An Evaluation of Alternate Means to Diagnose Chronic Inflammatory Response Syndrome and Determine Prevalence

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This study was completely funded by Whole World Health Care. The author has no conflicts of interest.

Keywords: CIRS, inflammation, diagnosis, prevalence, cluster analysis, cytokine overproduction, haplotype

Abbreviations used

ACLA	anticardiolipin antibody
ACTH	adrenocorticotrophic hormone
ADH	antidiuretic hormone
AGA	antigliadin antibody
C4a	split product of the complement protein C4
CD	case definition
CIRS	chronic inflammatory response syndrome
CPD	cycles per degree of visual arc
GAO	United States Government Accountability Office
HLA	human leukocyte antigen
MARCoNS	multiply antibiotic resistant coagulase negative *Staphylococcus*
MMP-9	matrix metalloproteinase 9
MRI	magnetic resonance imaging
MSH	melanocyte stimulating hormone
TGF-β1	human transforming growth factor – beta1
VCS	visual contrast sensitivity
VEGF	vascular endothelial growth factor
VIP	vasoactive intestinal polypeptide
WDB	water-damaged building

Abstract

Chronic inflammatory response syndrome is an illness of unreported prevalence first described in 1997. This study is the first reporting prevalence and evaluating alternate methods of diagnosis vs. the existing two case definitions in the literature. Both case definitions require improvement with therapy to confirm the diagnosis. Compliance is difficult and improvement may take months. Definitive diagnosis, via case definition, requires time. 1061 consecutive patients of all ages assessed at a chronic inflammatory response syndrome specialty clinic were retrospectively evaluated using case definitions. 371 met diagnostic criteria. Cluster analysis, a series of 10 lab tests and 3 screening tests were applied to these 371 patients in 4 age groups. Clusters demonstrated high sensitivity. The number of abnormal lab tests and failing 3 screening tests each demonstrated good sensitivity. Applying Clusters with Screens or Labs demonstrated excellent diagnostic accuracy with combined age group error rates ranging from $1.24 \times 10 \times 10^{-3}$ to 1.10×10^{-6}. These approaches were applied to the 690 patients failing case definition criteria. 302 additional patients achieved one or both Clusters and Screens or Labs raising the total to 673 confirmed chronic inflammatory response syndrome cases. Partnership with a pediatric practice revealed 246 of these confirmed cases were from that practice yielding a minimum pediatric prevalence of 7.01%. Adult prevalence is likely even higher. At a prevalence of $\geq$ 7.01%, chronic inflammatory response syndrome is one of the greatest public health dilemmas in existence.

1. Introduction

Chronic inflammatory response syndrome (CIRS) is an emerging illness of unreported prevalence. It was first described in 1997 (Shoemaker 1997). In adults, CIRS presents as a multi-system, multi-symptom illness. In children < 11 years, CIRS may parade as single system illness such as chronic headaches, recurrent abdominal discomfort or chronic fatigue, persistent bedwetting after 6 years of age, prolonged "growing pains" or inattention (unpublished data). In all patients, environmental exposures to biologically produced toxins (Smoragiewicz et al. 1993, Suihko et al. 2009, Butte 2002, Hirvonen et al. 2005, Pestka et al. 2008, Kettleson et al. 2013) trigger chronic innate immune cytokine overproduction (Gonzales-Rey et al. 2007, Heiman et al. 1997, Qin et al. 2004, Roeder et al. 2004, Magaki et al. 2007, Perry 2004, Vojdani et al. 2009) in the genetically susceptible (Shoemaker, Rash, Simon 2006). Hyperflexibility, inflamed sclerae, pallor, tremors, facial rash and weakness of the anti-gravity muscles of the dominant shoulder are typical physical findings. CIRS patients routinely possess an HLA predisposition, depleted neuroimmuno-regulatory peptide levels (VIP and MSH), elevations of innate immune system markers (TGF-β1, MMP-9 and C4a), dysregulation of multiple hypothalamic-pituitary-end organ axes (ACTH/cortisol and ADH/osmolality), abnormal VEGF levels, presence of ACLA and/or AGA antibodies and nasal carriage of coagulase negative Staphylococcus resistant to multiple antibiotic classes (Shoemaker 2008). Visual Contrast Sensitivity (VCS) testing is usually abnormal (Shoemaker 2001). Volumetric brain MRI evaluation of untreated patients frequently demonstrates increased volumes in the forebrain parenchyma, cortical gray, and pallidum with decreased volume of the caudate nuclei (Shoemaker et al. 2014, McMahon et al. 2016). Genomics testing reveals sarcin-ricin loop ribosomal, mitochondrial and Ikaros family abnormalities (Ryan et al. 2017). Symptoms, physical findings and lab/imaging abnormalities are largely reversible if treated properly and in timely fashion (Shoemaker and House 2006, Shoemaker, Rash and Simon 2006, McMahon et al. 2016). CIRS is a chronic, progressive, debilitating illness from which cognitive and physical disability may ensue. Recent publications in the field of Alzheimer's disease (Bredesen 2016) and inflammatory bowel disease (Gunn et al. 2016) show a link between these illnesses and CIRS. More links with other illness of inflammatory origin are sure to come.

