

Inflammation Induced Chronic Fatiguing Illnesses: A steady march towards understanding mechanisms and identifying new biomarkers and therapies.

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Abstract

Illnesses characterized by chronic fatigue are often defined by symptoms and not by objective biomarkers that support both diagnosis and treatment. Without readily obtained biomarkers, clinical management can be compromised by lack of certainty. This uncertainty creates a wide spectrum of possible therapies that in many cases is reduced to trial and error medicine, resulting in patient frustration and resource exhaustion, with little improvement in health status. Modern medicine must leverage modern science to bring common research tools into the clinic for patient diagnostics. Using biomarkers previously confirmed as useful in diagnosis and treatment of chronic inflammatory response syndrome (CIRS), including transcriptomics, the authors present evidence of benefit in assessment of a “symptoms-only illness.” These immune biomarkers, such as transforming growth factor beta (TGFβ), vasoactive intestinal peptide (VIP), melanocyte stimulating hormone (MSH), split products of complement activation, and many others discussed here, are now available for use as clinical diagnostics, but rarely ordered in cases of chronic illness. In cases of cognitive decline, new technology for brain MRI analysis, NeuroQuant, can pick up small changes in brain structures that are frequently missed by radiologists, but consistently shown in CIRS. By focusing on persistent symptoms seen in antibiotic-treated Lyme disease (Post-Lyme Syndrome, PLS), CIRS-biomarkers have utility to define both an initial infectious process and a subsequent inflammatory illness. Genomic testing can determine predisposition to chronic stages of Lyme after acute illness and use of Next Generation Sequencing now brings transcriptomics to the Lyme community, to assess remaining abnormalities at any given treatment stage of PLS. Application of these new, objective testing offerings will reveal the molecular pathophysiology of illness, avoiding over-reliance on symptoms and antibody testing alone. This will help providers direct highly targeted therapies on an individual basis, in this era of personalized medicine.

Key words: CIRS, inflammation, Lyme disease, fatigue, transcriptomics, biomarkers

Section 1

Background:

Fatigue is a normal part of life, but it can also be a symptom of disease, including serious illnesses. It is a common complaint in primary care, exceeded only by complaints of cough. Five to seven per cent of patients seeking primary care have a primary complaint of fatigue, with this proportion being remarkably consistent across Western countries (1, 2).

General practitioners perform investigations in only half of patients (1, 3, 4) complaining of fatigue, and few of these tests yield abnormal results. Even so, the high incidence of the fatigue complaints means that laboratory tests for fatigue account for almost 5% of the total number of laboratory tests ordered by general practitioners (1, 3, 4).

A diagnosis is made in less than half of patients with fatigue; furthermore, many of the diagnoses are descriptive, such as stress, or are one of the many synonyms for fatigue itself. Patients understand that an underlying identifiable disease may not be present, though patients' and doctors' beliefs are sometimes mismatched, with a higher proportion of doctors than patients considering the particular problem to be psychological (4).

Section 2

The Evaluation of Fatigue: Challenges of Differential Diagnosis

The differential diagnosis of persistent fatigue encompasses all medical disciplines. For example, anemia, hypothyroidism, Addison's disease, chronic liver disease, neuromuscular diseases, sleep disorders, and depression can all present as idiopathic fatigue (5). Thus, Fukuda *et al.* (6) called for a consensus on a clinical definition of persistent fatigue, as well as

inclusive and exclusive criteria for diagnosis.

Exclusion criteria for persistent fatigue illness includes:

- Untreated hypothyroidism
- Respiratory/food allergies
- Sleep apnea
- Narcolepsy
- Drug abuse
- Adverse effects of medications
- Severe obesity

Previously diagnosed medical conditions with uncertain resolutions (such as Hepatitis B or C). Fatigue illness, or persistent fatigue, is associated with a multisystem, multi-symptom illness, demonstrating a correlation with various infectious diseases, immune disturbances, hypothalamic-pituitary axis (HPA) or autonomic dysfunction, and psychiatric disorders. It is prevalent in 0.2% to 1% of the population worldwide, occurring in women 3-7 times more than in men, rarely diagnosed in children less than 10 years of age, and amongst all races and socioeconomic strata (7, 8). It is characterized as debilitating fatigue with impaired concentration, short-term memory and sleep, as well as widespread musculoskeletal pain (9, 10).

Persistent fatigue lacks clearly defined etiological mechanisms, mainly because of the dualistic model of medical illness, which assumes the mind and body are separate entities (5); however, it typically develops acutely following the onset of a respiratory or gastrointestinal infection (8). The prevailing etiologic hypothesis regards the disorder as a multifactorial condition, whereby genetic and environmental factors, including infections, interrelate to impede the ability to control and manage chronic stress, pain, and fatigue (7).

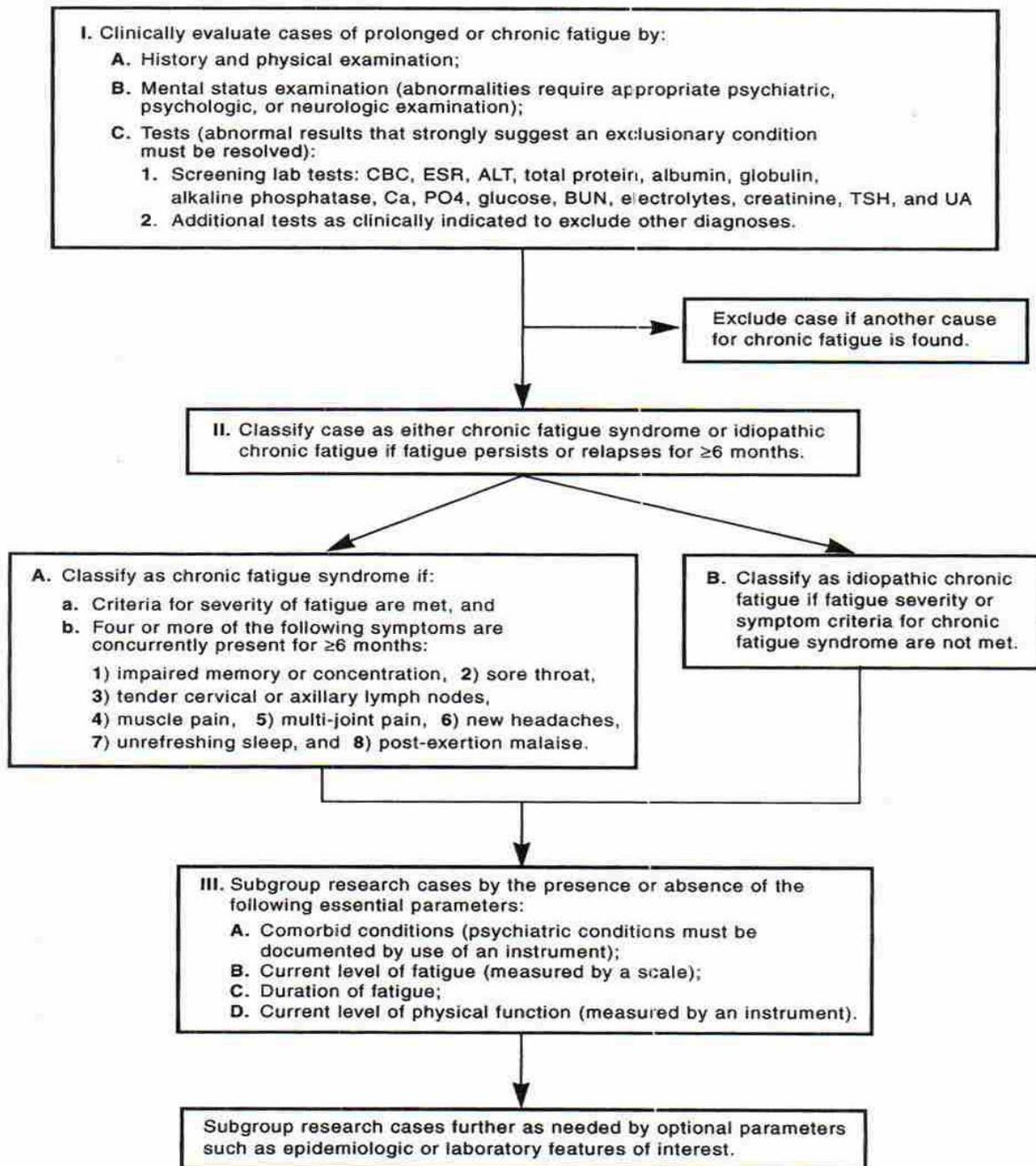


Figure 1 Evaluation and Classification of Unexplained Chronic Fatigue (Fukuda et al., 1994)

