem is psychiatric in origin.

Decreased VIP and MSH levels, elevated TGF-beta1, C4a and MMP-9 tests, multiple objectively demonstrated disruptions of hypothalamic-pituitary-end organ axes, abnormal brain imaging and distorted transcriptomics argue, however, that there is nothing "functional" about this illness. Objective data, not "functional ideation," define the illness and define the effectiveness of treatments.

Gastroparesis is rarely found in anyone except older diabetics with a history of poor control of blood sugar. Except for CIRS patients in whom delayed gastric emptying can be shown by a nuclear emptying study to be abnormal in 5-10% of cases. Do not guess: do the study.

Section 6. Environmental history

Environmental exposures can be hard to confirm. Given the Internet, one can find many non-biotoxin exposures called environmental. Could pesticides count? Which ones? Exposed for how long? Was the pesticide exposure from harvesting, application, doused by adjacent aerosol drift?

How about golf courses, known users of pesticides, herbicides, fungicides and fertilizers? Is playing golf considered to be a dangerous environmental exposure?

And what about Round-Up ready crops? Are exposures to genetically altered organisms a source of illness? Some practitioners preach that it is so (still waiting for data).

Dietary sources of exposures remain talked about frequently, but if someone is telling the public that drinking coffee equals being poisoned by mycotoxins, let us see the data. Less important than how many people buy special coffee (insert mushrooms, bleu cheese, bread, chocolate, beer and wine, among others) into this hysteria-based sales pitch, claiming how great they feel is the data supporting their claims.

In the sections that follow, exposure is a key factor in assessment of the case definition of CIRS. It remains one of the hardest elements to show conclusively.

A variety of factors underlie exposure risk. Well person or CIRS? HLA-based susceptible or not? Skin versus inhalation contact? Exposure to a water-damaged building for a non-HLA susceptible patient essentially means no risk. Lyme? Dinoflagellate? Cyanobacteria?

The history must include location and type of exposure, mechanism of exposure, with duration less important than intensity. Some exposures are simple: "I swam in a lake later confirmed to have bloom conditions of Microcystis." Some are not: "I walk by a retention pond every day. I never heard of any problems with any algae there." Be very careful with Public Health pronouncements regarding cyanobacteria, especially Microcystis in Lake Erie. Since the source of most illness from cyanobacteria comes from inhalation of droplets of water, when one hears that the water from Lake Erie in Toledo is unsafe to drink but safe to use for showering, do not believe it.

As this consensus moves forward to specific illness categories below, remember that there is no state in the US that does not get deliveries of reef fish. There is no state that does not have problems with Harmful Algal Blooms at some time.

Over 80% of CIRS cases reported stem from exposure to the interior environment of water-damaged buildings (WDB). What is

not known is the effect microbial toxins versus microbial particles have on pathophysiology. Studies have shown that for every spore found, over 500 fragments are present (18). Fragments of amplified molds, bacteria, actinomycetes and mycobacteria may possess toxins and certainly contain inflammagenic material which, once the fragments are inhaled, are detected by the innate immune system starting the CIRS process in the genetically predisposed.

It may seem odd that, until recently, only fungal DNA measurements were used to assess human health risk. The EPA adopted the research advances coming from Vesper and colleagues, selling a license to use Vesper's methods commercially, beginning in 1996. This test, called the Environmental Relative Moldiness Index (ERMI) was found to be cumbersome in interpretation, as fungi counts from benign species added to supposed risk assessment. A follow-up test, HERTSMI-2, also based on spore equivalents/mg of dust, was found to be more specific and sensitive (19, 20).

As time has passed, use of molecular methods (21), especially including transcriptomics, is complementing proteomics in assessment of causation of patient illness following exposure to WDB. With a distinctive molecular pattern marking gene activation by trichothecenes and actinomycetes (16), together with clusters of differentiation for endotoxin exposure (CD14, ref 16) and beta glucans, (CD 248; ref 16), the era of reliance on urinary mycotoxin testing, uncompromisingly exposed as unreliable by the CDC (22) has passed.

Both fungal QPCR measures necessarily failed to identify risks from bacterial endotoxin and filamentous bacteria, actinomycetes. Now that advanced testing for endotoxin and actinomycetes is available (EnviroBiomics and Mycometrics), as well as with quality-assured molecular methods, the future of accurate microbial assessments is bright. WDB are the most important exposures that results in CIRS.

Section 7. Environmental history specifically applied to WDB

OVERVIEW

An Indoor Environmental Professional's (IEP) perspective

Michael Schrantz, Tucson, Arizona

CIRS is surrounded by the primary concern of being exposed to a water-damaged-building (WDB). One of the first steps in the published Shoemaker Treatment Protocol is to remove the diagnosed patient from exposure to a WDB. While ERMI/HERTSMI-2 scoring offers a reliable, medically-backed and patient-driven index that SP-following physicians' use in their practice, such an index does not explain how and why a building may be labeled as a WDB. Understanding the environmental history of a building can provide additional evidence that it may, in fact, be a WDB.

The focus of this section is to help physicians better understand what makes a building a WDB, and more accurately recognize when exposure to their patients may be occurring.

What is a Water-Damaged Building (WDB)?

For our case definition, as used by the Surviving Mold community, any building (including residence, school, and workplace) that is suffering, or has suffered one or multiple events from damp conditions, or water damage is a WDB. Simply stated: uncorrected water intrusion lasting more

than two days will be followed by microbial growth.

The World Health Organization estimates that up to fifty percent of buildings have suffered damp conditions (1). In an analysis of previously collected data on a nationwide sampling of office buildings, Mendell and Cozen found correlations between building conditions and worker symptoms that, if causal, "would suggest an increase in symptoms among the very large proportion of the U.S. workforce that is employed indoors" (2). Another analysis of office building data indicated that thirty to fifty percent of built office environments in the U.S. have suffered from water damage (3). A separate analysis of office building data found that 34% had current water damage in occupied spaces, 71% had past water damage in occupied spaces, and overall, 85% of the buildings had past water damage and 43% had current water damage (4). The economic and health impacts of WDBs are considerable, warranting closer observations (5).

In 2003, the Institute of Medicine reported finding causal links between WDBs and allergies, asthma and respiratory infection (6). We contend that there now needs to be concern for the causal link between WDBs and CIRS. Professional investigation of WDBs should not settle for questions about allergies and respiratory illnesses alone as older literature would suggest. The presence of a multi-symptom, multi-system illness in one or more occupants warrants referral for medical evaluation to see if the occupant meets the case definition for CIRS-WDB.

Given that brief, low-dose exposures can initiate systemic inflammation in susceptible people, it makes sense that remediation is likely to be more challenging when WDBs are occupied by one or more persons with CIRS-WDB when compared to WDBs whose occupants are healthy. The "healthy occupant" routinely uses a rated furnace filter to *help* remove most pollen and intact spores. Removing smaller (ultra-fine & nano-sized particles) particles from the air/indoor environment, however, presents greater challenges and utilization of air filtration media not typically seen in residential settings (7). Further, excluding certain clean room operations, many buildings lack laminar air flow conditions indoors (8) resulting in decreased removal of total particulates in the occupied spaces (*example: office*) as a significant number of particles succumb to gravity and their own settling velocities (Stokes's Law). CIRS-WDB patients raise the bar on what constitutes remediation to a safe exposure level. We may learn that the higher standard of post-remediation safety and general cleanliness also helps patients with treatment-resistant asthma.

When moisture or unbound water (available water = water activity **(a_w))** reaches a level high enough to support microbial growth, such growth usually occurs (9). Roof leaks, plumbing leaks, floods and even elevated levels of moisture in the air can create these opportunistic environments. Ecological conditions indoors can be both independent and dependent of conditions outdoors.

The science of water is complicated. We know that our ecology on earth means that many microbes (fungi, bacteria, amoeba, for example) surround us; they are in abundance (10). They are also needed for survival on earth. Their abundance, combined with the correct amount of moisture (damp conditions) in our buildings, however, provides an environment conducive for microbial growth. These damp conditions do not just amplify the potential of microbial growth, they can increase the presence of

other vectors (i.e., insects, rodents, and other carriers of exposure concerns) (11).

We understand that most fungi, for example, can grow on wetted drywall, wooden framing, or composite cabinetry (*i.e. cellulose-based materials*). Most of these wetted materials would have been exposed to enough moisture to sustain an a_w long enough to support growth (~>.68 a_w). Such conditions are easily met when a building has been impacted/damaged by water. These conditions are not subject to linear dose-response ideas. The ecological environment in a building after a massive acute water event is trivially different from a much smaller but chronic event. Both events warrant concern.

While it may make a difference acutely, whether the home has a "small" water leak under the kitchen sink versus a whole-house flood, does that suggest that the kitchen sink leak should be of no concern and not addressed? No. Does a lesser quantity of water leaking under the kitchen sink guarantee that there will be less microbial growth? No. Any water intrusion or identified damp condition should put physician and patient on alert. In many of these situations, the consultation/guidance of a CIRS-knowledgeable Indoor Environmental Professional (IEP) is recommended.

