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RESEARCH ARTICLE

Transcriptomics and Brain Volumetrics Define the Causes of Cognitive Impairment in Patients with CIRS and Support the use of VIP in Treatment

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ABSTRACT

Problems with executive cognitive function, including recent memory, concentration, word finding, confusion, decreased assimilation, and disorientation, can have a variety of sources of disease, including inflammation, metabolic disturbances, and degenerative processes that are typically found in diseases where chronic fatigue is present. The problems presented by multiple coexisting cognitive symptoms are finding: 1) a single diagnostic test that has a history of use by clinicians working with brain-injured patients, such as NeuroQuant (NQ); 2) that is affordable, accurate, and reliable to use as a measure of benefits, or lack of same; and 3) screening, causation, and sequential features of therapy. Furthermore, the complexity of brain injury shows us the limitations of human versus machine-based testing, with advances in transcriptomics leading the way in a new world of diagnostics and therapies that follow gene activation studies.

The objective of this report was to retrospectively look at results of the transcriptomic testing of white blood cells, combined with a brain volumetric imaging study, in an observational study to provide a basis to identify the specific causation of brain injury caused by exposure to the interior environment of water-damaged buildings (WDB). By comparing transcriptomic abnormalities with known volumetric patterns of injury to cortical grey matter, enlargement of superior lateral ventricles and atrophy of grey matter nuclei, we demonstrate the feasibility of treatment of brain injury employing noninvasive methods in preparation for novel treatments previously shown to be effective. We intend to use these tests sequentially in a before-and-after fashion to show correction of metabolic and inflammatory conditions found in chronic inflammatory response syndrome (CIRS) in a subsequent study.

ACRONYMS:

CFI:	CHRONIC FATIGUING ILLNESS
CG:	CORTICAL GREY ATROPHY
CIRS:	CHRONIC INFLAMMATORY RESPONSE SYNDROME
NA:	GREY-MATTER NUCLEAR ATROPHY
SLV:	SUPERIOR LATERAL VENTRICLE
VDAC:	VOLTAGE-DEPENDANT ANION CHANNEL
WDB:	WATER-DAMAGED BUILDINGS
VIP:	VASOACTIVE INTESTINAL POLYPEPTIDE

BACKGROUND:

Chronic inflammatory response syndrome (CIRS) is a chronic, progressive, multisystemic, multisymptom syndrome characterized by exposure to biotoxins, HLA genetic predisposition, altered innate and adaptive immunity, peripheral hypoperfusion, and multiple hypothalamic-pituitary dysregulation[1]. This inflammatory dysregulation can affect virtually any organ system; if left untreated, it can become debilitating. In many cases of CIRS, even when patients are removed from the offending environment and treated with a stepwise protocol, their condition does not improve to the premorbid state. The most prominent feature of these treatment-resistant patients is their abnormally low plasma VIP[1]. However, many of these patients (>90%[4]) show a marked improvement and a reduction in symptoms after using VIP applied intranasally as the decisive step in the sequential treatment protocol.

In CIRS, executive cognitive symptoms abnormalities are found in more than 90% of patients[2]. As a single test, the previous use of NQ for (i) diagnosis and (ii) treatment of executive cognitive abnormalities [3, 4], but NQ only reflects volumetric abnormalities identified on a single sagittal view that show nuclear atrophy, cortical grey atrophy; and/or superior lateral ventricle dilation. Furthermore, NQ cannot show day-to-day changes based on therapy or recrudescence of brain injury. NQ cannot show the physiological basis of the observed injury.

The most significant physiological benefit, in particular of brain-injured patients and namely those with CIRS, is derived from a transcriptomic assay called Gene Expression: Inflammation Explained (GENIE). The results of GENIE and NQ tests have been separately studied in CIRS, resulting in multiple peer-reviewed publications. To date, evaluation of combining both tools into a single approach to CIRS has yet to occur. NQ and GENIE can function as screening tests; and both show value in diagnosing and following the treatment of executive cognitive dysfunctions. GENIE alone provides a mechanism in the peer-reviewed literature to assess recent relapse after therapy. Changes in NQ such as cortical grey atrophy (CG), superior lateral ventricle enlargement (SLV), and an increased number of grey matter nuclear atrophy (NA) have been identified as showing prior exposure that led to the causation of the disease in CIRS. Specific causation has been difficult to confirm until the advent of the use of GENIE in a diagnostic model[11] previously derived.