Other pathogenic mechanisms have been implicated. For example, a number of viruses including enteroviruses, particularly Coxsackie B., herpes virus infections, influenza, retroviruses, Epstein-Barr virus (EBV), and human T-cell lymphotropic

virus-2 (5, 7, 8). Other infections include chronic Lyme disease, as well as other pathogens such as *Brucella abortus*, *Campylobacter jejuni*, *Mycoplasma pneumoniae*, and *Toxoplasma gondii* (5).

Jones & Jenson (8) note the similarities between persistent fatigue symptoms and those experienced by patients suffering from autoimmune disease and other inflammatory disorders, underscoring a potential primary mechanism within the immune system for the pathogenesis of chronic fatigue. Further, they report on additional immunologic alterations including hypo- and hyper-gammaglobulinemia, immunoglobulin subclass deficiencies; together with dysfunctions of both NK cells and monocytes. Other biological bases of illness hypotheses include energy metabolism alterations related to exercise and post-exertion malaise, stress, sleep responses and lack of regulation in HPA axis.

Section 3

Fatigue: From Symptoms to Biomarkers

After completing a medical history and performing a careful physical exam, physicians quickly look to see what could cause the fatigue by searching for diagnostic biomarkers, often found in standard blood tests as well as an EKG or chest x-ray, for example.

Increasingly, we are finding these standard diagnostic tests are normal in ever-enlarging groups of patients with chronic fatiguing illnesses. Adding to the medical problem is the concern that if “all tests are normal,” is there really an illness after all? This current paradigm leaves attending physicians to show what a patient has in part by showing what he or she has; and does not have.

Far too often, as the march of scientific advance has shown, “all tests normal,” simply means, “all the tests that we knew about were normal.” There are 20,000 protein coding genes and another 30,000 non-protein coding genes that underlie our physiology. Now that we know so much more about regulation of differential gene

expression (transcriptomics) than even two years ago, the support for claims that “all tests are normal” disappears.

We often learn more about fatigue by looking at concomitant symptoms and conditions of the fatigued patient. Fatigue from a malignancy may have different associated features from those seen in fibromyalgia or Chronic Fatigue Syndrome/Myalgic Encephalitis (CFS/ME) (9, 10), but fatigue alone does not define any of these illnesses. Cancer might be shown to be present by biopsy, but what biomarkers do we see that define CFS/ME objectively? There are none.

Here is the problem: symptoms-based diagnoses lack (i) specificity in differential diagnosis and (ii) definition of relevant pathophysiology. With no physiologic mechanisms available to measure responses to treatment, attempts to correct illness more likely than not (a) lead to anecdotal improvement in symptoms, if any; (b) provide no mechanism that supports early detection, effective intervention and prevention; (c) demonstrate no mechanisms to identify what therapeutic targets have been reached; (d) inform us what abnormalities remain uncorrected.

Fatigue has features that help us stratify illnesses diagnosed by symptoms in the absence of biomarkers. Loss of restorative, restful sleep is a dominant feature of inflammatory illnesses, which can also disrupt normal circadian rhythms affecting sleep, especially with the reduction from normal levels of regulatory neuropeptides (see MSH and VIP sections). Fatigue that is worsened after trying to do more than normal, when “normal” days are dominated by lifestyle-altering fatigue, has been given several jargon names. “Post-exertional malaise,” or “delayed recovery from normal activity,” and “push-crash,” come to mind. This symptom usually implies defective production of energy

molecules (ATP) from mitochondrial breakdown of glucose. As we have recently learned (see transcriptomics, below), the mitochondrial problem can possibly be due to decreased capillary blood delivery of oxygen, or abnormal control of mitochondrial genes encoded in our *nuclear* DNA as shown by RNA Seq. Others feel that metabolic abnormalities in mitochondrial function are key (11).

Of note, Ryan (12) has shown that transcriptomics can also show marked abnormalities in ribosomal gene expression using RNA Seq, as a separate but dominant feature of chronic fatiguing illnesses. This exciting, but previously unreported finding, casts a new bright light on chronic fatigue.

Capillary hypoperfusion, abnormalities in activity of nuclear encoded mitochondrial genes or ribosomal gene abnormalities are readily used as biomarkers. Having these validated biomarkers creates the potential for enhanced precision in subsequent patient evaluations. For example, if one is looking for possible mitochondrial dysfunction, knowing that there are abnormalities in nuclear encoded mitochondrial genes provides the capability to stratify new conditions by the pre-existing gene abnormalities.

Section 4

A New Model of Inflammation: CIRS

When fatigue is associated with a multisystem, multi-symptom illness, the differential diagnosis narrows, with inflammatory illnesses leading the way. Among the possible diagnoses often given weight are Lyme disease and a chronic inflammatory response due to exposure to environmental sources of biotoxins.

Chronic Inflammatory Response Syndrome (CIRS) is an acute and chronic, systemic inflammatory response syndrome, most frequently acquired following exposure

to the interior environment of a water-damaged building with resident toxigenic organisms, including, but not limited to fungi, bacteria, actinomycetes and mycobacteria, as well as inflammagens such as endotoxins, beta glucans, hemolysins, proteinases, mannans and possibly spirocyclic drimanes; as well as volatile organic compounds (VOCs) (13). Other sources of CIRS could be consumption of reef fish containing neurotoxins produced by marine algae (14) or exposure to cyanobacteria in fresh water ponds lagoons and lakes (15).

We note the absence of confirmed biomarkers for other symptom-only syndromes: Post Traumatic Stress Disorder (PTSD), fibromyalgia, CFS/ME, depression and autism spectrum disorder, for example. What biomarkers create therapeutic targets for new interventions? Many proteomic tests have been used for more than 20 years without confirmation of benefit as biomarkers.

Section 5

The New World of Objective Biomarkers

Measuring levels of selected proteins is critical to understanding physiology, and is typical for most medical exams and essential for diagnosis and monitoring CIRS. However, it becomes difficult to measure multiple proteins simultaneously, limiting deployment of proteomic markers in a clinical setting. We now are expanding upon use of proteomics to include new biomarkers found through CIRS research on gene expression. Symptom-only diagnoses are being investigated for unique abnormalities in differential gene activation. We can no longer say all tests are normal. Even better than just aiding diagnosis, transcriptomics can identify targets of interventions, which in turn have brought new therapies to publication (12).

Additionally, with abnormalities in executive cognitive functions found in over 90% of CIRS illnesses (Table 1), what biomarker documents these abnormalities

and confirms an organic cause for cognitive deficits?

Table 1 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 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

While still in its infancy, use of volumetric central nervous system software programs (16, 17) shows remarkable detail of the brain structures that led to the ability to detect and treat atrophy of multiple grey matter nuclei (18). Said another way, if short term memory is deteriorating or if individuals can't remember what they just read or where they left their car, objective abnormalities in brain volumes can be demonstrated to define an underlying illness. These findings have effectively ended the idea that "it is all in your head." The illness can now be detected with brain testing.