Water does not just become a concern if it leaks into a home. Water (or moisture) is present everywhere, including crawlspaces and basements. By location, a crawlspace is almost always a part of the building, and thus a contaminated crawlspace can also define a home as a WDB. Most crawlspaces are unfinished, meaning efforts have not been made to seal, dry, and/or "contain" it. Unfinished crawlspaces often provide the ideal environment for microbial growth amplification. Due to various driving forces (pressure/temperature/airflow differentials) and available pathways (gaps/cracks/penetrations) between a crawlspace and occupied spaces above (including soil gases below), we know that crawlspace-originating contaminants invariably can make their way indoors.

While dampness and water intrusions play a big role in identifying a WDB, even "musty odors" can offer evidence to occupants that suggests an active (*growing/decomposing organic matter*) microbial source is present. One of the challenges with identifying a WDB is the fact that the building may not visually or historically provide evidence of being affected. A leak may have occurred years ago that resulted in microbial growth, but later dried up (naturally) leaving a significant portion of that growth in its place. Under the right conditions, this growth (source) of microbial growth may communicate into the occupied spaces of the home creating exposure concerns for a CIRS patient, yet the building may appear "perfect" or otherwise non-WDB.

This leads to the importance of not making assumptions, positive or negative, towards defining a WDB. Today, the Surviving Mold community has painstakingly built a small network of vetted IEPs that understand the built environment, the related building sciences, the physiology of microbial growth, and the comprehensive approach needed when working with CIRS patients.

Unfortunately, the supply of such vetted IEPs is low and they are in high demand. Most local "mold inspectors" have shown potential, but lack (in fairness) a much more needed understanding of CIRS, and the differences between the traditional ("industry-standard") and inflammatory sciences and methodologies used to identify a WDB.

While various mold analysis methods are available to help IEPs, patients, and physicians identify a "mold issue" or even location of contaminant sources, extreme caution is advised when interpreting the results. Here are a few considerations:

- Settle plates (Petri dishes) only identify viable spores that are supported by the agar on the plate. Non-viable spores, non-ideal growing conditions, and mold fragments (representing a majority of the exposure concerns) are NOT identified using this method of collection.
- Spore trap sampling can detect and identify both viable and non-viable spores but cannot identify certain smaller, fungal species, nor can they identify mold fragments especially those beyond the resolution limits offered by light microscopy. Spore trap sampling typically represents a very small sample size and inadequate representation of exposure when compared to QPCR analysis. Spore trapping is limited in time and location; never identifies Wallemia; can miss less aerodynamic Stachybotrys; never identifies the > 99.8% of indoor particulates that cause inflammation. Any effort to use spore-trapping to tell a CIRS patient that all field tests are normal and (thus) the "home is safe" is incorrect.
- Tape lift or swab sampling techniques can be used to help identify mold or unknown substance
- None of the methods above replace the ONLY current metric (ERMI/HERTSMI-2 performed by QPCR on settled dust) used in the Shoemaker Treatment Protocol to assess the average exposure in a home for someone with CIRS-WDB.

For physicians, repeated exposure to a WDB is evidenced by blood markers and symptoms. At this point, some physicians have already suggested that the patient collect their own ERMI/HERTSMI-2 dust sample to validate exposure. A potential issue with that suggestion is when the dust sample results come back showing a "safe" building. This data would conflict with the patient's blood markers and/or symptoms. The complexities of dust sample collection, however, may have resulted in a false-negative (or false-positive) result. It is possible that another contaminant source/s exist in the home that the ERMI/HERTSMI-2 method is unable to detect, especially bacterial endotoxin and/or actinomycetes. Further, there are concerns regarding where the sample was collected (i.e. near past/current water damage, near windows/plumbing, basement, and more). These are just a few reasons IEPs suggest that the patient consider consultation with a vetted IEP. Involving this IEP would help reduce the chance of sample error, and even be able to help identify other issues or contaminant sources that are affecting the patient.

In the case of an **incoming/new patient**, many physicians will have them initially collect their own ERMI/HERTSMI-2 dust sample. Typically, one sample is collected per floor of the home/building to provide an average exposure assessment (score). The results of this sample may show evidence that a WDB exists, and that exposures need to be further assessed. Two laboratories currently provide kits that the client can use and follow the instructions to collect this initial sample:

- Mycometrics LLC https://www.mycometrics.com/online.html #1-732-355-9018
- EnviroBiomics, Inc. https://www.envirobiomics.com/shopping #1-210-233-6162

Editor's note: additional documentation is available. See the IEP consensus from

4/2016 found on the home page of www.survivingmold.com.

Section 8. Post Lyme Syndrome (CIRS-PLS)

Chronic Inflammatory Response Syndrome-Post-Lyme Syndrome (CIRS-PLS) occurs after the acute phase of Lyme disease, marked by an end of antibiotic use. Lyme disease is the most common vector-borne disease in the United States and Europe (1). It is a systemic tick-borne infection caused by the bacteria *Borrelia burgdorferi*. In the acute phase of Lyme disease, symptoms reported in older literature include arthritis, meningitis, facial palsy and myocarditis (2). More recently, however, acute Lyme creates systemic inflammatory illness, with transcriptomic and proteomic markers seen within 48 hours of tick bite (3). The erythema migrans (EM) rash is a target lesion that is seen in fewer than 70% of acute cases (4). In the acute phase, patients develop increased complement split products of the third and fourth elements of complement. (5) These elevations are present in both EM-positive and EM-negative acute Lyme patients which make C4a and C3a useful biomarkers for Lyme disease.

For an estimated 20% of patients treated with antibiotics for acute Lyme disease, a post-treatment Lyme disease syndrome develops (6). This post-treatment Lyme disease is best understood as a chronic inflammatory response syndrome because of its parallels to other varieties of CIRS subtypes (7). These similarities include a strong genetic predisposition based on HLA haplotypes (15-6-51, 16-5-51, 4-3-53, 11-3-52B). Additional proteomic parallels to other chronic inflammatory syndromes are lower levels of regulatory peptides, especially MSH, higher than normal levels of transforming growth factor beta-1, split products of activation of the third and fourth element of complement and disrupted regulation of feedback control of osmolality by ADH and cortisol by ACTH. Various other diagnostic modalities are disrupted including deficits in visual contrast sensitivity (VCS) testing; stress echocardiograms revealing acquired pulmonary hypertension; pulmonary function tests revealing restrictive lung disease; and brain volumetric studies (NeuroQuant®) findings of excessive atrophy of gray matter nuclear material in the bilateral putamen and right thalamic swelling. The notable divergences between the proteomic and diagnostic differences between CIRS-PLS and CIRS-WDB, the predominant chronic inflammatory response syndrome, are some of the HLA genetic haplotypes particular to increased Lyme but not WDB sensitivity; elevations of C3a, a complement pathway specific to bacterial sources of innate immune system perturbation and brain volumetric findings.

The use of transcriptomics using RNA-seq most strongly affirms the existence and pathophysiology of CIRS-PLS (7). While the clinical complaints of post-Lyme patients might be disregarded as subjective and the proteomic changes considered non-specific, RNA-seq detects unique patterns of differential gene activation. These patterns have been sometimes referred to as a "transcriptomic signature." It demonstrates changes in the acute, post-treatment and chronic phases of Lyme disease. Bouquet, et al (7) assessed transcriptomics at three-time points: at the initial date seen and diagnosed with Lyme disease, after three weeks of antibiotics treatment and six months post-treatment. His study found marked differences in gene activation and suppression between cases and controls in 1,235 genes in the initial period. After three weeks of antibiotic treatment, 1,060 genes remained abnormal. The persistence of perturbed differential gene activation after antibiotic treatment offers genomic

validation of persistent illness and the concept of CIRS-PLS. After 6 months, 636 genes remained abnormal. Notably, there were no differences in abnormal gene activation between subjects who reported they were "fully recovered" and those who remained persistently symptomatic. The symptoms were not predictive of the persistence of perturbed differential gene activation. This finding advances the notion of the persistence of post-Lyme disease: patients presumably thought to have recovered from antibiotic treatment are experiencing objective subclinical pathology at the transcriptomic level.

The transcriptomic signature of post-treatment Lyme disease is unique compared with other inflammatory and immune-mediated conditions. (7) There are two disturbed gene pathways specific to Lyme: glutathione-mediated detoxification and IL-6 signaling pathways. Pathway analysis in the six-month post-treatment group with publicly available transcriptome data sets from patients with chronic illnesses shows varying overlap of differentially expressed genes up to 60%. These other chronic illnesses include chronic fatigue syndrome, systemic lupus erythematosus and rheumatoid arthritis. A notable gene that is suppressed at all three-time points is eIF2. eIF2 is a gene pathway that modulates ribosome-transfer binding, the process underlying the start of translation. Disruption of translation interrupts the vital function of protein synthesis from messenger RNA. Down-regulation of eIF2 is not specific to Lyme disease. This suppression is also seen in patients colonized with MARCoNS, SLE and RA. Restoration of eIF2 has been documented after use of exogenous VIP as part of the CIRS protocol (8).