The most common CIRS trigger is chronic exposure to the interior of water-damaged buildings (WDB). Shoemaker published a CIRS-WDB case definition in 2006 (Shoemaker and House 2006). The U.S. Government Accountability Office (GAO) created a second, similar, CD in their 2008 report on indoor mold (GAO 2008). Both case definitions require demonstration of an exposure to WDB, signs and symptoms consistent with CIRS and improvement with appropriate therapy. Dr. Shoemaker's definition is more specific providing a list of 37 specific symptoms and requiring abnormalities in 3 of 6 objective tests.

Case definition usage is helpful to define CIRS, in research and in legal pursuits, but becomes unwieldy in day to day patient management. Specifically, both

definitions require patient compliance to therapy and documenting subsequent improvement. The initial step of the therapeutic protocol often requires patients remediating or moving from their residence or water-damaged workplace or school. Resource limitations make this step unachievable for many, hence they cannot be compliant and typically do not achieve sustained improvement. By definition, non-compliant patients do not meet the criteria established in the CD, even when history, environmental history, physical findings and lab testing all suggest CIRS. It is equivalent to diagnosing an otitis media if and only if improvement from antibiotics is recorded, regardless of symptoms, initial physical exam and ear culture results. For such reasons, a need for alternate means to establish the CIRS diagnosis accurately is required.

2. Methods

A retrospective review of the charts of 1061 consecutive patients evaluated for CIRS-WDB was IRB approved. Patients were included if they passed Dr. Shoemaker's and/or the GAO case definition. Compliance required initial evaluation with history and physical exam, diagnostic labs, eliminating exposures to WDB, taking 1-2 months of a bile sequestrant medication, a follow-up visit and follow-up labs. 371 patients were compliant and met the case definition. Their charts were reviewed for cluster analysis (Clusters), three screening modalities (Screens) and the total number of abnormal lab tests of 10 standard diagnostic CIRS labs (Labs). All ages were included and patients were subdivided into 4 age groups (0-4.9 years, 5-10.9 years, 11-18.9 years and adults, respectively).

2.1 Clusters

Cluster analysis divided CIRS symptoms into 13 clusters (provided by Dr. Shoemaker). One point was awarded if one or more symptoms from a cluster were noted. In children < 11 years, the diagnosis of CIRS-WDB was made if 6 or more clusters received a point. In older patients, 8 points constituted the diagnosis. The clusters used were as follows:

1. Fatigue
2. Weakness, new knowledge 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

2.2 Screens

The three screening tests were a positive roster of symptoms, a failed VCS test and active weakness in shoulder anti-gravity muscles.