As we will present, sources of CIRS are diverse and are readily identified by examination of exposures, symptom clusters and abnormalities in innate immune responses. Now that sophisticated testing modalities, such as innate immune proteomics; NeuroQuant (FDA-cleared since 2006); and transcriptomics, which accurately demonstrates the enormous complexity of differential gene activation; are available, we can reliably leave behind diagnosis by symptom recording alone.

For example, use of transcriptomics can reliably demonstrate presence of gene expression patterns in patients that match those known to occur following deoxynivalenol (DON) exposure in cell culture and animal models. If a patient is sickened by exposure to a water-damaged building (WDB), we want to show actual abnormal gene activation referenced by published pathways involving trichothecenes, especially by MAP kinases (19), and not simply show possible exposure by finding those mycotoxins in body fluids. We know for example, that ingestion of a wide variety of foods will cause urinary excretion of mycotoxins, with such findings found universally and not just in a few sickened patients (20), and thus cannot be equated with causation of illness (13). The converse also applies, trichothecene exposure is not

likely to be extant in the absence of up-regulation of MAP kinases. As with all biomarkers, reliance on published, solidly peer-reviewed literature must form the basis for validation.

Without use of biomarkers, the possibility of mis-diagnosis in symptom-only illness is quite large; frequency of mis-diagnosis of sub-divisions of the same illness can become overwhelming. For CIRS-WDB, we already have a case definition that demands use of published, objective biomarkers and effective therapies:

2008 US Government Accountability Office case definition:

- (1) Potential for exposure**
- (2) Symptoms similar to those seen in published literature**
- (3) Labs similar to those seen in published literature**
- (4) Response to therapy**

Contrast CIRS-WDB to symptoms-only diagnoses. CIRS-WDB has an extensive peer-reviewed literature on symptoms, ancillary testing, proteomics, CNS volumetric studies, transcriptomics and the above clinical case definition published by a Federal agency. Moreover, CIRS has survived multiple legal challenges to admissibility in state and Federal courts. CIRS diagnostic and treatment protocols are peer-reviewed and published, with treatment effectively correcting proteomics (21), genomics (12, 22) and grey matter nuclear atrophy (18). Understanding that there are no studies showing dramatic or effective treatment of symptom-only diagnosed illness, like fibromyalgia, Post Traumatic Stress Disorder, (PTSD) or CFS/ME, and that CIRS is readily shown to have effective treatment protocols, we can ask if there are similarities. Take look at the symptom rosters of known CIRS illnesses (Table 1). Now look at symptom rosters for CFS/ME and fibromyalgia (Table 2).

Table 2. Symptoms of CFS and ME (10)

Symptom	Fukuda CFS	ME
Persistent chronic fatigue	Yes	Yes
Six or more months	Yes	Yes
Post-exertional neuro-immune exhaustion	No	Yes
Cognitive	Yes	Yes
Sore throat	Yes	Yes
Lymph nodes	Yes	Yes
Muscle pain	Yes	Yes
Multi-joint pain	Yes	Yes
Abdominal pain	No	Yes
Headaches	Yes	Yes
Unrefreshing sleep	Yes	Yes
Post exertion malaise	Yes	Yes
Chemical sensitivity	No	Yes
Orthostatic intolerance	No	Yes
Air hunger	No	Yes
Impaired thermal regulation	No	Yes

Let's now ask if exposure to the interior environment of WDB occurs in fibromyalgia patients or CFS/ME patients. According to the National Institute for Occupational Safety and Health (NIOSH) (23), such exposure happens in about 50% of US buildings. Does fibromyalgia or CFS/ME or PTSD protect patients from adverse environmental exposures or personal lifestyle effects? No. What biomarkers for a fibromyalgia patient newly found to have CIRS separates him/her from a classic CIRS-WDB patient?

If a CFS/ME patient is exposed to a WDB, and his illness is made worse, do we still call the illness CFS/ME? Even though the recommended CIRS labs are covered by all major insurance carriers, they are rarely requested in CFS/ME or fibromyalgia cases in primary care practices (unpublished; data from practices of Drs. Shoemaker and Heyman). When a CFS/ME patient finds a

provider who performs a CIRS battery of labs, with confirmation of CIRS, there is no objective baseline for the original diagnosis: there no longer is any way to determine if the original illness was in fact CIRS and not CFS/ME or fibromyalgia.

Laboratory studies are the required foundation for CIRS. These labs reflect the physiology of the illness. Here, however, is an important point: They are *rarely specific* for any one source or precipitating cause of CIRS – it can certainly be from a WDB, but also Lyme disease, ciguatera, Pfiesteria, VOCs and others. Therefore, it is important for the clinician to identify the cause of CIRS, as well as confirming the diagnosis of CIRS itself.

Controlling for known variables-1

We know there are risk factors for CIRS beyond simple exposure. First, we can demonstrate individual susceptibility, a

concept based on the epidemiologic construct of relative risk (RR). If the incidence of an abnormality in the case population is twice the incidence in the control population, there is increased relative risk and individual susceptibility is postulated, even when we don't necessarily know the precise cause of the increased relative risk.

One of the first questions in the early days of clinical management of biotoxin illnesses was, "What was going on such that three out of ten people got sick swimming in an area of the Pocomoke River (Maryland) where fish-killing dinoflagellates were found in 1997 (24, 25)?" Was the confounder race, gender, age; use of alcohol, cigarettes, or other medications? Duration of exposure? Route of exposure? Underlying illness?" No, to all these reasonable ideas. The answer simply was immune response genes stratified by HLA DR.

Section 6

HLA DR by PCR: genetic susceptibility in CIRS

Found on chromosome 6 in the Human Leukocyte Antigen complex, HLA DR has been widely used for years in tissue-typing patients in preparation for organ transplants but it also underlies the mechanism by which antigen presenting cells identify antigens as "foreign." When foreign antigens are presented to lymphocytes by HLA DR, the complex process that leads to antibody production begins. If the antigen presentation process is defective (26), as we see in PLS, there will

be no production of protective antibodies. Without antibody production, there is nothing to stop the ever-expanding inflammatory cascades responding to the ongoing carriage of antigen. The normally protective innate immune response becomes destructive, as described in a classic short essay by Lewis Thomas in 1972, "the host becomes the disease." (27)

This permissive of HLA DR becomes vitally important when a physician is searching to make a diagnosis by demonstrating presence of an antibody. If defective antigen presentation is present, as may be the case in acute Lyme disease, laboratory confirmation of presence of an antibody is problematic.

For 95% of patients with known CIRS-WDB, increased relative risk (> 1.9) for acquisition of illness is associated with just 6 of 54 major HLA haplotypes (unpublished practice data). These 6 haplotypes are found in roughly 24% of the population at large (28). Similarly, only four HLA haplotypes are associated with roughly 95% of patients with chronic symptoms following antibiotic treatment for Lyme disease, or Post Lyme Syndrome (PLS). These four haplotypes were found in 22% of all our patients initially infected by Lyme.

HLA DR haplotypes consist of HLA alleles that will be passed on to offspring. Use of HLA typing becomes important for epidemiologic risk assessment but it also is important in considering who else in a family might be predisposed to heightened inflammatory responses following exposure to biotoxins and inflammagens.

CIRS HLA Sequences

Table 3. HLA DR haplotypes associated with elevated relative risk for the CIRS categories shown

	DRB1	DQ	DRB3	DRB4	DRB5
Multisusceptible	4	3		53	
	11/12	3	52B		
	14	5	52B		
Mold	7	2/3		53	
	13	6	52A, B, C		
	17	2	52A		
	*18	4	52A		
Borrelia, post Lyme Syndrome	15	6			51
	16	5			51
Dinoflagellates	4	7/8		53	
Multi Antibiotic Resistant Staph epidemidis (MARCoNS)	11	7	52B		
Low MSH	1	5			
No recognized significance	8	3,4,6			
Low-risk Mold	7	9		53	
	12	7	52B		
	9	3/9		53	

Controlling for known variables-2

The next concept involves hormonal abnormalities that affect metabolic changes. Our focus is not on insulin, thyroxine or cortisol, but instead we look at regulatory neuropeptide hormones, especially vasoactive intestinal polypeptide (VIP) and alpha melanocyte stimulating hormone (MSH). Direct measurement of VIP is possible but is misleading, as the crucial problem with VIP physiology is variable production of one of its two receptors (29). MSH testing is readily available in commercial labs, but replacement therapy is not available for use in humans. VIP is available as a therapeutic agent; exogenous administration has shown great benefit in

CIRS patients and in those with grey matter nuclear atrophy.