Consistent with the theory that Post-Lyme Syndrome is a chronic inflammatory syndrome is the detection of upregulated pro-inflammatory cytokines. There are eight notable up-regulated inflammatory genes: interferon gamma, interleukin-1 beta, tumor necrosis factor alpha, interleukin-6, transforming growth factor beta-1, anti-inflammatory cytokine interleukin-4, colony stimulating factor 2, cell surface and marker ligand CD40L. Genes upregulated after treatment of antibiotics include Toll like adapter molecule 1 and nuclear factor kappa-B.

Ryan and colleagues have found disruptions in microRNA (unpublished) not covered in the Bouquet study. The significance of perturbed microRNA is the implication that post-Lyme syndrome involves disruptions of the mechanisms of regulation of DNA expression. This finding extends the model for understanding PLS beyond the simplistic model of varying up-regulated and down-regulated gene pathways. These transcriptomic deficits suggest the need for therapeutic approaches that eventually correct the abnormal gene expression. A future direction of research is assessing if the CIRS protocol resolves the gene activation abnormalities. Prior data has demonstrated the ability of the CIRS protocol to normalize the proteomic disturbances that remain post-treatment with antibiotics.

Measure	Control	Before abx	After abx	After CIRS RX
N=	13	34	29	31
TGFB	3621	6782	8967	4890
C4a	3886	8149	6710	4120
C3a	1124	1284	384	410
i-Treg	4.66	2.94	3.02	4.16
t-Treg	4.25	2.44	2.98	3.86

Table 3. CIRS biomarker test values for different stages of Lyme disease. All data from private practice of RS.

The above table demonstrates biomarkers that measure innate immune system inflammation and immune function. At baseline, the patients with Lyme disease have perturbations in all five of these biomarkers. The proteomic abnormalities persist after antibiotic treatment excepting C3a which decreases to normal levels. One biomarker (TGF-beta1) worsens after antibiotics. Only after CIRS treatment do all five of these biomarkers approximate the levels found in controls. This finding has three significant implications. First, post-treatment Lyme syndrome is a demonstrable disease entity. Two, post-treatment Lyme syndrome is best characterized as a chronic inflammatory syndrome with elevations of innate immune system biomarkers consistent with inflammation. Third, the use of antibiotics followed by the CIRS protocol can restore patients with this condition, leading to clinical and objective metabolic improvement.

It must be noted that transmission of *B. burgdorferi* from person to person, or by nursing, has been suggested but never confirmed. Further, vectors other than ticks, including flies and mosquitoes, have been suggested to be vectors of *Borrelia*. As of the writing of this statement, no confirmation of this speculation has been produced.

Section 9. Chronic ciguatera (CIRS-Ciguatera)

Ciguatera, also known as icthyosarcotoxism, is the most common fish-borne illness world-wide (1). A precise estimation is difficult to assess due to under-reporting and the limited use of objective diagnostic methods. Predictably, incidence rates vary widely by geography. A survey of 17 Pacific areas found an incidence of 194 per 100,000 people (2), while another study focused on the mainland United States estimated a rate of 5.6 per 100,000 people (3). The biotoxins involved are polycyclic ether toxins (4) produced by a dinoflagellate, *Gambierdiscus toxicus*. The ciguatoxins can activate voltage gated sodium channels and affect the release of substance P, calcitonin gene related peptide and VIP (5). Ciguatoxin ascends the food chain when it is consumed by smaller reef fish that are consumed by larger fish which are, in turn, eaten by humans. Identification of ciguatoxin contaminated fish can be confirmed by a variety of methods including quantitative PCR assay to detect *Gambierdiscus* using cytotoxicity or using mass spectrometry to detect toxin (6). After consuming contaminated fish, acute ciguatera illness may last for a few days to three months. This acute disease is a toxin-mediated syndrome. The half-life of ciguatoxin in blood and tissues is on the

order of days. Neurologic and gastrointestinal symptoms predominate the clinical presentation. About 90% of patients reported from the Pacific have extremity paresthesia, circumoral paresthesia and reversal of hot/cold sensation. Among the gastrointestinal symptoms are diarrhea (75%), nausea (45%), vomiting (35%), and abdominal pain (45%). Muscle aching (80%), fatigue (60%), itching (45%), headache (60%), sweats (35%), and paresis (10%) are also reported (7). Identifying acute ciguatera is a clinical diagnosis based on the development of neurological and gastrointestinal symptoms within 24 hours after eating reef fish. In some circumstances, the fish flesh consumed can be assessed by assay for ciguatoxin. Even rarer is assessing for specific transcriptomic signatures of acute ciguatera (1). Treatment of acute ciguatera is limited to watchful waiting, supportive care and a trial of mannitol (8) which is of uncertain value.

Of all acute ciguatera cases, an estimated 5% evolve into a chronic syndrome (9). Despite the increasing incidence of appearance of ciguatoxin-contaminated fish in non-tropical areas, recognition of ciguatera is often delayed due to lack of recognition of the toxin-related syndrome. Chronic ciguatera is best understood then as a chronic inflammatory response syndrome (1). Chronic ciguatera shows a marked genetic propensity to two specific HLA haplotypes: DRB1-4; DQ-3, DRB4-53 or DRB1-11; DQ-3; DRB3-52B (3). Additional proteomic parallels to other chronic inflammatory syndromes are lower levels of regulatory peptides, especially MSH, higher than normal levels of transforming growth factor beta-1, split products of activation of the fourth element of complement and disrupted regulation of feedback control of osmolality by ADH and cortisol by ACTH (1, 10). Various other diagnostic modalities are disrupted including deficits in VCS testing; stress echocardiograms revealing acquired pulmonary hypertension; pulmonary function tests revealing restrictive lung disease; and brain volumetric studies (NeuroQuant®) finding of excessive atrophy of gray matter nuclear material in the bilateral pallidum, putamen and thalamus (1).

A transcriptomic case definition may also be viable. In a study of 11 patients with ciguatera-induced CIRS and 11 normal controls, transcriptomic analysis achieved 100% classification accuracy (11). There were characteristic effects on differential gene activation in regions involved with wound healing (CD9, CD36, vWF and Factor XIII); adaptive immunity (HLADQB1, DQB2, IL18R1, IL5RA); innate immunity (GZMK, TOLLIP, SIGIRR and VIPR2); and differential expression of long, non-coding sequences (lncRNA). The significance of a transcriptomic signature for chronic ciguatera is it may allow for differentiation amongst different subtypes of CIRS with one study. Since nearly all the proteomic and diagnostic modalities listed in the prior paragraph are indistinguishable from those found in CIRS-WDB and CIRS-Post-Lyme Syndrome, RNA-Seq allows for insights and diagnostic clarity that may be otherwise unobtainable.

Section 10. Pfiesteria (CIRS-Pfiesteria)

Though far less prevalent than CIRS from water-damaged buildings and post-treatment Lyme disease, biotoxins from other organisms are important to recognize as potential causative agents for CIRS. *Pfiesteria* is of unique historical importance as it was Dr. Ritchie Shoemaker's initial investigation into this organism and the illness it caused that laid the foundation for discovering CIRS. In response to his investigation of the ecological disaster that led to fish kills in the Pocomoke River and a mysterious human illness in 1996, Dr.

Shoemaker published the first case reports of human illness caused by exposure to Pfiesteria toxin in 1997 (1). He published the first article identifying an effective treatment for Pfiesteria six months later (2).

Pfiesteria related fish kills affected not only the Pocomoke River in Maryland but also estuaries in the Chesapeake Bay, Virginia and North Carolina. These fish kills generally occurred under ecological conditions including elevated copper in porewater (water found in sediment), rain events and run off from chicken manure applied to no-till fields. Human illness caused by *Pfiesteria* has undergone multiple nomenclature changes. In Dr. Shoemaker's 1997 article first identifying this disease, it was referred to *Pfiesteria*-human illness syndrome. The Centers for Disease Control and Prevention named this disease Possible Estuary-Associated Syndrome. With the advance in understanding of the mechanisms of the chronic phase of human illness from *Pfiesteria*, the most accurate name would be CIRS-*Pfiesteria.* The acute phase of *Pfiesteria*-related illness is toxin-mediated and transmitted through direct contact with estuarine water or inhalation of aerosolized or volatilized toxin. Significant toxins producers are included in *Pfiesteria* complex organisms with specific mention of *Pfiesteria piscicida* and *Pfiesteria shumwaye*.