However, the pathophysiology underlying these abnormalities is poorly understood. Inflammation has long been advocated as the cause of these brain injuries, but there is little evidence demonstrating direct benefit from the therapeutic use of anti-inflammatory therapies alone. Unlike the effects of inflammation, or 'brain on fire', if metabolic acidosis is found[5], the risk of increased nuclear atrophy, or 'brain on ice," appears as well. Correction of metabolic acidosis caused by oxidative glycolysis, as in the Warburg effect[6], corrects energy deprivation caused by blocking the uptake of pyruvate across the outer membrane of mitochondria, either with voltage-dependent anion channel closure (VDAC) or reduction of the function of transferases [7,8] and the delivery of pyruvate into the mitochondrial matrix, correcting metabolic acidosis can restore mitochondrial ATP production. Furthermore, aerobic glycolysis has been shown of be protective to neurons and initiates proliferative physiology in the injured state.[12] The twin engines of neuroinflammation and aerobic glycolysis appear to be underlying the mechanisms of injury and repair observed in the NQ CIRS population.[i]

With the advent of GENIE, a commercially developed transcriptomic assay, in 2019,[10] RNA measurement showed that cellular dysfunction could be identified by suppression, affecting mitochondrial and ribosomal genes. GENIE reports on 188 targeted genes involved in apoptosis, insulin resistance, coagulation, cytokine and TGFBR production, as well as mitogen activation of protein kinases (MAPK), cytoskeleton activity, T regulatory cell suppression, and problems with antigen presentation. Gene activation also showed genetic markers from environmental exposure to mycotoxins, endotoxins, and Actinobacteria. In addition, GENIE shows a specific causation of the disease for endotoxins and Actinobacteria. Furthermore, unique physiological measurements were considered in the brain injury problem, and new therapies have been developed, especially those exposed to Actinobacteria[11], to correct the abnormal physiologies demonstrated by GENIE.

The most important is molecular hypometabolism (MHM), where a reduced amount of RNA delivered to ribosomes that then affects protein production, compounded by a reduction in pyruvate delivery in the central nervous system, which affects the function of sodium-potassium pumps needed to continue proper brain signaling. Furthermore, MHM is often associated with exposure to nonspecific low molecular weight biotoxins, mainly produced by Actinobacteria,

which cause vascular inflammation and immune dysfunction associated with metabolic injury[11].

Combining GENIE with NeuroQuant gives us a new perspective on the brain. At the same time, the use of GENIE reveals a unique view of brain injury, showing that nuclear atrophy is highly associated with MHM. There are specific immune abnormalities related to exposure to Actinobacteria and a response of activation of the receptor for transforming growth factor beta (TGFBR-1, -2, -3) to the superior lateral ventricles and an inflammatory response of the activating bacteria, we can conclude a specific causation with immune reactivity for Actinobacteria[13].

An important insight has also emerged from NQ and GENIE. The degree of brain injury appears to be associated with a worsening of the MHM associated with Actinobacteria in the environments where patients live or work. Further diagnostic and therapeutic advances have accompanied a greater understanding of the role of exposure to endotoxins and enlargement of the SLV. Using NeuroQuant and GENIE, the tests provide clear indications for different therapies needed to improve the volume of brain structures and the physiology of brain mechanisms.

In 2017[4], we showed the ability of the intranasal vasoactive intestinal polypeptide (VIP) to correct multinuclear grey matter atrophy in patients with CIRS. We extend those findings in this article to show that other abnormalities, namely ribosomal gene activation and nuclear encoded mitochondrial gene activation, present as defining elements of CIRS in GENIE, but many more abnormalities in GENIE are also associated with volumetric changes in patients with CIRS by NeuroQuant.

We can now provide new information on the management of cognitive impairment in patients with CIRS by restoring regulation of gene expression and correction of nuclear atrophy using unique therapies, including therapeutic administration of vasoactive intestinal polypeptide (VIP) intranasally. Treatment with VIP has already been evaluated by transcriptomic sequencing (mRNA-Seq) demonstrating an improvement in differential gene expression that matched the improvement of the patient[9]. The most notable changes in gene regulation were found in ribosomal and mitochondrial activity. Ryan et al.[10] discussed the role of microbial toxins that cleave or modify ribosomal RNA in the conserved sarcin-ricin loop in the functional ribosome, rendering the ribosome ineffective. Furthermore, the authors posited that since mitochondria also have their ribosomes that contain the evolutionarily conserved sarcin-ricin loop, these mitoribosomes may also be vulnerable to microbial toxins.