Regulatory neuropeptide hormones affect (i) hypothalamic hormone function; (ii) pituitary hormone production; (iii) peripheral hormone regulation by pituitary hormones; (iv) immune cell and innate immune functions; (v) cytokine physiology, (vi) limbic system activity; (vii) genomic activity; (viii) pulmonary artery pressure; among other functions (21).

Section 7 MSH

MSH deficiency is important in CIRS. MSH is made in the hypothalamus and to a lesser extent in part of the pituitary. It is a regulatory neuropeptide. What this

means is that it is a protein that regulates inflammation and immunity; regulates other hormone functions, especially pituitary hormones and peripheral hormones; it has important regulatory features in the limbic system, circadian rhythms, pain perception and weight. MSH “patrols” the periphery of the skin, respiratory system, gastrointestinal tract and blood. In the gut, MSH is invested in just about every cell, including tight junctions between them. Deficiency of MSH will result in what others call leaky gut. MSH deficiency was one of the first elements found while looking for features important in the pathophysiology of CIRS (29).

The role of MSH in prevention of hormonal abnormalities is best seen in low MSH cases. Here we find lack of normal regulation in adrenocorticotropin hormone (ACTH) and cortisol in approximately 67% of patients. We find lack of regulation of antidiuretic hormone (ADH) and osmolality in 80% of patients. Androgen abnormalities, particularly including upregulation of aromatase, are found in 50% of CIRS cases. Understanding the impact of hormone dysregulation requires looking at feedback loops involving central and peripheral hormones.

Another correlation of MSH deficiency in CIRS is the presence of biofilm-forming, multiply resistant coagulase negative staphylococci in deep aerobic nasopharyngeal cultures, essentially found exclusively in those with low MSH.

Controlling for known variables-3

Innate immune inflammatory elements in CIRS act in an ever-expanding web of receptor-based responses. Th-1, Th-2 and Th-17 responses; together with coagulation and complement activation; all participate in amplified inflammatory responses to persistence of carriage of antigens.

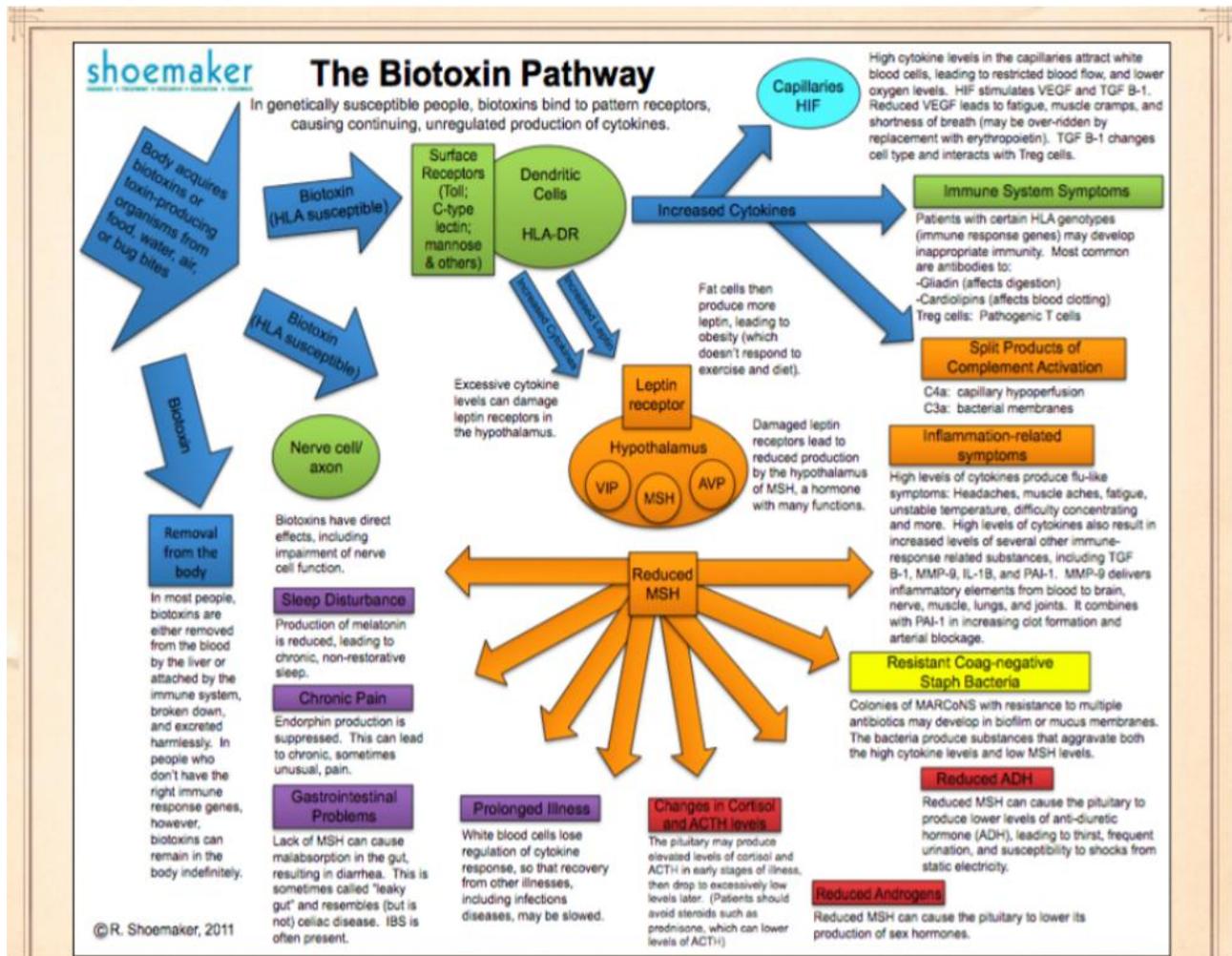
Section 8 MMP9, C4a, TGF beta-1

We use three separate labs in our approach to detection of innate immune inflammation. Remember that CIRS has its basis in systemic inflammatory response syndromes. In SIRS, acute patients will have activation of pro- and anti-inflammatory cytokines, Th-17 immunity, complement, clotting abnormalities and more. All of these entities are important in CIRS.

We use matrix metalloproteinase-9 (MMP-9), transforming growth factor beta (TGF beta-1) and split product of complement 4 (C4a) as the main diagnostic and prognostic variables to assess for inflammation seen in CIRS. The complement system can be activated to the point that some people with elevated C4a are suffering from auto-activation of MASP2, the enzyme that cleaves C4a (30). Removal from exposure doesn't stop production of C4a. This so called “sicker, quicker” process is recognizable with persistent elevation and “elevated elevation” of C4a.

Delivery of oxygen in capillary beds is reduced in CIRS. This reduced delivery sets off alarm signals through the activity of a nuclear transcription factor, hypoxia inducible factor (HIF). Low oxygen in tissue means HIF will be turned on. HIF being turned on means vascular endothelial growth factor (VEGF) will be released. VEGF is intimately linked to TGF beta-1, which in turn is linked to countless genomic pathways leading to fibrosis, differential gene activation, the “leakiness” of blood brain barrier and a host of effects on beneficial T-regulatory cells.