There are a host of nonspecific clinical symptoms that are also present in other forms of CIRS. In *Pfiesteria*, the most common of these nonspecific symptoms are cough, secretory diarrhea, headache, fatigue, memory impairment, rash, difficulty concentrating, light sensitivity, burning skin upon water contact, muscle ache and abdominal pain (3). Less common symptoms are upper airway obstruction, shortness of breath, confusion, red or tearing eyes, weakness or vertigo (4). VCS testing reveals sharply reduced scores for both the acute and chronic phase of this disease (4).

An early treatment protocol in 2001 described the use of cholestyramine and as-needed use of sorbitol and Prilosec to manage the possible side effects of constipation and reflux respectively (2). With advances in the understanding of the chronic phase of *Pfiesteria* induced illness as a CIRS, the application of the modern CIRS protocol is considered the updated standard of care.

Section 11. Cyanobacteria (CIRS-Cyanobacteria)

CIRS can also result from exposure to toxic blooms of cyanobacteria, also known as blue-green algae. Ubiquitous in all aquatic environments, they are a phylum of bacteria that obtains their energy through photosynthesis and are the only photosynthetic prokaryotes able to produce oxygen. Cyanobacteria, along with eukaryotic phytoplankton, form the basis of the aquatic food web. However, some cyanobacteria have the capacity to produce powerful toxic substances (1). Those known for their potential ability to produce cyanotoxins include *Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya, Microcystis, Nodularia, Nostoc* and *Planktothrix*. These cyanotoxins include neurotoxins: anatoxin-a, anatoxins-a(s) and saxitoxins as well as the hepatotoxins: microcystin, nodularin and cylindrospermopsin (2).

The first literature reference for toxic cyanobacteria was in 1878 from the brackish water cyanobacteria *Nodularia spumigena* when George Francis issued a report on sheep and cattle death in *Nature* called "Poisonous Australian Lake." (3). During the period of 2007–2011, Departments of Health and/or Environment from 11 states funded by the National Center for Environmental Health (NCEH), Centers for

Disease Control and Prevention contributed reports for 4534 events to the Harmful Algal Bloom-related Illness Surveillance System (HABISS) which included 458 cases of suspected and confirmed human bloom-associated illnesses and 175 animal morbidity and mortality events. However, the extent of human and animal exposures to these organisms or their toxins, and the public health impacts from acute exposures associated with recreational activities or chronic exposures associated with drinking water is unclear (4). It is possible that the incidence of CIRS from cyanobacteria will be on the increase as the EPA reports in their National Lakes Assessment (NLA) 2012, that the toxin microcystin is detected in 39% of lakes. There has been a significant increase in the percentage of cyanobacteria cell density, a measure of the density of cells that could produce cyanotoxins. In 2012 the NLA identified an increase of 9.5% in the detection of the toxin microcystin (5). At present, the US Federal government has not established exposure regulations for cyanobacteria or cyanotoxins, but several states have relied on guidelines published by the World Health Organization or have derived their own risk assessments to support public health decision-making for posting advisories or closing water bodies (6).

Exposure to cyanotoxins occurs via ingestion, inhalation or skin contact. Health effects associated with recreational water exposure to harmful algae blooms (HABs) include gastrointestinal, respiratory, eye, ear and dermatological effects with fever and neurological effects reported less frequently (7). As with CIRS-WDB and CIRS-PLS, exposures to HABs can lead to a chronic biotoxin-associated illness that is characterized by abnormalities in symptoms, VCS and multiple biomarkers that resolves with CSM therapy. A cohort of 10 people who experienced chronic illness after exposure to Microcystis blooms were assessed before and after 2 weeks of CSM therapy. Mean symptoms decreased from 19.7 (out of 37), to 3.2, equaling controls. VCS increased by about 40%. All biomarkers normalized except MSH. Two HLA DR haplotypes (7-2-53 and 14-5-52B) were significantly overrepresented in this small cohort (8).

The symptoms caused by cyanotoxins are like those of other biotoxin-mediated illnesses. As with other toxin molecules, the many cyanotoxins contain heterocyclic rings. The shape of the anion ring varies based on the species creating the toxin (9). The hepatotoxins are the most commonly found cyanotoxins.

Microcystin, named because it was first isolated from *Microcystis aeruginosa* (10), and nodularin, isolated from the cyanobacteria *Nodularia spumigena,* are cyclic peptides with seven amino acids and five amino acids respectively.

Over 60 different types of microcystin have been isolated from the species of *Anabaena, Hapalasiphon, Microcystis, Nostoc, Oscillatoria* and *Anabaenopsis.* The anatoxins and saxitoxins are alkaloids, containing ring structures with at least one carbon-nitrogen bond and have been isolated from the species of *Anabaena, Oscillatoria, Aphanizomenon, Lyngbya, Schizothrix, Cylindrospermopsis* and *Umezakia.* Species may or may not be toxic and even within a single-species bloom there may be a mixture of toxic and non-toxic strains (11). Cyanotoxins, like other toxin molecules, can be bound by CSM (12).

A unique cyanotoxin, BMAA, has been associated with neurologic deterioration following consumption on the island of Guam. Once thought to be related to consumption of fruit bats by the native

Chamorro tribes, the cyanotoxicity, formed by root-dwelling Nostoc species in cycads, may be due to concentration of BMAA in seeds. Cycads seeds are ground into flour. Sorting out illness causation on Guam is confounded by ciguatera and moldy buildings. Detection of BMAA is expensive and time-consuming (13).

Section 12. Differential diagnosis

The differential diagnosis of CIRS has been likened to a differential diagnosis of all medical illness. CIRS is a systemic problem: it is not organ-based so much as it is body-based. Practically any disease, any disease mechanism, any perturbation of inflammation can theoretically be seen in CIRS.

Differential diagnosis is a process essentially of sorting; taking what fits and separating out what does not fit. Since all inflammation can be seen in CIRS, the process of sorting can be quite complex.

Begin the sorting process by recognizing that CIRS cases must meet a published, peer-reviewed case definition. There will be potential for exposure identified. The first question in differential diagnosis is, is there exposure to a water-damaged building? Is there exposure to a source of biotoxins? If not, is there no *potential for exposure*? What this means is that it is very hard to remember in a 20-year illness what building one might have been in 20 years ago, as we have seen repeatedly in cases of chronic ciguatera. Often people will forget what they ate and when they ate it in relationship to a disease. The problem is even worse with Lyme disease in that some people will not remember a tick bite but still can have Lyme. Other people will have a tick bite but no Lyme rash (erythema migrans). In Lyme, we end up being forced to rely on an inherently flawed antibody test as opposed to a physiologic test to assist us in diagnosis.

If the potential for exposure can be satisfied, the next requirement is that we have a multi-system, multi-symptom illness. In turn, the symptoms must meet the criteria from cluster analysis. The sorting process is now becoming clearer in that, if one does not have a multi-system, multi-symptom illness, they do not have a CIRS. If they do not have eight symptom clusters as an adult, or 6 as a child, it is likely that they do not have a CIRS. They may have something else similar, but not CIRS.

Further, the case definition includes not just symptoms alone, but lab abnormalities seen in published peer-reviewed literature. We will see reduction of the normal levels of regulatory neuropeptides especially MSH. We will see elevation of at least of one of three inflammatory markers of TGF-beta1, C4a and MMP-9. We will see dysregulation of ACTH and cortisol; we will see dysregulation of ADH and osmolality with a frequency that is quite high (> 80%). In addition, we will see abnormalities in gliadin antibodies and VEGF. The issues we faced in the past with CIRS is that we did not know the transcriptomic signatures that these illnesses have. We hope to distinguish CIRS-PLS from CIRS-WDB readily with transcriptomics. We have published on transcriptomics of ciguatera; we have published transcriptomics of mold (5, in references, Sections 1-6).

We add NeuroQuant® findings to the differential diagnosis to quickly make things easier. Lyme has distinctive abnormalities as does CIRS-WDB. Other illnesses do not have those findings. One might simply say, well why not start with the NeuroQuant®? We often do!

The differential diagnosis process never stops in the sense that as we continue with therapy there will be resolution of individual physiological abnormalities, *one by one*, as we institute sequential treatments that fix physiological abnormalities. We make no

effort to do two things at a time; such "shotgun" medicine has no role in CIRS.

Differential diagnosis only ends when there is (i) near-100% exclusion of all possible causes of a given patient's illness or (ii) we have resolution of symptom abnormalities; VCS abnormalities; laboratory abnormalities; nasal culture abnormalities; and transcriptomic abnormalities. We must not abandon another vital element of differential diagnosis: simply recognizing the remote possibility of an alternative medical diagnosis is never exclusionary for the approach of a treating physician: "To a reasonable degree of medical certainty, it is my opinion that Mrs. XYZ has CIRS." Finally, while we want to have NeuroQuant® abnormalities resolved as quickly as transcriptomics that takes more time than what initial therapy alone requires.

Section 13. Physical exam

A carefully performed physical exam remains a second fundamental basis of medical diagnosis beyond history. Physical exams provide a basis for confidence in diagnosis with positive and negative findings, both important in the process of sorting out what illness or group of illnesses is present in a patient with complex multisystem, multi-symptom illness.