The roster of symptoms contained 37 symptoms (Shoemaker and House 2006) from 9 body systems. It was administered by a certified CIRS provider and was considered positive for children < 5 years old with ≥ 5 symptoms, children > 5 years but < 11 years with ≥ 8 symptoms, and older

children and adults with ≥ 13 symptoms. (Certification information found at www.survivingmold.com). Patient completed symptom checklists are not used. They are considered inferior and less accurate because patients often do not understand what many of the questions and short phrases on checklists mean. For instance, if the survey asks if a patient is fatigued, a simple "yes" is inadequate. Is the fatigue recent, i.e., after a cold 1 week ago, or has the fatigue been ongoing for years starting after a bad case of influenza? Is the fatigue every day or are there good and bad days? The answers to these questions mean the difference between a "yes" and a "no" and give prognostic value in the right hands. The 37 symptoms are: fatigue, weakness, aches, cramps, unusual pains, ice pick pains, lightning bolt pains, headaches, light sensitivity, blurry vision, red eyes, tearing, sinus problems, cough, shortness of breath, abdominal pains, diarrhea, joint pains, morning stiffness, numbness, tingling, metallic taste, vertigo, memory problems, inattention, confusion, difficulty assimilating new knowledge, word loss, disorientation, skin sensitivity, excessive thirst, excessive urination, static shocks, excessive sweating, mood swings, temperature dysregulation and appetite swings.

VCS testing used a standard APT VCS tester obtained through www.survivingmold.com and followed the previously published protocols for usage (Shoemaker 2001). A test was considered positive, or a failed screen, if the patient was unable to correctly answer up to the 7th item in Column C (6 cycles per degree of visual arc, or CPD) or the 6th item in Column D (12 CPD) with all tested eyes. Visual acuity of at least 20/50 was required for each tested eye. Cooperation and knowing one's right from one's left was required. Small children were not tested. Patients with adequate acuity in only one eye were tested for that eye only.

Anti-gravity muscle testing was performed by a certified CIRS provider. A history of significant trauma, neurologic illness or surgery to one or the other shoulder excluded patients from testing. The patient placed both arms in the fully extended position, parallel to the floor. The examiner placed his hands on the patient's hands. The patient was instructed to lift their hands upward with as much force as possible. The examiner opposed this movement with his hands. After attempting this technique one time, the patient was instructed to squeeze two of the examiner's fingers as hard as possible to assess for distal weakness in the arm. Then the examiner placed his hands on the patient's shoulders and instructed the patient to shrug their shoulders up with as much force as possible while the examiner opposed this movement, assessing for proximal weakness. Finally, the examiner repeated the first maneuver two more times assessing for fatiguing of the anti-gravity muscles.

The norm for testing is the non-dominant arm will dip initially, then rebound, when the examiner opposes the patient's anti-gravity muscles and that there will be no fatigue in either shoulder even on the third attempt. An abnormal test shows normal proximal and distal arm strength but weakness in one of the shoulders' anti-gravity muscles compared to the other and fatiguing in that shoulder. Typically, in CIRS patients, it has been noted that, for right-handed patients, the weakness is nearly always in their dominant right arm. For left-

handed patients, the weakness is more evenly split between the sides (unpublished data).

Once the three screens were performed, the reliability of two positive screens and three positive screens for making a presumptive diagnosis of CIRS-WDB was compared to cases confirmed by the case definition.

2.3 Labs

Lab testing assessed a set of 10 standard biomarkers evaluating the accuracy of labs alone in foretelling the diagnosis of CIRS-WDB. For children < 11 years, 4 abnormal tests were considered diagnostic. For patients 11 years or older, 5 abnormal lab tests were considered diagnostic. The predictive ability of using labs only to make a diagnosis was then calculated.

The lab tests used were: HLA haplotypes in the DRB1, DQ and DRB3, B4 or B5 loci, VIP, MSH, ADH with serum osmolality, ACTH with cortisol, TGF-β1, MMP-9, C4a, MARCoNS and the presence of significant ACLA or AGA antibodies.