The schematic entitled “The Biotoxin Pathway” (Figure 2) released in 2011, illustrates how many of these general principles are tied together. Our next release will include differential gene activation.



Controlling for known variables-4

Immunomodulatory responses can be affected by microbes resident in areas of the body distant from blood. It seems hard to imagine just what is going on in the gut, but given the onslaught of discussion on the intestinal microbiota and its effect on immune reactivity and brain activity, we can expect to know more soon.

Section 9 MARCoNS

No discussion of biomarkers in MSH deficient patients can possibly be complete without emphasizing the importance of multiply antibiotic resistant coagulase negative staphylococci (MARCoNS). Following the initial recognition of the

importance of MARCoNS in chronic fatigue and chronic pain (31) published by the group at Newcastle University (Australia) led by Henry Butt and Tim Roberts, special cultures that permitted these slow growing commensals to be identified (API-STAPH) became available to primary care providers in the US. Not only has subsequent research confirmed the Newcastle observations but we now think that extracellular compounds made by previously "benign" organisms are driving gene responses. Moreover, preliminary mass spectrometry data suggests that biofilm-forming MARCoNS make a polycyclic ether compound similar to palytoxin, a dinoflagellate toxin (data not published).

One point must be made: without eradication of MARCoNS, there will be no clinical improvement. For those who consider worsening of clinical status with use of antibiotics as suggestive of an occult infection, often reported by some as indicative of Lyme, do not forget that antibiotic treatment of MARCoNS also causes a similar syndrome. If no culture is done, an unsupported diagnosis might be made.

Controlling for known variables-5 Pertinent Negatives

As part of evaluation of objective parameters to define CIRS, we also look for objective parameters that are *not* abnormal. These would include commonly used tests as a CBC, CMP, immunoglobulins, thyroid studies, antinuclear antibodies (ANA) and many more. The differential diagnosis is activated by showing what is present in CIRS and what is not present in CIRS. Interestingly, a commonly used test of inflammation, the sedimentation rate, is invariably normal in CIRS. C-reactive protein, an acute phase reactant that activates interleukin-6 (IL-6), also is usually normal in CIRS.

Section 10 VCS

Visual contrast sensitivity (VCS) testing has been used clinically for years and remains the most accurate test for functional vision (25). Contrast is one of the seven main functions of the optic nerve that 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 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 step of what is now a 12-step protocol, reversed the visual contrast abnormalities. With re-exposure, however, visual contrast deficits reappeared, identical to the initial deficits, usually within 36 hours.

Section 11 Cluster analysis

When we use persistent health symptoms as a group of 37, recorded by a trained health care provider in a medical history (never use patient-completed checklists), we can collate individual symptoms into groups, called clusters. Statistically, these clusters of symptoms, 13 in number, comprising 35 symptoms, yield a diagnostic capability to separate out CIRS from essentially all others syndromes and diseases. If a patient is confirmed to have 8 or more clusters of symptoms, the likelihood of presence 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%).

Cluster Analysis of Symptoms

Individual categories:

1. Fatigue
2. Weakness, assimilation, aching, headache, light sensitivity
3. Memory, word finding
4. Concentration
5. Joint, AM 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

A positive cluster analysis for biotoxin illness is presence of 8 or more of 13 clusters.

Note: symptoms of unusual pain and tremors did not sort into individual categories

Even though the combination of symptom clusters with VCS is accurate for diagnosis, we use laboratory studies as an additional layer of assessment for diagnostic certainty and to provide a method for following the effects of treatment on clinical status. These tests of immune function help us hone in on (i) where the inflammation of the illness is active; (ii) what inflammation is doing; and (iii) how inflammation is doing. We use labs to follow patients, monitor their progress, document achievement of endpoints and to document relapse.

If we had the ability to use a few proteomic tests to tell us all we need to know about treatment, we wouldn't need transcriptomics. Since transcriptomics tells us so much more than proteomics, we employ both approaches to identify complex clinical syndromes.

Section 12 Transcriptomics

The most sophisticated genomic testing available today is transcriptomics. We look at differential gene activation using a Next Generation DNA Sequencer. 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 (don't forget demethylation and deacetylation!) can shut off and turn on gene expression. 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, from ciguatera and from PLS. The application of genomics to human illness is just in its infancy but it has catapulted us into the age of personalized medicine.

We will return to a discussion of transcriptomics as an important biomarker for diagnosis and treatment of Lyme disease below.

Section 13

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 14

VO₂ 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 use 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 our earlier discussion of CFS/ME, we discussed the absence of a biomarker for CFS/ME. In 2015, the Institute of Medicine (IOM) emphasized the importance of cardiopulmonary exercise testing (CPET) in their redefinition of chronic fatigue syndrome as Systemic Exercise Intolerance Disorder (SEID) (9). 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 VO₂ 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 VO₂ max of women and men. We also know that age has a role in normal ranges for VO₂ 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 VO₂ 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 VO₂ max as one of the key elements in assessing disability.

Functionally, even more important than VO₂ 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. Don't 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 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.

Section 15

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: 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 (DDAVP) that costs about a nickel. If the differential diagnosis didn't 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 16

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 17

Summary of biomarkers in CIRS

As opposed to chronic fatiguing illnesses that have no biomarkers, CIRS has (i) exposures; (ii) cluster analysis of symptoms; (iii) differential diagnosis; (iv) VCS testing; (v) proteomics; (vi) genomics; (vii) pulmonary hypertension; (viii) low VO2 max; (ix) depressed AT; and (x) specific objective findings showing specific brain injury.

Now that we know of the remarkable abnormalities of (xi) ribosomal and (xii) nuclear encoded mitochondrial gene expression, we have access to sophisticated markers that are abnormal at baseline, with therapy bringing interval improvement. Two tubes of blood and a few weeks will bring

the remarkable research discoveries to direct patient care.

We no longer need to guess about executive cognitive dysfunction: we follow NQ sequentially with treatment. The diagnosis is no longer complicated or drawn out: just take the history and do the testing.

Now that we can combine transcriptomics with proteomics, precision in diagnosis and treatment has never been higher.

Biomarkers lead the way to defined and published treatment protocols. If one wanted to decide to look for the evidence showing CIRS is present in a fatigued patient, no longer would speculation and clinical opinion be the gold standard for diagnosis and treatment. The era of symptom-only diagnoses applied to that patient would become past history.

Section 18

Applying the CIRS Model to Lyme disease

Few illnesses in American medicine over the last 30 years have created such bickering (and worse) among health professionals and patients alike as Lyme disease. Where else do we see patient advocates intensely hurling insults at physicians who say treatment of Lyme is easy? Or those same docs viciously attacking the “Lyme literate” docs who say the infection is difficult to clear and is often confounded by coinfections. Both sides of the Lyme issue will seemingly be quick to argue about any aspect of this increasingly common vector-associated illness. From the time needed to transmit a *Borrelia* spirochete from tick salivary glands to treatment of grey matter nuclear atrophy seen on NeuroQuant, there is just too little agreement.

In years gone by (Lyme was first named in 1975), no one argued that about 20% of patients confirmed to have Lyme

would go on to develop a symptoms-only illness called Post Lyme Syndrome (PLS). Those who advocated use of short courses of antibiotics as the only needed treatment started defining the PLS as myofascial pain and fibrositis. Mainstream medicine did not really accept these terms. By 1990, however, the American College of Rheumatology codified (33) a new entity that provided a ready explanation for PLS: fibromyalgia. With major manufacturers of a non-curative prescription medication making billions of dollars (Lyrica generated sales of \$5.1 billion in sales in 2016 alone) on treatment of fibromyalgia, now a diagnosis accepted by insurers for reimbursement, fibromyalgia fit nicely into modern American medical practice. Patients suffering from a vague diagnosis that lacked any objective biomarkers learned that they had to live with chronic pain, pay their therapists and try to

find some quality of life. And fibromyalgia wasn't just from Lyme. Auto accidents, especially whiplash, created a new breed of personal injury attorneys, collecting damages for the feared illness, initiated and caused by the 40-mile per hour rear end collision. Unfortunately, physical abuse too could result in classic fibromyalgia.