Vital signs are done routinely on all new patients to include blood pressure, temperature, pulse and respirations. Of these four vital signs, careful assessment of respiration is most often skipped. It only takes 30 seconds to count breathing and look for Cheyne-Stokes respirations in individuals with cognitive effects. Blood pressure tells us if we can use losartan down the road; it also tells us if we need to worry about problems with adrenoceptors if blood pressure is suppressed. The character of the pulse provides clues to ectopic beats. It only takes 30 seconds: do not skip. Short cuts in temperature taking are common but nothing replaces an old-fashioned mercury thermometer, when available, under the tongue.

Please use the tools of physical exam properly. Otoscopes are held upside down. Left hand, left ear. Right hand, right ear. Stethoscopes are placed on bare skin. When I see TV actors listening to lungs through a shirt or a sweater I cringe; even worse if a health care provider does this.

There are multiple possible tip-offs to the presence of a CIRS. Look for a resting tremor of the outstretched fingers, spread wide apart. Place a piece of paper on the top of the spread apart fingers to make a resting tremor obvious to the examiner and patient alike. Look for evidence of abnormal skin turgor, rashes, venous stasis changes and the like. Look especially for evidence of enlargement of turbinates and or polyps upon nasal exam. Transillumination of sinuses is frequently done with limited return. Look for evidence of gingivitis. One of the most important indicators of dental biofilm formers is refractory genitival abnormalities.

HEENT evaluation includes determining the presence or absence of pallor, erythema of the cheeks approximating a butterfly rash but sparing the nasal bridge ("mold facies" often misdiagnosed as rosacea), the presence of scleral injection and acne. The exam should also note the presence of goiter (thyroid illness frequently accompanies CIRS), the size of cervical lymph nodes and presence of any cranial nerve dysfunctions (often with CN VII weakness). Carotid upstroke should be full without any bruit. Look for jugular venous distention, especially in someone who has evidence of right ventricular hypertrophy on EKG.

Pulmonary exam takes at least 15 seconds. Do not skip. Listen for symmetry and for

evidence of mucous plugging with change after cough. Crackling rales and evidence of dullness, usually at the right base, possibly indicate a pleural effusion. Evaluate for wheeze, rhonchi, decreased diaphragm excursion, post-tussic rales. Ask for three maximal inspirations and then listen for inspiratory rales.

Cardiovascular exam looks for evidence of cardiovascular compromise. It seems straightforward, but we know that individuals who are disproportionally long in wingspan compared to height are a greater risk to develop abnormalities in thoracic and abdominal aorta that can lead to aneurysm formation. Listen carefully for wide splitting of S1 vs. a gallop rhythm. If you hear an S3 or an S4 (it is not necessarily your job to distinguish the two, but it is your job to hear one) look for a reason for cardiovascular compromise of either volume-overload and pressure-overload, both from right ventricle and pulmonary artery or systemically.
Abdominal exam is rarely helpful unless there is enlargement of liver and spleen. Trained observers can feel kidneys reliably, but this skill diminishes with extra patient weight (a problem commonly seen in leptin resistant patients with CIRS).

Extremity exam demands looking for evidence of true arthritis together with evidence of peripheral edema (pitting or not, unilateral or not) and venous thrombosis. Capillary refill may be diminished in fingers and toes if end organ profusion is diminished. Hands and feet should be evaluated for coolness, discoloration and perfusion. Proximal (shoulder shrug) and distal arm strength (grip) should be recorded. Handedness should be noted and dominant arm strength in anti-gravity muscles, compared to the non-dominant arm should be tested 3 times (for initial strength and fatiguing). Joints should be evaluated for pain, erythema, swelling and heat. Look also for sensory deficits.

Mental status is usually normal but there is often dropout of specific abilities. Most notably, math functions, performed without paper and pen, are frequently diminished. Asking questions such as, “What is 91 divided by 7” or, “What is 65 plus 17”, will frequently elicit delayed and/or incorrect answers.

The CIRS physical rarely requires rectal, pelvic or breast exams except as history indicates. Always have a chaperone if private area exams are planned.

There are a few tricks that go along with physical exam that CIRS docs will perform that other docs rarely do. Performance of measurement of wingspan compared to height takes 5 seconds but is associated with a more common finding of HLA 11-3-52B and elevated TGF-beta1. Similarly, stroking the skin, as if making a tic tac toe board on a patient’s back, is an easy way to look for dermatographia. While some providers think this shows evidence of mast cell activation, a positive finding simply reflects the presence of increased levels of C4a.

Ancillary findings are equally important as vital signs regarding oximetry, 12-lead EKG and pulmonary functions with spirometry. If for example, there is a discrepancy between the counted pulse and the recorded pulse with pulse-ox, you have a duty to explain the difference. Any oxygen saturation under 92% should create red flags immediately. Look on an EKG for evidence of abnormal rhythm, prior myocardial injury and voltage suggesting overload either of the right or left ventricle or both. Spirometry gives us two of the most important recordings at bedside, for primary care and CIRS. Low forced vital capacity (FVC) tells us about a restrictive lung disease; and low forced expiratory

volume in 1 second (FEV-1) tells us about obstructive lung disease. If a patient has given their very best effort and can only blow out for three seconds, be looking for restrictive lung disease or some. The spirometry computer will call the test inadequate; it is more important to sort out the mechanism of the inadequateness.

Section 14. Ancillaries - VCS

1. Either perform in person (better) or review VCS performed online at www.survivingmold.com (accept no substitutes).
2. Look for inability to see 7 in column C or 6 in column D.
3. Near visual acuity must be better than 20:50
4. Lighting must be greater than 70-foot lamberts (get a meter, do not guess)
5. If positive VCS (shows deficit) and positive symptom clusters, the likelihood of CIRS exceeds 98.5% (shown in 2005 and replicated routinely)

Visual contrast sensitivity (VCS) testing has been used clinically for years and remains the most accurate test for functional vision (25). VCS testing is best done in person at each office visit. Contrast is one of the seven main functions of vision facilitated by the optic nerve which provides the neurologic basis of vision. When we test for contrast, we control for other elements of vision such as near vision, far vision, static, motion, peripheral vision and night vision; we are looking only at contrast. Contrast is the ability to see an edge. What this means is that if I look at a door frame and I see a white background and a black door frame, I can identify what is background and what is frame very easily. Contrast sensitivity looks at the graded change of contrast at different light frequency (cycles per degree of visual arc) that we use to make a grid of five separate frequencies. This grid begins at 1.5 cycles per degree of visual arc extending in discrete intervals (3, 6, 12, 18) up to 18 cycles per degree of visual arc. Remember visual acuity is tested at 24 cycles per degree of visual arc.

Dr. Ken Hudnell, neurotoxicologist for the US EPA NHEERL in Research Triangle Park, NC, was the first to use VCS testing in biotoxin illnesses. His landmark work in 1997 (32) paved the way for others to follow. Our group was able to reproduce the observations of Dr. Hudnell of visual contrast being abnormal in that same fish killing dinoflagellate (*Pfiesteria*) illness, but treatment, beginning with cholestyramine, the first medication step of what is now a 11-step protocol, corrected the visual contrast abnormalities! With re-exposure, however, visual contrast deficits reappeared, identical to the initial deficits, usually within 36 hours.

If new symptoms are appearing during therapy, or if prior symptoms recrudesce, VCS can point the way to re-exposure (scores in columns C and/or D will fall). Do not use VCS to make nutritional-related diagnoses.

Section 15. Ancillaries - Transcriptomics

The most sophisticated genomic testing available today is transcriptomics. We look at differential gene activation using a Next Generation DNA Sequencer that performs RNA Seq. The Human Genome Project, completed in the early 2000's at the cost of billions of dollars, identified thousands of genes that code for proteins. What we saw then was the total genome structure, including duplicate copies called copy number variation (CNVs). Later, we learned that everyone had slight variations in their genes, called single nucleotide polymorphisms or SNPs. Many of these

SNPs are now known to be important markers of disease because they can indicate a change in protein function or activity. However, these SNPs are fixed and do not change throughout your life. The most impactful modulator of cellular activity is likely differential gene expression, since the amount of the gene expressed is ultimately in control of protein levels and cellular output. Based on current conditions, the genome will output a certain combination of genes, but when the conditions change, the gene output will change to best adapt to the new conditions or demands. This is generally what determines one's day to day, or even morning to night physiology.

What neither the first sequencing of the human genome, nor the later identification of various SNPs, could identify is this dynamic yet critical differential gene activity.

Remarkably, environmental stimuli, and there are many, can cause gene activation in minutes, if not faster. Such rapid changes in gene activity provide incredibly precise adaptations of the host to a rapidly changing environment. If the host is a one-celled creature, like bacteria or a fungus, it might be easier to conceive of the survival benefits that accrue from rapid responses to moisture, foodstuffs and chemical signals. Yet the same concepts apply to "higher", more complicated life-forms, like humans, as well.