HLA was considered abnormal if either haplotype was an "M" (mold) or a "D" (dreaded or multi-susceptible) haplotype (see Table 1, Shoemaker et al. 2010). VIP, MSH, TGF-β1 and MMP-9 were considered abnormal if either decreased or elevated levels were recorded. The normal range for C4a is 0-2832 ng/mL. Elevated levels were considered abnormal. Presence of significant ACLA and AGA antibodies (levels greater than in the "equivocal" range) were considered abnormal. While coagulase negative *Staph* found on nasal culture is considered normal flora, MARCoNS are only found in 2% of the population (Shoemaker et al. 2003). MARCoNS testing was considered positive if there was presence of coagulase negative *Staph* on deep nose culture and that *Staph* was resistant to at least 2 classes of antibiotics by routine gram positive sensitivities of 12 antibiotics. ADH and osmolality were considered abnormal if either measure was absolutely high or absolutely low. They were also recorded as abnormal if they were dysregulated. The parameters assessing normal regulation were for osmolality $\geq$ 292, ADH > 4; and if osmolality was $\leq$ 278, ADH < 2. ACTH and cortisol were evaluated in similar manner with absolute abnormals and dysregulated abnormals. For the latter, a cortisol $\geq$ 15 with ACTH $\geq$ 15; and a cortisol $\leq$ 8 with ACTH $\leq$ 40, were considered abnormal. When abnormal by absolute means and by dysregulation, ADH/osmolality and ACTH/cortisol pairs were counted as abnormal only once.

Table 1

"D" Haplotypes				
4-3-53	11-3-52B	12-3-52B	14-5-52B	9-3-53
"M" Haplotypes				
7-2-53	7-3-53	13-6-52A	13-6-52B	13-6-52C
17-2-52A	17-2-52B			

Key: HLA haplotypes written as DRB1-DQ-DRB3, DRB4 or DRB5. "D" = dreaded or multi-susceptible. "M" = mold.

Statistical evaluation looked at the accuracy of each method to predict a diagnosis in known cases. Two and three failed screens were compared with each other. Clusters, Screens and Labs were compared against each other to develop sensitivity, specificity, positive and negative predictive values and *p*-values at each of the four age groups. Fisher exact testing and Chi squared analysis were performed online to determine *p*-values (http://vassarstats.net/tab2x2.html and http://www.socscistatistics.com/tests/chisquare/Default2.aspx, respectively).

This author maintains a pediatric practice and a separate CIRS practice in the same building. Pediatric prevalence data was constructed by manually counting every chart in the pediatric group practice. The total number of patients seen by this author was determined by reviewing 350 of the 4342 counted charts solely to determine eligibility. 80.9% of the 350 charts had at least one visit with this author. Patients never seen by this author could never have been screened for CIRS and were excluded. The total of patients seen at least once by the CIRS certified author was determined to be 3511. This provided the denominator. The pediatric charts of the CIRS clinic (WWHC) were reviewed. 246 pediatric patients with CD and/or Clusters plus Labs/Screens proven CIRS were identified at WWHC and were also patients at the pediatric practice. This provided the numerator for the minimal prevalence of CIRS in children.

The error rate by age group was determined using the generated sensitivities and specificities, linear regression and the prevalence rate determined above with Fisher exact testing and Chi squared analysis to calculate *p*-values.

3. Results

1061 charts were reviewed. By age group (0-4.9 years, 5-10.9 years, 11-18.9 years and adults), there were 52, 185, 250 and 574 patients, respectively. Of these, by age group, 18, 65, 86 and 202 patients, respectively, met the case definition criteria for CIRS-WDB. Roughly 2/3 of each age group were excluded and almost always for non-compliance.

3.1 Sensitivity

Sensitivity varied by age group. The schemes with the best sensitivity by age group were: Labs (100%), Clusters (96.9%), 2 screens (100%) and Clusters (98.4%), respectively (See Tables 2-5). Age group combinations (0-4.9 years plus 5-10.9 years and ≥11 years plus adults) were evaluated for all measures. For sensitivity, Clusters had the best result for both combinations (92.7%, 98.5%, respectively, see Tables 6 and 7). For all age groups combined, Clusters was the most sensitive (97.2%).