The symptoms seen in fibro are familiar to all CIRS practitioners: pain, fatigue, mood swings, weight problems, sleep disturbance and even respiratory illness. These symptoms are consistent with what is recorded in cases of MSH deficiency (Table 1).

The problem with PLS (and fibro too) remains absence of reliable biomarkers. In CIRS-WDB, those objective parameters can be sorted by stage of treatment (before, after, with relapse, with re-treatment and off meds; all compared to controls (Table 3).

Table 3. CIRS biomarker test values for normal controls, untreated, treated and relapsed patients. Other column includes chronic ciguatera and cyanobacteria patients. All data from private practice of RS

	Control	Untreated	Treated	Relapse	Other/treated
N=	13	101	58	64	11
C4a	3886	6449	4710	8688	5846
MMP-9	327	279	270	339	329
VEGF	60	73	46	59	52
vWF	0.18	0.45	0.16	0.45	0.43
TGF	3621	8149	5369	7268	7452
CD4+CD25	4.66	2.99	4.06	3.05	3.86
CD4+CD25++CD127	4.25	2.68	3.72	2.84	3.33

In Table 4, the data show what happens when a proven Lyme patient with symptoms refractory to antibiotics is seen and treated by a CIRS doc. The persistent innate immune activation created by the known infectious disease is not successfully treated by antibiotics. CIRS treatments work just as well in CIRS-PLS as they do in CIRS-WDB. With both sides of the Lyme argument, (“Antibiotics forever versus

antibiotics for never”) not even willing to talk to each other civilly, we submit that the time has arrived to change the argument.

What remains remarkable is the paucity of published papers on Lyme and what lab abnormalities and symptom complexes are associated with (i) acute Lyme before antibiotics; (ii) Lyme after antibiotics; and (iii) persistently symptomatic Lyme.

Table 4. CIRS biomarker test values for different stages of Lyme disease. All data from private practice of RS.

	Control	Base	Post Abx	Post CIRS
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

Even though we don't have any large cohorts reported from the antibiotic arm of Lyme stratified by lab test results, we have even fewer clinical trials from the "antibiotics for never" group. Not surprisingly, MMP9 is listed by PubMed (accessed 5/10/2017) in three Lyme papers; TGF beta-1 in another 3; MSH none; NeuroQuant none.

Compare what we know about diagnosis, treatment and re-exposure in CIRS-WDB (Table 3) to sequential steps of intervention in Lyme (Table 4). Despite the thousands of Lyme papers available on PubMed (and many more not indexed on PubMed), what accounts for such an unexpected absence of delineation of the effects of treatment on inflammatory markers in Lyme patients? We see individual studies in animals; we see selected cases presenting before treatment, with the study authors discussing a new finding, call it "XYZ," but rarely do we see the results of treatment upon that finding. There is little clinical data in the Lyme literature that shows the benefit of any treatment using objective parameters. Without objective before and after clinical findings in Post Treatment Lyme, there is little the careful physician can rely on to show benefit (or not) of antibiotic therapy. Changes in patient-reported symptoms aren't biomarkers.

Symptom-only based studies touting benefit, or paradoxically no benefit, of long

term antibiotic therapy are not persuasive. Similarly, long term antibiotic use has its own troubling side effects, especially with antibiotic resistance and horizontal gene transfer taken into account.

How does a careful patient know what to do about an illness that might be Lyme? Do a test! We want a blood test or some other indicator that will be positive when *Borrelia* is living inside the human host that would then turn negative when the organism is gone. Labs have looked for such a marker for 40 years without satisfactory findings. Show us the physiology.

Further, is there an inflammatory marker that rises with exposure to *Borrelia*, creates genomic activation that in turn sickens the patient with illness persisting beyond death of the bacteria? Clearly, that is the case as noted in Table 4 above. If an inflammatory marker appears and is not responsive to antibiotics, as Table 4 also shows, what basis does the careful patient have to say treat with more antibiotics? If we had a biomarker that would help us decide what to do, that would be ideal. As we sit here now that biomarker has not been identified in published research.

A word on Lyme testing is in order. Currently, the only tests licensed by the US FDA and Health Canada Medical Devices Branch are two serologic assays: the two-tiered ELISA; and IgM and IgG Western blot. Other tests, like PCR, seemingly useful, still aren't licensed (an excellent

review and meta-analysis is found in (34). A large number of assays, mostly from CLIA-approved labs, are available commercially (see Tables and figures in (34)). Do any of these tests show a biomarker that changes with inflammatory responses? No.

Do any of the serologic tests available make much difference in treatment of putative Lyme patients either by (i) reducing incidence of symptomatic Lyme or (ii) reducing costs of diagnosis? The answers are the same but might be considered surprising. No (35). In Europe, attempts to use the two-tiered system employed by Canada and the US did not fare well. Specificity was 95% but sensitivity was variable and was too low for reliability (36). In the US, the 2-tiered sero-diagnostic testing algorithm that employs ELISA, followed by Western blot testing, performs well in later stage Lyme, but not for early erythema migrans patients [EM (+)]. This approach has been employed for over 10 years, despite missing 33% of convalescent cases. Newer improvements in modification of the two-tiered testing system (37) show promise but will still fail to show (i) presence or absence of living Lyme organisms and won't show (ii) inflammatory responses.

The poor performance of all commercially available Lyme tests was shown recently (38). Sensitivities ranged from 30.6% to 86.2%. These authors suggest that negative lab tests do not rule out Lyme but are unable to show what rules *IN* Lyme.

Section 19

Search for biomarkers for *Borrelia* in Lyme that will also show CIRS

Reliable testing to show living spirochetes in symptomatic Lyme patients has been sought for nearly 40 years. Aside from culture, countless labs have been offered, but to date, each has not been

substantiated over time. Antibody testing in long-term illness is limited by the absence of ability to tell a physician (i) when the exposure occurred; (ii) whether an illness occurred; (iii) whether the illness is still present; (iv) whether there were multiple exposures; (v) whether there have been multiple illnesses; or (vi) if the present illness is progressive.

Symptoms are not suitable for use as biomarkers; they are non-specific and reporting can be subject to multiple possible biases. We already know that symptoms of chronic fatiguing illnesses are essentially uniform across multiple diseases (Table 1). Symptoms of CIRS are also essentially the same as pre-treatment Lyme. Post-Lyme Syndrome, a diagnosis assigned to Lyme patients still sick despite reasonable use of antibiotics, is not associated with defining symptoms.

Finally, a newly codified case definition of Chronic Lyme Disease has appeared in peer-reviewed literature (39). Once again, we are not given any single method to show reliably that living Lyme organisms are responsible for symptoms. Instead, we are given directions to use clinical judgment as we use symptoms, maybe with culture, molecular testing (not specified) or “some other technology that *directly identifies presence*” of *Borrelia*.

What technology directly identifies presence of *Borrelia*? We need a biomarker!

Use of transcriptomics brings reason for optimism for a test that shows both active infection and provides a mechanism for monitoring progress of therapy. The answer lies in differential gene activation associated with infection; monitoring therapy in a patient is accomplished by sequential studies over time to show correction of differential gene activation.

The target is to restore gene activity back to normal control levels.

Bouquet is the lead author of the first “sizable” study on acute Lyme patients, defined by tick bite with (+) EM rashes, published in 2016 (40). This small case/control study (29 patients and 13 controls) measured transcriptomics at (i) initial date seen; (ii) after three weeks of antibiotics; and (iii) after six months. 15 patients were “fully recovered” from infection (by symptoms) by 6 months, with 13 still symptomatic. This study does not support the idea that three weeks of antibiotics alone will ensure a successful therapy. What we need is the biomarker that transcriptomics could provide to show resolution of inflammatory contribution to illness.