We now know that the static genome is actively manipulated, constantly increasing production of some gene transcripts and decreasing others in response to its environment. Regulation is complex: nuclear transcription factors and newly discovered long non-coding RNAs, together with microRNAs and circular RNAs, as well as methylation and acetylation (do not forget demethylation and deacetylation!) can shut off and turn on gene function. If this sounds complicated, it is. Research into the interacting complexities of so many layers of regulation has progressed beyond its infancy, but new discoveries are published every month.

We are at the beginning of a new era in science where we can use genomics, *transcriptomics* for those that are sticklers for words, to our advantage in that we could find a distinct fingerprint for CIRS from water-damaged buildings, another for ciguatera and another for PLS. Not only will transcriptomics aid diagnostically but will likely also revolutionize individualized therapy to many illnesses, CIRS included. The application of genomics to human illness is just in its infancy.

Section 16. Ancillaries - Pulmonary artery pressure

Additional objective indicators of physiologic complications due to an inflammatory response syndrome are obtained through echocardiography. "Echos" are usually done resting, most often performed to assess function of the left ventricle as well as to assess the pumping function of the heart. Each echo will assess function of multiple cardiac structures; we are interested in the velocity of the tricuspid regurgitant flow, also called the tricuspid jet. Blood can go backwards from the right ventricle to the right atrium passing "the wrong way" across the tricuspid valve. The rate of backwards flow is measured in meters/second. The velocity is recorded accurately by the machine on at least four separate views during a routine echocardiogram. Curiously, cardiac sonographers are trained to label the tricuspid jet qualitatively as either absent, trace, mild or moderate. This is an unfortunate problem in that the CIRS health provider needs to know whether or not there is elevated pulmonary artery pressure, a

result that must be calculated. Since the echo machine generates numbers for each of the four ways the jet is measured, an average can be generated.

We use the tricuspid jet velocity to calculate the pulmonary artery pressure indirectly. We square the tricuspid jet number and then multiply that number by 4. To that product, we add the right atrial pressure (usually between 5 and 10 mm) to give us a calculated pulmonary artery pressure. Any resting pulmonary artery pressure (PASP) greater or equal to 30mm of Hg is consistent with pulmonary hypertension. *Any tricuspid jet greater than 2.5 meters per second will arouse concern about pulmonary artery systolic pressure in people with CIRS.*

For individuals with normal pulmonary artery pressure at baseline or patients with health symptoms such as unexplained cough, shortness of breath or chest pain, it can be useful to perform stress echocardiography. In this modification of the basic echo technique, an individual has two sonograms done. The first is at baseline, as discussed. The second is done after maximal exercise, requiring a target heart rate of 90% of predicted.

Stress testing is most often performed to look for problems with performance of the left ventricle. Exercise stress testing is a fundamental diagnostic aid that can help identify the presence of coronary artery disease. In our example, we are not looking for left ventricular problems; we want to know the pulmonary artery pressure change with exercise. Any rise in PASP pressure over 8 mm of Hg is abnormal.

The mechanics of performing a stress echo can become problematic. Here is someone, following possibly 11 minutes of maximal exercise, for example, exhausted, breathing heavily and leaning forward after the stress portion of this stress echo. Now the echo sonographer will insist that within 30 seconds the patient lie down. The out of breath patient lies down on the exam table for a repeat measurement of tricuspid jet.

As you might imagine, most sonographers are not asked to interrogate the tricuspid valve after exercise. It helps to talk to your cardiopulmonary staff to make sure they know exactly where they are going to place their transducer before the exercise begins.

We use pulmonary artery pressure as an inclusion criterion for use of vasoactive intestinal polypeptide (VIP) as treatment. If PA pressure rises more than 8 mm, the indication for use of VIP becomes stronger.

Section 17. Ancillaries - VO_2 max and anaerobic threshold

Another important cardiovascular diagnostic test is a cardiopulmonary exercise test (CPET). While the name of this test sounds like a stress echo, it is different. This test measures oxygen usage and carbon dioxide production in performance of exercise, usually on a bicycle. The test is somewhat cumbersome in that a patient is strapped to EKG monitors and is peddling maximally on a bike all the while breathing with hoses, tubes and a mask used to record oxygen consumption.

In 2015, the Institute of Medicine (9) emphasized the importance of cardiopulmonary exercise testing in their redefinition of chronic fatigue syndrome (CFS) as Systemic Exercise Intolerance Disorder (SEID). This effort fell short, however, of making CPET a biomarker necessary to diagnose SEID. The IOM simply returned to an updated, but still inadequate, non-specific symptom-only definition, one that essentially applies to 100% of all CIRS cases.

Much is known about the importance of VO_2 max (milliliters of oxygen consumed per kilogram per minute) as this is an important mechanism used to classify possible disability. We know that there is a difference between VO_2 max of women and men. We also know that age has a role in normal ranges for VO_2 max. Based on our practice data (unpublished), it is not unusual in the face of chronic fatiguing illness for a 50-year-old woman to have a VO_2 max of approximately 20 ml per kilogram per minute (with slightly higher values for men) raising the diagnosis of chronic fatiguing illness.

The tables for Cardiovascular Fitness Classification are published in the AMA Guides to Evaluation Disability and Impairment; Social Security uses VO_2 max as one of the key elements in assessing disability.

Functionally, even more important than VO_2 max is a delineation of anaerobic threshold (AT). This is the maximum level of activity achieved through available oxygen (aerobic metabolism). Mitochondria, the energy powerhouses of the cell, need oxygen to break down fragments of glucose, releasing water, carbon dioxide and energy (ATP). For those with low AT, even walking slowly up a flight of stairs results in reduced oxygen delivery, in turn diminishing aerobic energy production. When AT is exceeded, as in the stairs example, oxygen is not available as needed for mitochondria to produce the full complement of 38 ATP from a single molecule of glucose. Without oxygen to supply the electron transport chain, a single glucose molecule will now just provide two molecules of ATP, or a 5% efficiency in the face of oxygen depletion. In turn, as glucose and glycogen stores are quickly exhausted, the energy depleted cell looks for additional sources of fuel. In the face of low MSH, leptin resistance is often present, preventing normal use (through direct beta oxidation) of fatty acids for fuel (the "second wind" most runners have experienced). In "desperation," lean body mass, our protein reserves, are broken down into amino acids, with direct conversion of amino acids (especially alanine and glutamine) to glucose. The demand for ATP may create protein wasting syndromes that conserve fat reserves (the more detailed physiology can get complicated).

If AT is depressed, even trying to do a few things extra when a patient has a day with a bit more energy than most, results in glycogen depletion. Do not forget, glycogen replenishment is a slow process: patients will feel exhausted until their "batteries are recharged." Terms for this commonly observed phenomenon include "push/crash;" "delayed recovery from normal activity;" and "post-exertional malaise." Simply stated: "the patient did too much."

But contrary to the IOM opinion, low AT is not uncommon, not just in SEID. In CIRS, the oxygen delivery problem is complicated by lack of normal blood flow into capillary beds, not to mention nuclear encoded mitochondrial gene problems. Still, capillary hypoperfusion is the mechanism that underlies deficits in VCS which is a hallmark of CIRS.

Performing CPET twice, 24 hours apart, can objectively document the push/crash syndrome. Maximal effort on Day 1 often demonstrates low VO_2 max and impaired AT. Because maximal effort was given (push) the first day, at risk CIRS patients will often perform even more poorly (crash) on Day 2. VO_2 max and AT are commonly even lower on the second day highlighting the push/crash phenomenon. CPET performed over two consecutive days can provide objective evidence of physical disability.

Section 18. Ancillaries - von Willebrand's factors

Additional problems in CIRS paradoxically include both excessive clotting and bleeding. Just like in sepsis, where multiple inflammatory mediators are activated including complement, Th1, Th2 and Th17, coagulation defects also appear. So too for CIRS: fully two thirds of CIRS patients will have abnormalities in a comprehensive von Willebrand's profile (data not published).

In CIRS, shortness of breath will be reported by over 80% of patients. Asthma might be involved, but restrictive lung disease, interstitial lung disease and pulmonary emboli are all primary features of the differential diagnosis. Similarly, when exposure to a building results in unexplained nosebleeds and hemoptysis, immediately think of acquired von Willebrand's disease (AvWD), an easily treated condition using a medication (desmopressin) that costs about a nickel. If the differential diagnosis did not include AvWD, uncontrolled hemorrhage might follow.

Conversely, elevated levels of vWF raise the risk of intravascular clotting, with deep vein thrombosis and pulmonary emboli possible. Whenever, for example, a patient suffers clotting around an intravenous catheter (especially PICC lines), make sure that elevated vWF factors are not the underlying problem.