Table 2

0-4.9 years	*p*-value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
GAO v Clusters	1	77.8	Undef	100	0
GAO v Labs	1	77.8	0	100	0
Dr. S v Clusters	0.623	72.7	14.3	57.1	25
Dr. S v Labs	***0.001032***	100	57.1	78.6	100

Key: Evaluation of p-values, sensitivity, specificity, PPV and NPV for the 2 case definitions in patients 0-4.9 years old. Screening tests not performed on children <5years old. GAO = case definition from the 2008 GAO report (GAO 2008); Dr. S = case definition from Dr. Shoemaker (Shoemaker and House 2006); Clusters = cluster analysis; Labs = lab testing; PPV = positive predictive value; NPV = negative predictive value; Undef = undefined (divided by zero). Significant *p*-values are ***bolded and italicized***.

Table 3

5-10.9 years	*p*-value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
GAO v Clusters	1	96.9	0	98.4	0
GAO v Labs	1	71.9	0	97.9	0
GAO v 3 Screens	1	31.3	100	100	2.2
GAO v 2 Screens	1	79.7	0	98.1	0
Dr. S v Clusters	0.175	95.7	0	71.4	0
Dr. S v Labs	***0.0003***	83	66.7	86.7	60
Dr. S v 3 Screens	***0.00653***	40.4	94.4	95	37.8
Dr. S v 2 Screens	1	80.9	22.2	73.1	30.8

Key: Evaluation of p-values, sensitivity, specificity, PPV and NPV for the 2 case definitions in patients 5-10.9 years old. 2 Screens = 2 of 2 or 3 failed screening tests; 3 Screens = 3 of 3 failed screening tests.

Table 4

11-18.9 years	*p*-value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
GAO v Clusters	1	98.8	0	96.5	0
GAO v Labs	1	69.9	33.3	96.7	3.8
GAO v 3 Screens	0.588	44.6	33.3	94.9	2.1
GAO v 2 Screens	1	100	0	96.5	Undef
Dr. S v Clusters	1	98.7	0	87.1	0
Dr. S v Labs	***0.0254***	78.7	90.9	98.3	38.5
Dr. S v 3 Screens	0.102	49.3	81.8	94.9	19.1
Dr. S v 2 Screens	1	100	0	87.2	Undef

Key: Evaluation of p-values, sensitivity, specificity, PPV and NPV for the 2 case definitions in patients 11-18.9 years old.

Table 5

Adults	*p*-value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
GAO v Clusters	1	98.4	0	89.9	0
GAO v Labs	0.320	84.1	5	89	3.3
GAO v 3 Screens	0.334	37.4	50	87.2	8.1
GAO v 2 Screens	0.260	78	10	88.8	4.8
Dr. S v Clusters	1	98.4	0	92.5	0
Dr. S v Labs	1.17×10^{-17}	89.8	73.3	97.7	36.7
Dr. S v 3 Screens	0.0513	40.6	86.7	97.4	10.5
Dr. S v 2 Screens	0.0904	80.7	40	94.4	14.3

Key: Evaluation of p-values, sensitivity, specificity, PPV and NPV for the 2 case definitions in adult patients.

3.2 Specificity

Specificity varied by age group. The schemes with the best specificity by age group were: Labs (57.1%), 3 Screens (100%), Labs (90.9%) and 3 Screens (86.7%), respectively. For age group combinations, Labs (64%) and 3 Screens (84.6%) respectively, were the most specific. For all age groups combined, 3 Screens (88.6%) had the highest specificity followed by Labs (72.5%).

Table 6

< 11 years old	*p*-value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
GAO v Clusters	1	92.7	0	98.7	0
GAO v Labs	1	73.2	0	98.4	0
Dr. S v Clusters	0.6628	91.4	4	68.8	16.7
Dr. S v Labs	$\boldsymbol{9.294 \times 10^{-6}}$	86.2	64	84.7	66.7

Key: Evaluation of p-values, sensitivity, specificity, PPV and NPV for the 2 case definitions in the combination of patients from 0-4.9 years and 5-10.9 years old.