Finally, successful treatment of CIRS can be measured by a combination of NQ and GENIE. NQ reveals: 1) correction of volumetric abnormalities of grey matter, 2) reduction in the size of the superior lateral ventricle (SLV); and 3) and correction of cortical grey atrophy (CG) by reducing the inflammatory proteomic response induced by exposure to biotoxins. GENIE further defines the transcriptomic changes associated with these volumetric abnormalities. This combination brings the use of GENIE to the bedside and reveals physiological abnormalities as the source of cognitive problems in patients with CIRS.

METHODS: Compounding the VIP

VIP was compounded by Hopkinton Drug, Hopkinton, Massachusetts, at 500 mcg/ml, providing 50 mcg/0.1 ml as a single dose. The peptide was dissolved in sterile saline for irrigation, to which 1% glycerin USP was added to prevent aggregation and help preserve protein structure. All glassware and equipment used were disinfected with 70% isopropyl alcohol before use and rinsed with a sterile saline solution. The nasal sprayer used is manufactured by Aptar Pharma and is compliant with all requirements of UDP <601>. Despite passing a 90-day stability study, the product is dispensed with a 60-day beyond the usable date (BUD) under refrigeration to guard against failures in patient compliance with handling requirements. Patients are instructed to keep the product under refrigeration at such a temperature that repeated freezing and thawing will not occur. The pH of the finished solution was between 6.1 and 6.2. To determine the consistency of the compounded preparation, the pH was evaluated over ten weeks while kept in refrigerated conditions as recommended to patients by Hopkinton Drug; Given the predicted isoelectric point of the peptide (9.82 calculated with ExPASy pI calc.), a pH of -6 is advantageous for both the stability of the peptide and the suitability of the solution as a nasal spray.

The VIP peptide is prepared by conventional solid phase chemistry, with a residual solvent content of DMF, TFA, methylene chloride, and acetonitrile well below the acceptable range according to the United States Pharmacopeia (USP) <497>. Therefore, many VIPs were pulled and these impurities were detected concurrently with the patient's dispensation. In addition, the lot was considered by circular dichroism for the confirmation of the peptide.

METHODS: Use of a transcriptomic test named GENIE:

The GENIE test demonstrates the amounts of mRNA for 188 genes found to be abnormal in CIRS cases compared to controls matched by age and gender, including ribosomal genes and nuclear encoded mitochondrial genes. Down-regulation of mRNA for these genes has been termed molecular hypometabolism (MHM) since 2016. Additional genes of interest in GENIE that have been reported in the peer-reviewed literature on CIRS include cytokines; TGF beta-1 receptors 1, 2, and 3; coagulation elements; genes involved with apoptosis and necroptosis; CD3D and CD48 associated with T-cell receptors; beta tubulins TUBB1 and TUBA4A; and ribosomal stress response genes, including specific indicators of exposure to Actinobacteria and endotoxins, MAPK; and genes CD14 and Toll receptor 4, respectively. Although the treatment of CIRS cases with a published protocol results in resolution of these gene abnormalities, it is unknown what role these genes play in cases of central nervous system injury.

Standard transcriptomic methods used since 3/19 were used in all study subjects. Statistical analysis was performed according to the Excel package at docs.google.com. An alpha score of <.05 was considered significant.

METHODS: Transcriptomics Assay
RNA extraction

Venous blood was drawn from the arms into PAXgene RNA blood collection tubes (http://www.preanalytix.com/product-catalog/blood/rna/products/paxgene-blood-rna-tube/), incubated for four hours at room temperature, then frozen at -80 ° C until RNA extractions were performed. Total RNA was extracted with the Qiagen PAXgene Blood miRNA system kit according to the manufacturer's protocol. Total RNA was analyzed using an Agilent 2100 bioanalyzer (Agilent Technologies, USA) for RNA integrity and then quantified using a NanoDrop NS-2000 (Wilmington, DE). Only samples with Agilent RIN scores $\geq$ 8 were used for sequencing.