Section 19. Ancillaries NeuroQuant

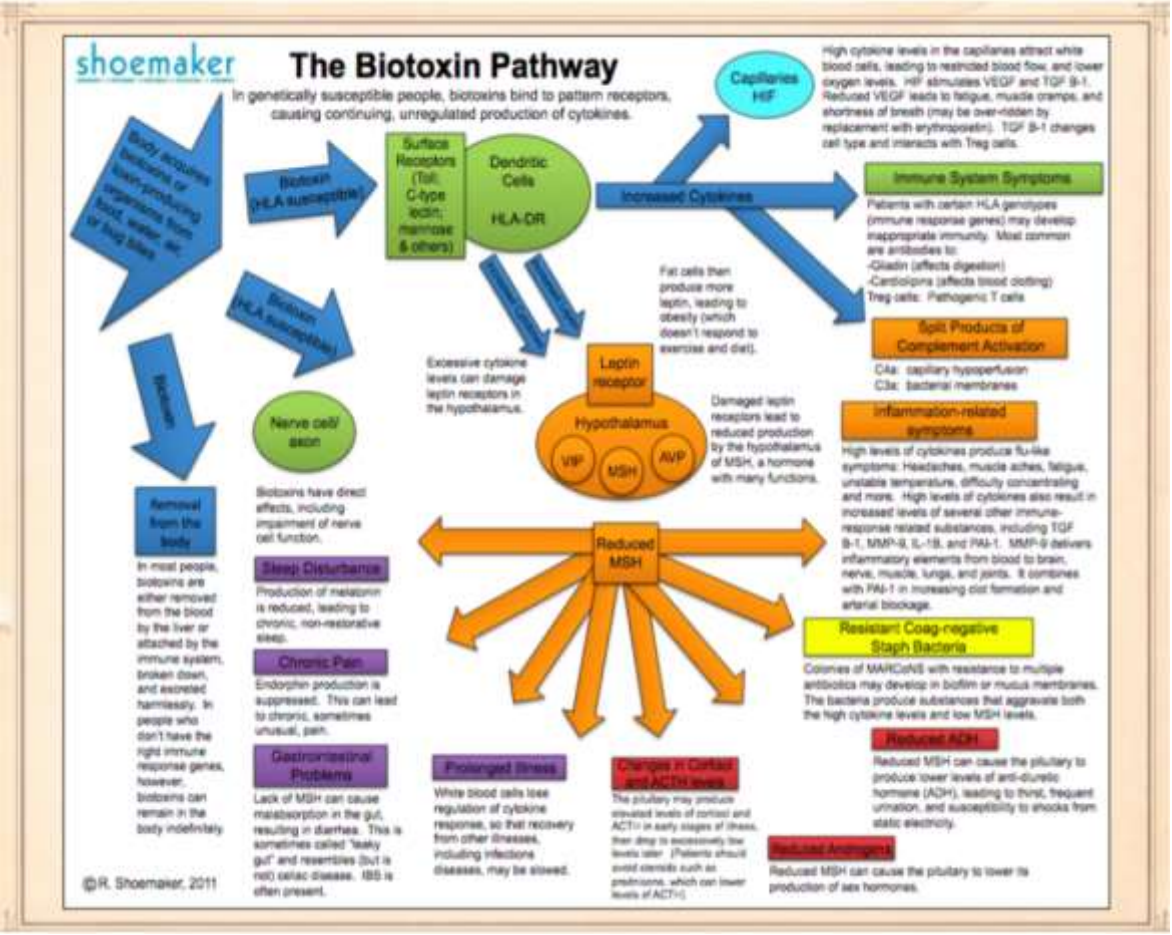
For most medical practices, NeuroQuant® (NQ) might appear to be just be a spin-off of MRI of the brain, but for CIRS providers, NQ has made (i) identification and (ii) separation of CIRS-WDB, CIRS-PLS, traumatic brain injury, PTSD, ciguatera and multi-nuclear atrophy straightforward. When added to an MRI of the brain, NQ is found to be an illness-specific indicator. Use of sequential NQ testing has shown there is much more plasticity of an injured brain to heal than once thought (18). With low cost, rapid turnaround times and no need for contrast dyes, NQ adds powerful weight to assessment of cognitive dysfunction, including evaluation of possible risk for development of dementia.

As all research efforts to date in the CIRS movement, data driven studies involving institutional review board approval (IRB), good statistics and good science have carried the day. We can look at a General Morphometry Report (GMR) produced by NQ, rapidly identify microscopic interstitial edema, atrophy and patterns of brain injury accurately. Much of the unsupported ideas about PTSD being purely a psychiatric condition will need to be re-evaluated now that we have indications of a unique volumetric measure that correlates with symptoms. Now that we can use NQ to identify and correct grey matter nuclear atrophy, we hope a new era will arrive in the field of treatment of neurodegenerative illnesses.

Section 20. Proteomics

Basic concepts have been published in multiple papers. We are looking at multisystem dysfunction, with genetic susceptibility; deficiency of neuroregulatory peptides; inflammatory involvement of Th1, Th 17, complement, coagulation; deficiency of T regulatory cells. The basics of the role of innate immune inflammatory pathways have been summarized in a schematic called the Biotoxin Pathway.

Biotoxin Illness Pathway: A Description of the Progressive Stages of CIRS Pathophysiology



Stage 1: Biotoxin Effects

Within normal hosts, exposure to biotoxin will result in an innate immune response via the production and release of innate inflammatory markers (1). This will in turn signal an adaptive immune response with resultant specific antibody formation to the offending agent (2). The antibody tags the biotoxin, signaling it for destruction and subsequent removal from the body (3). With activation of the adaptive immune system, inhibitory signals are released to downregulate the innate immune response, preventing chronic innate immune activation (4).

In contrast, hosts predisposed to chronic inflammatory response syndrome (CIRS) exhibit disruptions in their adaptive and innate immune mechanisms when exposed to biotoxins (5), precluding the removal of the offending agents. The resultant, persistent biotoxicity creates an upregulation of the innate immune system (6, 7), which if left unchecked will manifest as CIRS (8). Specific HLA immune response genes (HLA-DR genes) have been linked to an increased risk for CIRS (9, 10). The biotoxins bind to certain cell surface receptors such as Toll (11), mannose (12), and L-type lectin (13). Recognition and binding of the biotoxin at these receptors leads to specific upregulation of inflammatory pathways, resulting in an abnormal rise in inflammatory markers such

as cytokines (12), TGF-beta1, and split products of complement (14).

In addition to triggering systemic inflammation, biotoxins can also have a direct neurotoxic effect (9). In most patients, visual contrast sensitivity (VCS) testing can detect deficits caused by biotoxicity affecting the neurologic function in the visual system (15).

Stage 2: Cytokine Effects (MMP-9, Leptin, MSH, Coagulopathies, Hypoperfusion)

The upregulation of the innate immune system results in increased cytokine levels, which in turn bind to receptors on certain white blood cells (16), signaling the release of MMP-9 into the bloodstream (17). MMP-9 enzymatically degrades the proteins found in the protective extracellular matrix of blood vessel walls, allowing other inflammatory markers originating in the bloodstream to penetrate sensitive tissues such as the brain (18). Elevated MMP-9 has been linked to increased risk of atherosclerotic plaque formation, progression, and rupture (19). Additionally, high MMP-9 adversely affects joints (20), muscles (21), and nerves (22).

Leptin also plays a key role in the biotoxin illness pathway. Leptin is primarily produced in adipocytes; acting as both a hormone and cytokine, it links the neuroendocrine and immune systems (23). Leptin's cytokine action is exerted upon macrophages resulting in synthesis of additional pro-inflammatory cytokines (23), such as TNFα, IL-1 and IL-6 (24). These cytokines can in turn stimulate further production of leptin from adipocytes, creating a positive feedback loop that perpetuates the innate inflammation (24).

As opposed to leptin's driving force on the innate immune system during CIRS, leptin's normal physiologic hypothalamic influence is numbed due to competing cytokines that block the leptin hypothalamic receptor (23). Certain cytokines such as interleukin (IL)-6, IL-11, IL-12, and oncostatin M are capable of such a feat due to their structural similarity to leptin (24). The leptin receptor blockage creates leptin resistance (25), which in turn causes upregulation of leptin production (23). In normal physiology, leptin binds receptors located within the arcuate nucleus of the hypothalamus (26). This signals for enzymatic cleavage of the preformed prohormone proopiomelanocortin (POMC) into the following hormones: alpha melanocyte stimulating hormone (MSH), adrenocorticotropic hormone (ACTH), and endorphins (26). Inevitably, the leptin receptor blockade in CIRS leads to loss of hormonal regulatory control due to disruption of the POMC pathway with decreased levels of the hypothalamic hormones (23). Leptin resistance also leads to a decreased ability to mobilize fat stores for energy, resulting in recalcitrant weight gain in some patients (27) that does not respond to typical measures of diet and exercise (28).