Table 7

≥ 11 years old	*p*-value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
GAO v Clusters	1	98.5	0	91.9	0
GAO v Labs	0.2705	79.6	8.7	90.9	3.6
GAO v 3 Screens	0.2721	39.6	47.8	89.7	6
GAO v 2 Screens	0.5477	84.9	8.7	91.5	5
Dr. S v Clusters	1	98.5	0	90.8	0
Dr. S v Labs	$\boldsymbol{1.098 \times 10^{-12}}$	86.6	80.8	97.8	37.5
Dr. S v 3 Screens	***0.005971***	43.1	84.6	96.6	13
Dr. S v 2 Screens	0.2388	86.3	23.1	91.9	14

Key: Evaluation of p-values, sensitivity, specificity, PPV and NPV for the 2 case definitions in the combination of patients from 11-18.9 years old and adults

3.3 Positive predictive value

Positive predictive value varied by age group. The schemes with the best PPV by age group were: Clusters and Labs (100%), 3 Screens (100%), Labs (98.3%) and Labs (97.7%). For age group combinations, Clusters (98.7%) and Labs (97.8%), respectively, had the highest PPV. For all age groups combined, 3 Screens (96.4%) had the highest PPV. Of note, all measures had high PPV (>86%) at all age ranges except the youngest children (n=18).

3.4 Negative predictive value

Negative predictive value varied by age group. The schemes with the best NPV by age were: Labs (100%), Labs (60%), Labs (38.5%) and Labs (36.7%), respectively. For age group combinations, Labs (66.7%) and Labs (37.5%), respectively, had the highest NPV. For all age groups combined, Labs (46.3%) had the highest NPV.

3.5 *p*-values

p-values varied by age group and case definition. Most evaluations, i.e., Clusters vs. patients who met the GAO criteria, did not generate significant *p*-values (<0.05). At all age groups, comparing cases meeting Dr. Shoemaker's case definition with Labs, there were significant p-values (p = 0.001, 0.0003, 0.0254 and 1.17×10^{-7}, respectively). 3 Screens vs. Dr. Shoemaker's case definition had significant values at the 5-10.9 year group (p = 0.00653). Age group combinations for Labs vs. Dr. Shoemaker case definition patients developed *p*-values of 9.29×10^{-6} and 1.10×10^{-12}, respectively. This grouping for all ages was significant at $p = 1.24 \times 10^{-17}$. Age group combinations for 3 Screens vs. Dr. Shoemaker case definition patients were significant for the ≥11 year combination at p = 0.00597. This grouping for all ages combined demonstrated a *p*-value of 4.63×10^{-5}.

3.6 Best approach

The best approach to using these schemes was evaluated. Having few Clusters suggests a patient is unlikely to have CIRS (high sensitivity). Having 3 of 3 abnormal Screens is a better indicator than 2 of 3 abnormal Screens. Having 3 of 3 abnormal Screens or 4 abnormal Labs (<11 years) or 5 abnormal Labs (≥11 years) suggests a patient is unlikely to have a different disease (high specificity). Combining these approaches by evaluating Clusters plus Screens or Clusters plus Labs provides high sensitivity with high specificity. When these calculations were performed, a prevalence rate was unknown. Using different prevalence rates provides different *p*-values. Calculations were made at the highest possible prevalence of 25% (unpublished data calculated from the population prevalence of HLA frequencies obtained from the National Marrow Donor Program, at http://bioimformatics.nmdp) and 1%. The p-values for Clusters plus 3 Screens, for all ages combined, at these two prevalence rates, were 3.97×10^{-147} and 0.0011, respectively. For Clusters plus Labs, the values were 7.36×10^{-95} and 0.0010, respectively (See Table 8). These data demonstrate that using Clusters plus Labs or Clusters plus Screens, even at a prevalence of 1%, are indistinguishable from using case definitions for diagnosing CIRS.