METHODS: Transcriptomic Analysis Using Nanostring

We used a Nanostring digital analyzer to measure gene expression with a custom probe set developed by ProgeneDX as a research-use-only assay called GENIE. The GENIE test was designed to test for CIRS. Specific metabolic gene names were anonymized due to confidentiality restrictions. GENIE contains 174 genes of research interest and 14 housekeeping genes for normalization. Approximately half of the research genes (80) in the assay comprise a metabolic panel with multiple probes averaged using a geometric mean to describe gene expression for the following elements: large ribosomal subunit (17 probes), small ribosomal subunit (14 proves), large mitoribosome subunit (8 probes), small mitoribosome subunit (7 probes), ATP synthase (8 probes), Cytochrome C oxidase (8 probes), mitochondrial inner and outer translocases (8 probes) and NADH dehydrogenase; ubiquinone (10 probes). Two hundred nanograms of total RNA were used as input material, and the GENIE assay was performed according to standard protocols for the Nanostring digital analyzer platform.

METHODS: DATA ANALYSIS

The samples for GENIE were compared with a control database of 70 healthy, normal adult GENIE results. All samples were normalized using the geometric mean of the 14 housekeeping genes. The results of the metabolic panel were achieved using a simple ratio of the metabolic scores (geometric mean of the probe groups of probes above) divided by the normal, healthy, and control average. For the remaining 94 genes assayed in GENIE, the standard deviation of the control group was used to generate a z score for subjects.

Patients with GENIE were deidentified, known to researchers only by numbers, matched to NQ studies using the same unique identifier number used for GENIE.

The results were derived by entering the data into an EXCEL file from the results of the GENIE and NQ testing. GENIE was performed by identifying a specific Stage, i.e., 1 was untreated; 2 was treated according to a published protocol; 3 was after VIP treatment; 4 was off all medications with resolved illness; and 5 was confirmed relapse. Patients in Stage 1 were stratified into NQ parameters that could be associated with specific GENIE findings. Environmental testing was not available for all patients.

RESULTS:

Table 1:

GENIE					
CASES N= 1576	**Female Gender**	%	**Male Gender**	%	
	967	64.1	537	35.6	
NQ CASES N= 580	354	61.0	226	39.0	
Hypometabolism					
	NOT PRESENT		**PRESENT**		
1576	488	35.6	876	64.4	
	492	**31.2**	667	**42.3**	
GENIE patients - Age Distribution (Years)					
0-17	**18-30**	**31-45**	**46 - 65**	**66-70**	**>71**
77	**198**	**398**	**563**	**267**	**85**
4.9%	**12.6%**	**25.3%**	**35.7%**	**16.9%**	**5.4%**

Table 2 :

Superior Lateral Ventricle	**NORMAL**		%		**Enlarged**		%
	441		75.5		143		24.5
Nuclear Atrophy	**0**	**1**	**2**	**3**	**4**	**5**	**6**
	14	71	136	130	120	59	44
	2.4%	24.5%	23.4%	22.4%	20.6%	10.9%	7.5%.

Cortical Grey Atrophy N=1576 NUCLEAR ATROPHY	**0**	**1**	**2**		
	376	124	67		
	65.8%	21.7%	11.7%		
	1	**2**	**3**	**4**	**5**
	663	358	103	45	22
	55.2%	29.8%	8.5%	3.7%	1.8%

Taking selected findings, in Category 1 of cortical grey atrophy, endotoxin is positive in 41 subjects (CD14 + 28 and Toll-like Receptor 4 13 subjects, respectively). Positive cytoskeleton, TUBABA + present in 101 subjects and TUBBI + in 104. Of + cytoskeleton and + nuclear atrophy, 2-4 represented all subjects. Of the cytoskeleton (-), N= 376, nuclear atrophy 2-4 accounted for 119. Risk of 2-4 nuclear atrophy from cytoskeleton +/ (-) = 100/3.

In cortical grey and SLV, 257 subjects had (-) CG, 1 having superior lateral ventricle enlargement. Eight of the + CG group of 84 subjects had enlarged SLV. For cortical grey atrophy and grey matter nuclear atrophy counted as 5 or 6, N = 66, or 25.6%.

Within the cohorts of GENIE tested, there was a consistent percentage of subjects with nuclear atrophy sorted by age. We also saw that the distribution of cortical grey was similar in various cohorts at 65.8% for 0, 21.7% for 1, and 11.7% for 2.

DISCUSSION:

GENIE and NeuroQuant are two different diagnostic tools used in clinical practice.