Marked differences in gene activation/suppression in cases/controls was noted in 1,235 genes at baseline, with 1,060 genes still showing differences after three weeks of antibiotics. At 6 months follow-up, 636 genes were still abnormal compared to controls, but surprisingly, there were no differences in abnormal gene activation at six months between “fully recovered” versus patients with ongoing symptoms. This finding returns us to the question of (i) whether symptoms remain a better clinical indicator of health compared to objective biomarkers; (ii) and of possible greater importance, what is the source of persistent gene activation left uncorrected by antibiotics alone? Without use of CIRS biomarkers, we are left to perform another study to identify the reason for the persistent transcriptomic abnormalities.

Almost lost in the discussion in Bouquet’s paper is downregulation of eukaryotic initiation factor 2 (eIF2) signaling at each of the three-time points. eIF2 modulates ribosome-transfer RNA binding, or the initiation of translation, this

first step in production of protein from messenger RNA. We have also seen downregulation of ribosomal genes in patients with nasal colonization of biofilm-forming multiply antibiotic resistant coagulase negative staphylococci; eIF2 downregulation is not a finding specific for Lyme patients. Ryan, et al (12) showed a decrease in ribosomal gene activity after the final step in a CIRS treatment protocol with use of exogenous vasoactive intestinal polypeptide (VIP). We feel the sequential tracking of ribosomal gene activity throughout illness and treatment can be highly indicative of health status.

Bouquet also notes overlap of gene pathways in Lyme with lupus, rheumatoid arthritis and Chronic Fatigue Syndrome. No attempt was apparently made to compare genomics of Lyme to ciguatera genomics (22). In addition, pathway data will be strengthened by recording in larger sample sizes, owing to multiple test corrections needed when analyzing tens of thousands of genes simultaneously.

With 20,000 protein-coding genes and even more non-coding regulatory genes, each having the potential for significant roles in Lyme versus other inflammatory illnesses, enhanced data mining from white blood cell gene expression may hold promise for greater benefit in our search for a reliable dual functioning Lyme biomarker.

Bouquet noted a group of eight genes found upregulated in acute Lyme compared to controls, most were pro-inflammatory cytokines: interferon gamma (IFNG), interleukin-1 beta (IL-1B), tumor necrosis factor alpha (TNF), interleukin-6 (IL-6), transforming growth factor beta (TGF beta-1); anti-inflammatory cytokine interleukin-4 (IL-4), colony stimulating factor 2 (CSF2) and cell surface marker CD40L (a costimulatory protein found on antigen presenting cells).

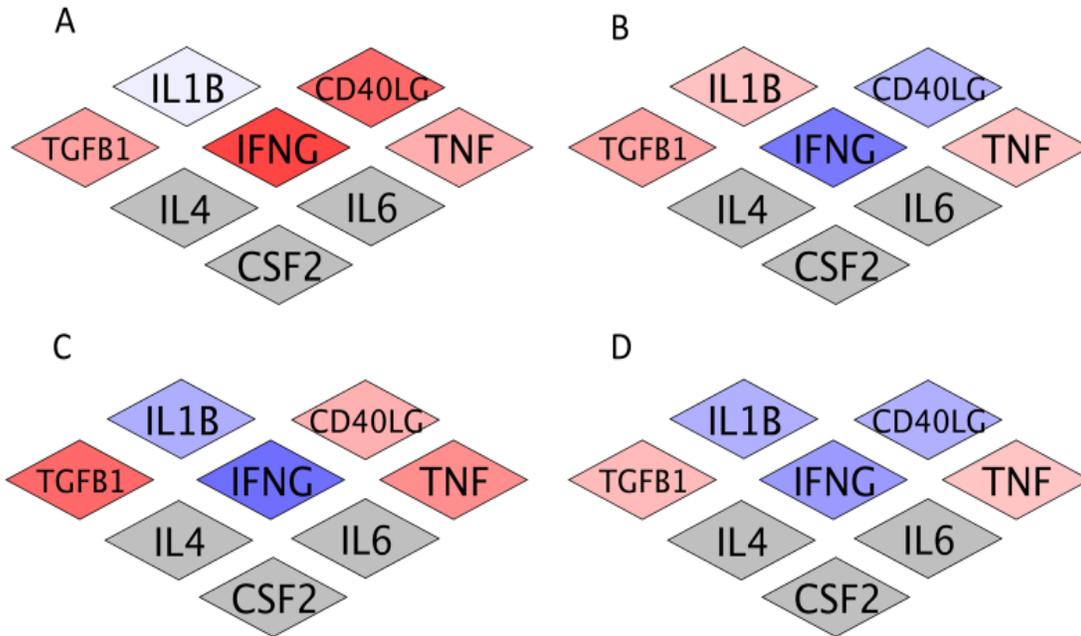


Figure 3

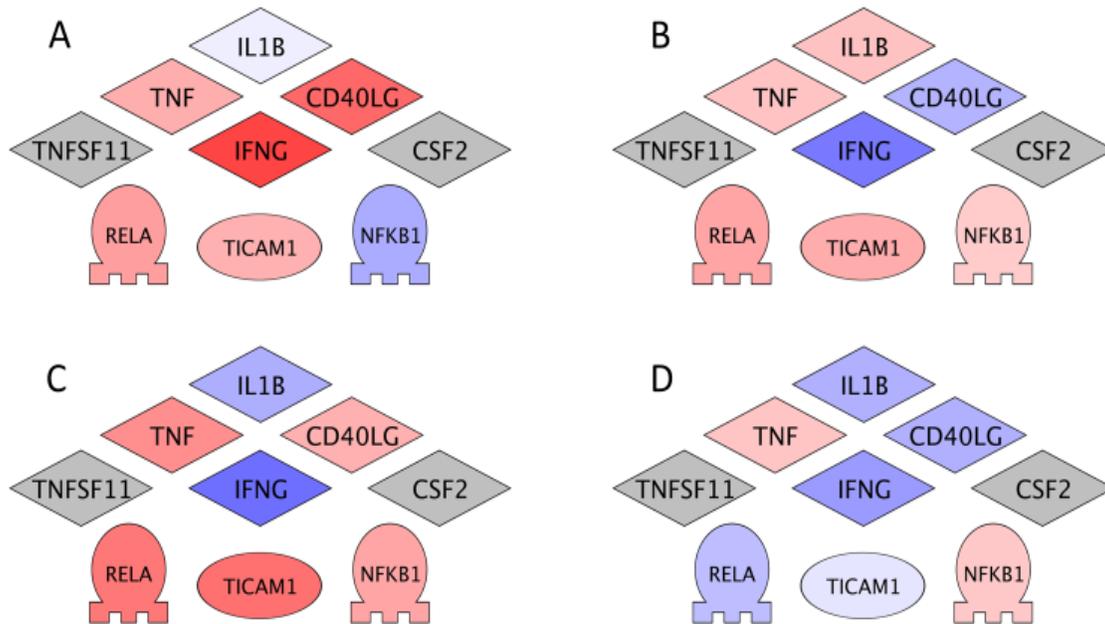


Figure 4

Figures 3 and 4. Selected genes that have been reported to be up regulated in Lyme disease at acute exposure (Figure 1) and after a 3-week course of antibiotics (Figure 2). Four patients are represented at different times post exposure: A - 53yo F at acute exposure, B - 48yo F at 10 years post diagnosis, C - 20yo M at 6 months post diagnosis, D - 38yo M at 1 year post diagnosis. Patient gene expression is shown with red indicating elevated and blue indicating depressed gene expression relative to a control database with greater intensity of color indicating greater differential expression. Gray indicates expression levels below limit of sensitivity.