Table 8

Alpha Error Rate at different prevalences, all ages	Clusters + Labs	Clusters + Screens
Est Prev = 1%	***p = 0.0010***	***p = 0.0011***
Est Prev = 25%	***p = 7.36 x 10^{-95}***	***p = 3.97 x 10^{-147}***

Key: Comparing error rates using Clusters plus Labs and Clusters plus 3 Screens at all ages and estimated CIRS prevalence of 1% and 25%. Est Prev = estimated prevalence; *p* = alpha error rate

3.7 Prevalence

Re-evaluating the 1061 charts using Clusters plus Labs or Screens confirmed an additional 302 patients with CIRS, raising the total to 673 from 371. Of these additional patients, 67 were children and 235 were adults.

Minimum pediatric prevalence was calculated as above using 246 children meeting CD and/or Clusters plus Labs or Screens who were also FHL Pediatrics patients. There were 3511 patients this author had seen at least once from FHL Pediatrics. This generates an astonishing minimum pediatric prevalence of 7.01%. Minimum is emphasized because most of the 3511 were not screened for CIRS and many were only seen once (often for a 2 month well check - long before CIRS symptoms manifest). Astonishing is emphasized because the calculated minimum prevalence of pediatric CIRS is on the order of pediatric asthma, the most common chronic illness in children. Since CIRS is a progressive disease and since 7.01% is the minimum prevalence for the studied pediatric population, it is assumed that the prevalence in adults is at least 7.01%.

3.8 Error rate

Alpha error rate was calculated after the pediatric prevalence of 7.01% was established. Using this prevalence, linear regression and previously calculated sensitivities and specificities, the *p*-value at each age group for Clusters plus Labs were 0.0706, 2.31 x 10^{-7}, 0.024 and 7.36 x 10^{-6}, respectively (See Table 9). The *p*-value at each of the 3 older age groups for Clusters plus Screens were 0.4, 5.98 x 10^{-4} and 4.28 x 10^{-3}, respectively. Age group combinations of 0-10.9 years and ≥11 years had *p*-values of 1.10 x 10^{-6} and 1.96 x 10^{-4}, respectively, for Clusters plus Labs and 1.24 x 10^{-3} for Clusters plus Screens in those ≥11 years (See Table 10).

Table 9

Alpha Error Rate with Prevalence 7.01%	*p*-value
Clusters + Labs (0-4.9 years)	0.0706
Clusters + Labs (5-10.9 years)	$\boldsymbol{2.31 \times 10^{-7}}$
Clusters + Labs (11-18.9 years)	0.024
Clusters + Labs (adults)	$\boldsymbol{7.36 \times 10^{-6}}$
Clusters + Screens (5-10.9 years)	0.4
Clusters + Screens (11-18.9 years)	$\boldsymbol{5.98 \times 10^{-4}}$
Clusters + Screens (adults)	$\boldsymbol{4.28 \times 10^{-3}}$

Key: Evaluation of alpha error rate by age group with Clusters plus Labs and Clusters plus Screens at the calculated prevalence of 7.01%.

Table 10

Alpha Error Rate with Prevalence 7.01% (Combined age groups)	*p*-value
Clusters + Labs (0-10.9 years)	$\boldsymbol{1.10 \times 10^{-6}}$
Clusters + Labs (>11 years)	$\boldsymbol{1.96 \times 10^{-4}}$
Clusters + Screens (>11 years)	***0.00124***

Key: Evaluation of alpha error rate in combined age groups with Clusters plus Labs and Clusters plus Screens at the calculated prevalence of 7.01%.