GENIE is a transcriptomic platform for medical practitioners that was developed by the company ProgeneDx. It is primarily used for the diagnosis and management of CIRS patients. GENIE can integrate various types of medically significant molecular genetic data, including to provide physicians with detailed information about the patient's clinically significant genetic profile. This information is invaluable for differential diagnoses of overlapping clinical pictures when separating mold-related illnesses from more bacterially related syndromes and even clarifying pictures such as seen in post-Lyme, plus defining other manifestations such as molecular hypometabolism (MHM).

NeuroQuant, on the other hand, is a software tool developed by CorTechs Labs that is specifically designed to analyse brain magnetic resonance images. It uses advanced algorithms to automatically segment different regions of the

brain and quantify their volumes. It can also detect subtle changes in brain structure that may be associated with certain neurological disorders, such as Alzheimer's disease, multiple sclerosis, or traumatic brain injury. NeuroQuant can provide clinicians with quantitative data on brain structure that may be useful for making a diagnosis, monitoring disease progression, or assessing the effectiveness of treatments.

Both GENIE and NeuroQuant are examples of how software tools are being used to improve medical imaging and improve patient care. By providing clinicians with more detailed and quantitative information about a patient's anatomy and brain structure, these tools can help improve the accuracy of diagnoses and treatment planning and ultimately lead to better patient outcomes.

In this study GENIE and NeuroQuant showed consistent abnormalities among each group, leading to (i) diagnosis; and (ii) treatment. These interventions were directed by the physiologic abnormalities demonstrated. For example, given that nuclear atrophy > 3, TGFBR 1, 2, or 3 in association with MAPK, or one or more cytoskeleton abnormalities are indicators of the specific immune reactivity of Actinobacteria, which should direct a specific sample of suspect buildings for Actinobacteria. Similarly, in cortical grey atrophy, the probability of specific immune reactivity caused by endotoxins (+ CD 15 or + TOLL 4) increases markedly in the presence in settled dust of specific measurement of elevated levels of bacterial endotoxins. SLV dilation was an uncommon finding, with endotoxins and Actinobacteria equally reported. We have tracked therapies designed to correct GENIE for efficacy in correcting NQ. In parallel, our correction of nuclear atrophy was marked by a correction of GENIE.

In the field of indoor industrial hygiene, there is little focus on sick patients compared to focus placed on remediation and correction of water intrusion. With our findings of association with exposure and brain injury to specific markers, vague but persistent symptoms of “brain fog” can be reduced using sophisticated tests such as GENIE and NQ. Patients with persistent symptoms no longer have to endure being diagnosed with a psychiatric illness or being labeled as manipulative, i.e., making up their illness.

The importance of this retrospective observational study is to bring order to the diagnosis and treatment of CIRS associated with exposure to moist or damp buildings, as many unsupported claims for therapies have been advocated, especially the use of antifungals[13, 14] despite the absence of a case definition or confirmation of causation using prospective study designs. Use of antifungals for unproven reasons runs the risk of inducing drug resistance in fungi[15], particularly Candida auris[16].

Finally, those individuals who have been exposed to the interior of WDB and have suffered personal injury, but have not been treated for CIRS, no longer have to file a lawsuit to recover their losses. Reports of legal awards in the millions of dollars are not uncommon. If a careful delineation of the physiological basis of the disease is performed, using peer reviewed protocols for initial treatment, followed by correction of brain injury, such massive awards would be far less common.

We have seen the correlation of GENIE and NQ with specific microbiological contaminants found in WDB. This finding has expanded the definition of CIRS cases to include both NQ and GENIE as objective biomarkers. We propose that the identification of markers of brain injury, followed by definitive treatment, with verification of successful treatment, be included in all patients with CIRS.

Finally, we propose that patients with an unproven diagnosis of dementia, in the face of exposure to a WDB, have both NQ and GENIE performed so that seemingly untreatable cases of dementia, including Alzheimer’s, may be separated from treatable cases of dementia.

CONCLUSIONS:

In this retrospective observational study, we observed patterns of transcriptomic abnormalities that were seen to match abnormalities in NQ. This study revealed previously unreported evidence supporting the conclusion that these gene abnormalities responded to VIP therapy in patients with NQ abnormalities. These data will be presented in a subsequent study.

