A Measurable Immune Abnormality

Dr. Ritchie Shoemaker is on the cutting edge of biotoxin illness research. He made a brilliant presentation at the 2009 IACFS/ME conference in Reno, Nevada:

When C4A complement Activation factor shoots through the roof after a known exposure to mold toxins, unrestrained
cytokine response is now a measurable immune abnormality.

Background: Our understanding of the pathophysiology of human illness acquired following exposure to the interior environment of
water-damaged buildings (WDB-illness) has been transformed over time by advancements in unveiling the role of innate immune
mechanisms contributing to human illness (1, 2, 3, 4). Initial emphasis on mycotoxins alone as causative agents of WDB-illness has
given way to recognition that multiple members of the indoor microbial world make toxins and other antigenic compounds that initiate
inflammation and cause illness (5, 6). Recognition of the "chemical stew" that exists as a unique ecosystem inside WDB, with multiple
elements capable of initiating multiple interacting cascades of host responses through differential gene activation and activation of
innate immune responses following pattern recognition of microbial antigens now dominates current literature on WDB-illness.

Fundamental to the ultimate target of the research effort to define WDB-illness is a need to explain
(i) the genetic basis of differential susceptibility to initial illness
(ii) absence of recovery following removal from exposure and
(iii) accentuated inflammatory responses ("sicker, quicker") seen in those previously ill but then re-exposed to WDB.
Results of research in WDB-illness now implicate physiologic mechanisms including capillary hypoperfusion and chronic inflammatory
response syndromes (CIRS) demonstrated in affected patients but not in controls (7). Understanding the basis of capillary
hypoperfusion and CIRS has already led to better understanding, treatment and prevention (primary and secondary) of WDB-illness
(8).

Previously presented data on WDB-illness supported the role of capillary hypoperfusion induced by innate immune responses as a
basic physiologic mechanism in the illness (8). Application of the recognition of capillary hypoperfusion could provide data to support
use of diagnostic markers for impaired executive cognitive function using magnetic resonance spectroscopy measurements of
lactate, and the ratio of glutamate to glutamine (G/G) in the selected areas of the brain.

Prior work from this site (8, 9) marked CIRS by the near-universal deficiency of regulatory neuropeptides melanocyte stimulating
hormone (MSH) and vasoactive intestinal polypeptide (VIP), as well as reduction of ADH response to hyperosmolality and reduction
of VEGF which in turn is correlated with low VO2 max seen on pulmonary stress testing. Elevated levels of C4a; MMP9 and
autoimmune markers of antigliadin and anticardiolipins in CIRS have been identified. Clotting

abnormalities are not uncommon in
CIRS. This occurrence may correlate with commonly observed clinical problems of hemoptysis and epistaxis in cases.

TGF beta-1 has provided a new window of opportunity to study abnormal T-regulatory cell function and autoimmunity in this illness
as well as the unexplained occurrence of unusual rheumatologic syndromes often seen in WDB-illness (9). The newer understanding
of innate immune responses is bringing salutary new therapies to patients previously disabled by WDB-illness. Here we present a
database on 1000 consecutive patients (850 WDB-illness cases; 150 controls) seen since 2007 at one site.

Methods:
Our first major hypothesis was
(1) the same differences documented previously between symptoms, VCS, HLA, MSH, ADH/osmolality, ACTH/cortisol, MMP9 between
cases and in controls would be replicated;

our second major hypothesis was
(2) elements of activated innate immune response known to reduce capillary perfusion, i.e. low VEGF, high C4a and high TGF beta-
1 would be enhanced in cases compared to controls.

Our minor hypothesis predicts
(2a) differences between cases and controls would be highly associated with HLA DR haplotype.

If (2b) TGF beta-1 were elevated, we would see evidence of abnormal T regulatory cell function as manifested by increased
presence of autoimmunity.

Finally, minor hypothesis (2c), if C4a were elevated, we would see the same elevation of lactate in frontal lobes and hippocampus
and reduction of G/G ratios as seen on MR spectroscopy previously that in turn would correlate with symptoms of executive cognition
impairment recorded on a severity scale of 0 to 4, with zero being absent and 4 being severe.

1000 consecutive adult patients seen at a single medical clinic site signed IRB- approved HIPAA waivers (Copernicus IRB, Cary, NC)
that permit use of baseline data in research studies. Patients were labeled as being a case if they met a restrictive, two-tiered case
definition for WDB-illness published previously (2). Controls were identified as patients coming to the site for well adult physical exam
without known untreated acute or chronic illness. Data was extracted from charts retrospectively including symptoms (individual, from
a roster of 37; and total); visual contrast sensitivity testing (VCS); lab studies: HLA DR by PCR, MSH, VIP, leptin, ADH/osmolality,
ACTH/cortisol, MMP9, PAI-1, CBC, CMP, CRP, ESR, lipid profile, testosterone, DHEAS, androstenedione, GGTP, VEGF,
erythropoietin, ACLA (IgA, IgM, IgG), AGA (IgG, IgA), TGF beta-1, C3a, C4a, IgE, TSH, von Willebrand's (vWF) profile; and deep
aerobic nasal culture were also compared for cases compared to controls. MR spectroscopy data on N-acetyl aspartate, choline,

creatinine, myoinositol, lactate and ratio of glutamate and glutamine (G/G) measured in left and right frontal lobes and hippocampus
were also analyzed. Individual measurements and test results were compared using two-sample T-test.