4. Discussion

Case definitions are needed in medicine but can be cumbersome in day to day practice. The intent of this study was to find a single screening scheme which had sufficiently high sensitivity and specificity at each age group to substitute for case definitions when making the diagnosis of CIRS. Each of 3 schemes proved more likely to have high sensitivity OR high specificity. Combining a scheme possessing high sensitivity with a second scheme demonstrating high specificity produced significant results. The analysis demonstrated combining Clusters plus Labs

in children under 11 and Clusters plus Labs or Clusters plus Screens in older children and adults are just as effective in making a CIRS diagnosis as either of the CIRS case definitions found in the literature. Clusters and Screens can be performed in an initial visit and an immediate diagnosis made or ruled out. Clusters and Labs can be determined from a chart review if a sufficient number of symptoms were reviewed and appropriate tests were abnormal. Labs can be obtained at an initial visit and take up to a month to return. This delay is far less onerous than that of the case definitions, however.

PPV and NPV followed a similar pattern as sensitivity and specificity. Typically, one scheme would be high in one value but low in the other. Combinations of schemes were not evaluated using these measures.

p-values were significant at all age groups for the Dr. Shoemaker CD versus Labs analysis. Since Dr. Shoemaker's case definition requires 3 of 6 objective tests to be abnormal, and 5 of those are amongst the 10 tests in Labs, this finding is to be expected. It merely adds further support to the validity of his groundbreaking case definition.

Age groups were subdivided because CIRS can present differently at differing ages. Children under 5 often have only a single body system involved, such as chronic headaches, fatigue or abdominal issues. These three symptoms are amongst the most common chronic complaints in pediatric practice. Children 5-10.9 years have one or more systems affected and tend to have more of the 37 diagnostic symptoms than younger children. Patients 11-18.9 years present similarly to adults, always display multi-system illness and typically have more symptoms than children in the 5-10.9 year age group (unpublished data). The 0-4.9 year age group had only 18 members. Combining with the next older group however, produced statistically significant results. Combining Clusters with Labs in the combined age group would lead to roughly one diagnostic error in a million patients < 11years old. Combining the two oldest age groups also produced significant results. Clusters plus Labs' diagnostic error rate was around 2 in every 10,000 patients while Clusters plus Screens would produce 1.24 errors in 1000 patients.

Presence of ACLA and/or AGA antibodies was used as a part of Labs. Many adult and pediatric patients have been on a gluten free diet for considerable time before testing occurred. The presence of either antibody occurs significantly less often than abnormal VEGF values (unpublished data). Future evaluations should consider the use of abnormal VEGF instead of ACLA/AGA.

Two strengths of this study included the novel approaches to diagnosis and the ability to determine a minimum prevalence. In addition, all interviews and exams were performed by one experienced and certified examiner. Finally, the size of all age groups, except the youngest children, was large enough to demonstrate statistical significance. The major weaknesses are the retrospective method and small number of children < 5 years old. Further prospective studies should be undertaken.

Chronic inflammatory response syndrome is an emerging illness with a minimum pediatric prevalence of 7.01%. The prevalence in adults is almost assuredly

higher due to the progressive nature of CIRS. As such, one could conclude there are at least 21 million CIRS sufferers in the United States making CIRS one of the very largest U.S. public health concerns. CIRS is linked to Alzheimer's disease, inflammatory bowel disease and likely a host of functional disorders (fibromyalgia, chronic fatigue syndrome, irritable bowel syndrome, functional abdominal pain syndrome in adults and children, functional neurological disorders, chronic headaches, chronic regional pain syndrome and more). Many patients see 20-30 physicians before the proper diagnosis is made. Many unnecessary invasive procedures, lab tests and imaging exams are performed, in futility, because they seek wrong diagnoses.

To date, much CIRS research has been completed by private practitioners. Because of the human suffering and financial impact of 21 million patients on health care resources, governmental and private funding agencies should begin appropriating resources to further elucidate the nature of CIRS. Since environmental exposures to biologically produced toxins are required to trigger CIRS, the illness could theoretically be prevented in the vast majority of future sufferers. Screening of children for HLA haplotypes, or genomic testing, could be a useful strategy to minimize, or even prevent, the effect of CIRS in the next generation. Ignoring the CIRS epidemic will be disastrous. Researching CIRS and training practitioners will prevent an even larger epidemic than already exists.

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