In addition, we conclude that use of NQ as a diagnostic and screening test should be correlated with GENIE, in patients with complex diagnostic pictures and on the basis of the evidence these diagnostic procedure contributes, the use of VIP may be indicated. Furthermore, the unconfirmed benefits of antifungals for CIRS can be discarded before excessive financial expenses occur.

A weakness of this report is the lack of NQ data for each patient with GENIE results.

Furthermore, we did not have access to treatment results for the majority of patients.

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Competing interests: Dr. Shoemaker is a shareholder in ProgeneDx, the company that developed and sells GENIE.

REFERENCES:

[1]Shoemaker R, Heyman A, Mancia A, Ryan J. Inflammation induced Chronic Fatiguing Illnesses: A steady march towards understanding mechanisms and identifying new biomarkers and therapies. *Internal Medicine Review* 2017; 3: 1

[2]Shoemaker, R, Johnson K, Jim L, Berry Y, Dooley M, Ryan J, McMahon S. Diagnostic process for Chronic Inflammatory Response Syndrome (CIRS): A Consensus Statement report of the Consensus Committee of Surviving Mold. *Internal Medicine Review* 2018. 4: 1-47.

[3]Shoemaker R, House D, Ryan J. Vasoactive intestinal polypeptide (VIP) corrects chronic inflammatory response syndrome (CIRS) acquired following exposure to water-damaged buildings. *Health* 2013; 396:401

[4] Shoemaker R, McMahon S, Ryan J, et al. Intranasal VIP safely restores volume to multiple grey matter nuclei in patients with CIRS. *Internal Medicine Review* 2017; 1: 1-21.

[5]Shoemaker R. Metabolism, molecular hypometabolism and inflammation: Complications of proliferative physiology include metabolic acidosis, pulmonary hypertension, T reg cell deficiency, insulin resistance and neuronal injury. *Trends in Diabetes Metab* 2020; 3: 1-15.

[6] Vander Heiden M, Cantely L, Thompson C. Understanding the Warburg Effect; The metabolic requirements of cell proliferation. *Science* 2009; 324: 1029-1033.

[7]Shoshan V, Keinan N, Zaid H. Uncovering the role of VDAC in the regulation of cell life and death. *Biochem Pharmacol* 2020; 40: 183-91.

[8]Heslop K, Rovin A, Hunt E, Fang D, Morris M, et.al. JNK Activation and translocation to mitochondria mediates mitochondrial dysfunction and cell death induced by VDAC opening and sorafenib in hepatocarcinoma cells. *Biochem Pharmacol* 2020; 171: 113728.

[9] Ryan J, Shoemaker R. RNA-Seq on patients with chronic inflammatory response syndrome (CIRS) treated with vasoactive intestinal peptide (VIP) shows a shift in metabolic state and innate immune functions that coincide with healing. *Medical Research Archives*, 2017, 4: 1.

[10] Ryan J, Wu Q, Shoemaker R. Transcriptomic signatures in whole blood of patients who acquire a chronic inflammatory response syndrome (CIRS) following an exposure to the marine toxin ciguatoxin. *BMC Medical Genomics* 2015; 8:15.

[11] Shoemaker R, Neil V, Heyman A, van der Westhuizen M, McMahon S, Lark D. Newer Molecular methods bring new insights into human and building health risk assessments from water-damaged buildings: Defining exposure and reactivity, the two sides of causation of CIRS-WDB illness. *Medical Research Archives* 2021; 9:

[12] Jones, Williams and Bianchi, Kanisicak. Aerobic Glycolysis: Beyond Proliferation. *Frontiers in Immunology*. 2015; 6: 227.

[13] Kawamoto M, Page E. Notes from the field; use off unvalidated urine mycotoxin tests for the clinical diagnosis of illness-US 2014. *MMWR* 2015;64: 157-158.

[14] Shoemaker R, Lark D. Urinary mycotoxins: A review of contaminated buildings and food in search of a biomarker separating sick patients from controls. Int Med Rev 2019; 5(6): 1-35.

[15] Toda M, Beer K, Kuivila K, Chiller T, Jackson B. Trends in agricultural triazole fungicide use in the United States, 1992-2016 and possible implications for anti-fungal-resistant fungi in human disease. *Env Health Perspect* 2021; 129::055001-1-2.

[16] Rubin R. On the rise, Candida auris outwits treatments and travels incognito in health care settings. *JAMA* 2023; 329: 197-199.

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