MSH is a crucial neuroregulatory peptide hormone with anti-inflammatory actions achieved through numerous signaling pathways (23). By inducing cyclic adenosine monophosphate (cAMP) and inhibiting nuclear factor κβ (NF-κβ) (29), MSH is capable of downregulating expression of pro-inflammatory cytokines such as TNFα, IL-1 beta, IL-6 (which are all typically upregulated by leptin) and interferon gamma (23). Through these means, MSH regulates both peripheral cytokine responses and intracerebral inflammation (23, 29). MSH deficiency leads to unchecked cytokine effects, manifesting as numerous symptoms such as muscle aches, temperature instability, headaches, and decreased ability to concentrate.

Additionally, cytokines can cause elevated levels of plasminogen activator inhibitor-1 (PA1) (5), along with abnormal levels of von Willebrand's factor (VWF) and Factor VIII (30) leading to coagulopathies in some CIRS patients (31).

Capillary hypoperfusion can occur due to cytokine effects, including those produced by TNFα (23, 32). It has been suggested that this decrease of microvascular perfusion could be from either vasoconstriction due to direct cytokine effects or recruitment of leukocytes causing obstruction of vessels (33). The latter is based on a phenomenon occurring at times of inflammation: leukocytes are marginalized to the endothelial cell (EC) wall of a venule; a weak adhesion occurs between the leukocyte and the EC wall via a tethering and rolling process; the adhesion is strengthened through leukocyte activation resulting in a stationary attachment (34).

Stage 3: Reduced VEGF & Elevated TGF-beta

In response to cytokine-induced hypoxia, upregulation of hypoxia inducible factor (HIF) genes occurs, which in turn promote the increase production of vascular endothelial growth factor (VEGF) (35) and transforming growth factor beta (TGF-beta (36). VEGF is known for vasodilation (37), angiogenesis, and neuroprotection (38). VEGF can be conspicuously high or low in patients with CIRS (23). With VEGF deficiency, there is loss of neuroprotection with noted increased permeability of blood brain barrier (39), as well as capillary hypoperfusion (23). Symptoms associated with low VEGF include shortness of breath, cognitive dysfunction (28), fatigue, and muscle cramps (40).

TGF-beta induces VEGF production (41), and promotes stiffening of soft, pliable epithelial cells (42). This latter process is known as epithelial mesenchymal transformation (EMT) (42). Such remodeling in the lung tissue can result in a restrictive airway pattern and fibrosis (43). Like VEGF (44), TGF-beta can increase blood brain barrier permeability (45).

Stage 4: Immune System Effects (Tregs, TGF-beta**, Antibodies, C4a)**

Persistent innate inflammation leads to abnormal T cell responses (46) and dysregulated complement cascade (5, 14, 47).

In a normal host, there is tight regulation over the production of mature effector helper T (Th) cells and regulatory T (Treg) cells (46). Each of these mature T cells have specific functions in the immune system: Th1 and Th17 are implicated in autoimmune diseases; Th2 are involved in allergies; Tregs modulate inflammatory responses through regulation of Th cells (48). Cytokines play a role in dictating maturation of naïve CD4+ T cells into the various functional subtypes: IL-12 and interferon gamma induce TH1 cells; IL-4 induces Th2 cells; IL-6, IL-23, and elevated TGF-beta induce Th17 cells; IL-2 and normal levels of TGF-beta induce Treg cells (46). Additionally, specific intracellular proteins known as "suppressors of cytokine signaling" (SOCS) 1 and SOCS 3 influence cytokine effects on both maturing effector helper T cells and Tregs (46).

The forkhead box P3 (FOXP3) gene is the "master of gene control" for Tregs (49). In SOCS1 deficient Tregs, there is a loss of FOXP3 expression, leading to production of pathogenic Tregs (46). Although CD4+CD25+ Treg cells normally suppress autoimmunity (49), once they become pathogenic T cells in the face of ongoing inflammation they can induce autoimmunity (46) and to create tissue inflammation (50). In CIRS patients, levels of circulating CD4+CD25+ Tregs were noted to decrease on Day 1 upon re-

exposure to a water damaged building (WDB) without spontaneous return to normalcy (51). This finding supports the notion that the innate inflammation incited by inhalation of particulates from the WDB exposure induced conversion of CD4+CD25+ Tregs into pathogenic Tregs, thereby decreasing measurable levels of the CD4+CD25+ Tregs (51).

Additionally, TGF-beta regulates pathways that initiate and maintain expression of FOXP3, thereby manipulating production of Treg cells (52).

CIRS patients can develop autoimmunity as evidenced by the following antibodies: anti-gliadin antibodies (gluten sensitivity), anti-cardiolipin antibodies (ACLA) (53), antineutrophil cytoplasmic antibodies (ANCA) (28), and more.

CIRS can also involve derangements in the complement cascade, indicated by elevated C4a levels (5, 14, 47). C4a elevations occur through activation of the classical and mannose-binding lectin pathways (10) and trigger an amplified release of downstream-signaling proteins, promoting a swift inflammatory response (1). Additionally, some patients may experience auto-activation of the C4a protease enzyme known as mannose-binding protein-associated serine *protease* (MASP2), giving rise to markedly elevated C4a levels (10). The MASP2 auto-activation results in a "sicker quicker" phenomenon upon re-exposure (28). Symptoms of elevated C4a include fatigue, musculoskeletal issues (54), capillary hypoperfusion, and cognitive impairment (31).

Stage 5: Low MSH and Endorphins

Chronic leptin resistance limits production of MSH. Low MSH levels lead to further immune system dysfunction, sleep issues, and gut malabsorption (28). The MSH driven conversion of T helper cells into CD4+CD25+ Treg cells, leading to suppression of hypersensitivity and autoimmune diseases (55), is diminished in MSH deficiency. With loss of leukocyte regulation over cytokine responses, patients may succumb to opportunistic infections (28) and have slower recovery from infections (56). MSH tightens junctions in the gut lining and has anti-inflammatory effects in the colon (57). As observed in CIRS (28), loss of intestinal epithelial tight junctions can lead to increased intestinal permeability (aka leaky gut), allowing foreign material such as toxins, bacteria, and food antigens into the body (58). Leaky gut has been linked to predisposition to autoimmunity (58), which is seen in some cases of CIRS.

Leptin resistance also causes low endorphin levels, resulting in loss of modulation of pain perception which leads to chronic pain and unusual pain (28).

Stage 6: Antibiotic Resistant Staph Bacteria

Multiple antibiotic resistant coagulase negative *Staph* (MARCoNS) bacteria colonize in mucosal membrane surfaces (59) with minimal difficulty due to the MSH deficient state in CIRS patients (28). MARCoNS evade host defenses through biofilm formation (59). Additionally, they can secrete exotoxins A and B (60) which split MSH molecules apart, causing further reduction in MSH levels. Coagulase negative *Staph* are known to secrete hemolysins which can increase inflammation in the host (61). MARCoNS can also alter genomic expression of host genes (14).

Stage 7: Pituitary & Hypothalamic Hormone Effects

As the chronic inflammation continues, further hormonal dysregulation ensues.

Released in response to stress, cortisol is typically tightly regulated by ACTH through the hypothalamic-pituitary-adrenal (HPA) axis and is a good reflection of neuroendocrine wellbeing (62). Unfortunately, 50% of CIRS patients with low MSH will experience loss of cortisol regulation (28). During the beginning stages of CIRS, simultaneous measures of ACTH and morning cortisol are often high with minimal symptoms (28). However, as CIRS progresses, ACTH and morning cortisol levels fall resulting in a marked increase in symptoms (28). Decreased ACTH production due to hypothalamic leptin resistance results in altered sleep regulation (63).

Androgen production is downregulated in 40-50% of CIRS patients (28). Additionally, in patients deficient in vasoactive intestinal peptide (VIP), estradiol levels may be elevated due to an overactive aromatase enzyme (28) which converts androgens (i.e. *dehydroepiandrosterone* (*DHEA*), androstenedione, testosterone) into estrogens (i.e. estrone, estradiol) (64).

Antidiuretic hormone (ADH) is secreted by the posterior pituitary and is involved in regulation of sodium and water balance (65). The hypothalamus responds to the concentration of solute (i.e. serum osmolality) in the blood through osmoreceptors (65). If the serum osmolality is too high, the hypothalamus signals to the pituitary to secrete ADH into the bloodstream (65). ADH subsequently induces the kidneys to reabsorb free water, thus diluting the blood (65). Approximately 60% of CIRS patients will have dysregulation of ADH/serum osmolality levels (28). Resultant symptoms include static electrical shocks, migraine-like headaches, excessive thirst with frequent urination, and dehydration (28).

VIP is a neuroregulatory peptide hormone produced in the hypothalamus that is often diminished in CIRS (14). VIP deficiency can lead to shortness of breath with exercise (28), and pulmonary hypertension (66) that is reversible with exogenous administration of VIP (67). Lastly, VIP can downregulate cytokines (56), making it invaluable to the CIRS treatment protocol for refractory patients.

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