Bouquet also reported genes up-regulated in Lyme patients after antibiotics include toll like adapter molecule 1 (TICAM1), nuclear factor kappa-B (NF- κ B), tumor necrosis factor super-family 11 (TNFSF11), triggering receptor expressed on myeloid cells 1 (TREM-1) and transcription factor RELA proto-oncogene, NF- κ B subunit (RELA), as well as IL1B, TNF, IFN γ , CSF2 and CD40L.

When used as biomarkers for acute Lyme and Lyme after antibiotics, respectively, as shown in Figures 3 and 4, the markers correctly identify one patient with acute Lyme (patient A) but would not support the diagnosis of acute Lyme made in three other (patients B, C, D) cases diagnosed previously with Lyme. Patients B and C were diagnosed clinically without biomarkers of ECM, positive ELISA or tick bite followed by flu-like illness, with duration of illness being 120 months and 6 months respectively. Patient D also did not have a consistent presentation of acute Lyme with a tick bite, followed by flu-like illness and physician-witnessed ECM rash. The question remains as to how long a patient should be treated with protocols for acute Lyme after that diagnosis is no longer supported by recognized transcriptomic biomarkers. Each of the three not confirmed to have acute Lyme by transcriptomics, but still receiving antibiotics, were subsequently shown to have CIRS-WDB with multiple confirming biomarkers and excellent recoveries obtained using CIRS treatment protocols (21). Each of these three patients had NeuroQuant consistent with CIRS-WDB but not with CIRS-PLS.

Bouquet does not report any microRNA data. Based on preliminary data from our group we feel that microRNA may yield important clues to the pathophysiology of acute Lyme, an illness that is possibly defined better by transcriptomics than by antibody formation. Moreover, identification

of microRNA abnormalities poses an intriguing opportunity for transcriptomically active interventions shown to be beneficial in CIRS-WDB.

We are now poised to apply the work of Bouquet to our own group of EM (+) patients. We will look at messenger (mRNA) and microRNA at baseline diagnosis; after antibiotics; after use of our patent pending CIRS treatment protocols (Patent 131/961642)); and after 6 months of no Rx. Perhaps CIRS treatment can normalize the genes that Bouquet found to remain abnormal despite antibiotics and passage of time. By looking at microRNA at each stage of the Lyme illness, we hope to define mechanisms of regulation of DNA expression possibly gone awry in PLS.

Given that Lyme is a high-profile illness, as manifested by inter-physician group arguments and public awareness, the role of Next Generation Sequencing (NGS) has societal merit, but the role of NGS is no less important for other chronic fatiguing illnesses. Fibromyalgia and CFS/ME demand reliable diagnostic measures that will also serve as indicators for evaluation of treatment.

We consider treatment successful when (i) symptoms of treated cases equal controls; (ii) VCS of treated cases equals controls; (iii) proteomics of treated cases equals controls; (iv) MARCoNS assays of treated cases are no different from controls; and (v) transcriptomics of treated cases equal controls.

As a further word on the concept of “high profile,” CIRS-WDB affects far more people than Lyme in the US; causes far greater financial burden than Lyme in the US and results in far more litigation than Lyme in the US. The basic need of access to a safe school, a safe work place and a safe residence is not questioned. CIRS-WDB already has multiple biomarkers that demonstrate presence of illness and

progression of treatments. Let the use of objective biomarkers, as in CIRS-WDB, become a similar goal for Lyme, CFS/ME and fibromyalgia.

Section 20

CIRS Treatment Protocol

Treatment for CIRS is a lengthy process designed to (i) first remove the patient from the exposure(s); (ii) modulate both stress responses and inflammatory responses; (iii) decrease the inflammatory burden and allostatic load; (iv) repair damage to organs systems.

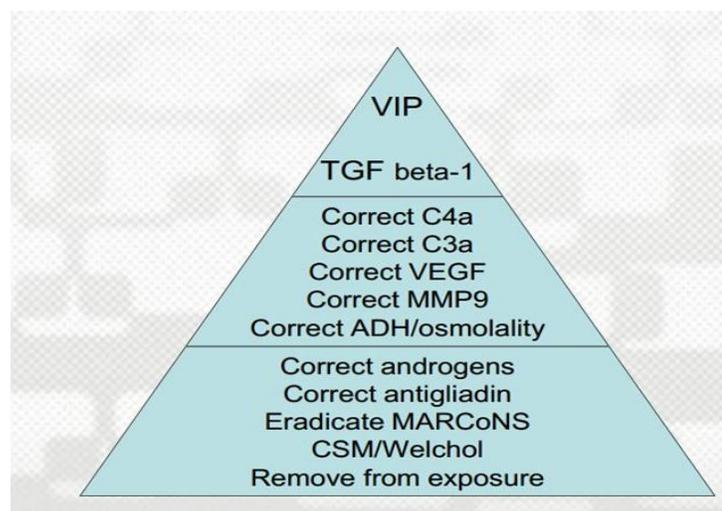
Allostatic load is defined by McEwen (41) as “wear and tear” or overload in response to being in a chronic state of allostasis (change). Sleep deprivation, maladaptation, poor nutrition, and exercise habits contribute to this weathering effect. Initial increase in stress and change is good for the body and the brain, but chronic stimulation and increased allostatic load can be detrimental. Patients with CIRS have an increased allostatic load due to the chronicity of the inflammatory response and consequent metabolic derangements. Reduction of internal metabolic resources leads to loss of physiologic and psychologic

resiliency as a result, and creates vulnerability for more permanent injury to the brain, organ and microvascular systems.

An 11-step treatment protocol for CIRS has been developed over the last two decades. Proper diagnosis relies on a combination of detailed history, physical examination and diagnostic data highlighted throughout. Evaluation needs to include determining the potential biotoxin exposure, length of exposure / re-exposure and co-morbid conditions.

The treatment process is staged and progressive. Detailed patient education and encouragement are paramount. CIRS is a complex immunological disorder resulting from uncontrolled inflammation involving primarily the innate immune system. Some patients move quickly through the protocol while others do not. Some individuals can “get stuck” in one treatment step or often even “backslide” into an already completed step. Treatment is highly individualized depending on exposure and concurrent disease states but in each patient the basic format of the protocol is invariant. Therefore, the CIRS treatment protocol will take time and requires a significant amount of patient education and support.

Shoemaker Protocol



Section 21

Conclusion

CIRS is a neuroregulatory-inflammatory disease process found in genetically susceptible patients, initiated by exposure to a biotoxin(s). The chronic inflammation that ensues leads to a multi-symptom, multisystem condition that presents as a fatiguing illness. CIRS is commonly found in the general population. While often not well recognized in the general medical community, the recognition of the CIRS patient has implications for reducing health care costs and solving a debilitating disease complex for which there is now evidence-based diagnostic and treatment criteria.

Future research should focus on refining the treatment protocol, determining the role of transcriptomics in chronic inflammatory processes and exploring the relationship and overlap between CIRS and other common conditions such as cardiovascular disease, diabetes and obesity, chronic pain syndromes, concussion and brain injury, and neurodegenerative disorders.

Section 22

Summary:

Taken together, for all diagnoses made by symptoms only, there is a need for biomarkers of pathology that aid in diagnosis and lead to interventions that yield effective therapeutics. Each step of CIRS treatment is based on published, peer reviewed literature originally produced from the CIRS-WDB community. The concordance of proteomic features of these illnesses of diverse origin is not surprising in that the innate immune responses are limited. The diversity of protein/gene functions are served by different combinations of gene activation/suppression that eventually yield a final common proteomic pathway. Although the Bouquet

work on Lyme transcriptomics has made the first mark, as of yet, no one has presented adequate data to bring a transcriptomic case definition to any phase of Lyme. Understanding that there is still a substantial need for broad-based studies using NGS and newer concepts, including all biomarkers for CIRS, as we take the next step beyond antibodies, we can begin to bear down on the inflammatory illness called Lyme disease.

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