Patients were excluded from the data set if they had untreated, active alcohol abuse with abnormal liver functions, ongoing cocaine
use, uncontrolled diabetes, anemia, active hepatitis, occupational exposure to hydrocarbons, petrochemicals, metal fumes and metal
dusts as well as undiagnosed neurologic conditions. Individuals requiring acute intervention for illness other than that acquired from
WDB were excluded.

Results: There was no difference between groups in gender, age or ethnicity. Mean total symptoms were 21.4 in cases and 2.6 in
controls. 37 individual symptoms were assessed in all patients (N= 1000); results for all symptoms were all different in cases
compared to controls except for sinus congestion (p=.137) and tremor (p=0.0064). VCS was different in cases from controls in all
frequencies tested, as shown by multivariate analysis; visual acuity was no different. Six HLA DR haplotypes were present in cases
compared to controls with relative risk > 2.0 for 4-3-53; 7-2-53; 11-3-52B; 13-6-52A; 14-5-52B; and 17-2-52A. Labs with no
differences ($p > 0.001$; N=1000) between cases and controls were leptin, PAI-1, CBC, CMP, CRP, ESR, lipid profile, testosterone,
DHEAS, androstenedione, GGTP, erythropoietin, C3a, IgE, TSH. Labs with differences ($p < 0.001$; N=1000) were MSH, VIP,
ADH/osmolality, ACTH/cortisol, MMP9, VEGF, ACLA, AGA, TGF beta-1, C4a, vWF and presence of multiply antibiotic resistant,
biofilm-forming coagulase negative staphylococci (MARCoNS) in deep aerobic nasal spaces. von Willebrand's profiles were abnormal
in 67% of patients compared to < 5 % of controls ($p < 0.001$). There were statistically significant differences ($p < 0.001$) between
cases (N=759) and controls (N=86) for lactate and G/G ratio, averaging a total of 5.2 abnormalities in eight measurements (added
four each for lactate and G/G) in cases and 0.9 in controls. A weighted symptoms score (out of 24 possible) for six symptoms of
executive cognitive function showed an average of 23, 20, 16 and 13 for the highest to lowest C4a quartile. NB: results table not
presented.

Discussion: ***Our major and minor hypotheses were confirmed.***
(1) Total and 35/37 individual symptoms, and VCS are again shown to be markedly different in cases compared to controls, with
results essentially identical to prior published findings (1, 2, 3, 8, 9).
(2) Markers of capillary hypoperfusion (C4a, TGF beta-1 and VEGF) from innate immune activation are present in cases but not in
controls.
(2a) Finding relative risk > 2.0 for haplotypes of patients, found in a total of 24% of well patients, replicates earlier published relative
risks.
(2b) TGF beta-1 elevation was associated with autoimmunity
(2c) C4a elevation was associated with impairment of executive cognitive function.

The result of untoward innate immune activation is systemic capillary hypoperfusion that can be measured directly in brain using CNS
lactate and indirectly in lung using VO2 max and anaerobic threshold. It remains likely that the underlying reason for ongoing
dysregulated innate immune response is deficiency of regulatory neuropeptides MSH and VIP, a finding seen in > 90% of cases (10).
In control patients who invariably had normal MSH and normal VIP, increased TGF beta, high C4a or low VEGF is rarely seen.
Deficiency of both MSH and VIP was not seen in controls but was common in cases. Given the not-infrequent history of epistaxis and
hemoptysis in this cohort of WDB-illness patients the abnormal vWF findings are consistent with a similar acquired coagulopathy
commonly seen in systemic inflammatory illness due to endotoxemia (12).

Persistent elevation of C4a, an otherwise short-lived anaphylatoxin, suggests ongoing activation of mannose binding lectin pathway
of complement activation, thought to be due to ongoing autoactivation of the enzyme MASP2 (14), continuing despite absence of an
environmental source of antigenic stimulus of the MBL pathway.

Studies of WDB-illness patients have noted neurologic symptoms (5), but other than hyperacute trials of re-exposure, in which rising
C4a correlates with increasing cognitive dysfunction (7, 8), no studies have been published that document a mechanism of illness
acquisition. Finding the clear link between peripheral inflammation (i.e. rising C4a) and central metabolic disturbances (elevated
lactate) provides a plausible mechanism of hypoperfusion to explain cognitive impairment. Unpublished studies presented previously
confirm that reduction of C4a alone, using low dose erythropoietin injections, simultaneously resolves the CNS hypoperfusion and
cognitive symptoms (13).

Conclusions: These results are consistent with the hypothesis that WDB-illness is a CIRS with ongoing capillary hypoperfusion.
Symptoms taken as a whole create a distinct cluster that classifies cases accurately without providing mechanisms. Lab results show
a dense, unregulated innate immune inflammatory response without yielding symptoms. Linking labs and symptoms, especially when
linked together with VCS, a neurotoxicologic measure; provides a landscape approach to a definable illness seen repeatedly in WDB-
illness patients.

Treatment of this complex syndrome will involve sequential (1) removal from exposure; (2) correction of toxin carriage, using VCS
monitoring to assess endpoints; (3) eradication of biofilm-forming MARCoNS; (4) correction of elevated MMP9; (5) correction of
ADH/osmolality; (6) correction of low VEGF; (7) correction of elevated C4a (8) reduction of elevated TGF beta-1 and (9) replacement
of low VIP. Each of these steps using FDA-approved medications is available to practicing physicians.

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