

## 1 **Are You Missing Mold Illness?**

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## 2 **The Testing Conundrum**

- Are You Missing Mold Illness In Your Patients?
- Dr. Jill Crista

## 3 **Testing Conundrum**

- Diagnostic Assessments
- Mycotoxin Discussion
- Shoemaker Panel Highlights
- Colonization, Allergy, Infection
- Advances In Imaging

## 4 **Diagnostic Assessments**

## 5 **Conundrum ~ Which? When? Reliability?**

- Decision points ~
- Info req'd to guide tx
- Baseline
- Pt request
- Buy-in ~ pt/fam
- "Proof" ~ Ins/occup/landlord

- Comfort w existing models
- Accuracy
- Cost
- Reassess tx progress
- 

## 6 **Diagnostics ~ Tier 1**

- Tier 1 purpose - is it mold?
- DIRECT
- Urine mycotoxin - LC/MS
- Comprehensive stool test
- INDIRECT+HIGH CORRELATION
- Visual Contrast Sensitivity (VCS)
- Serum mycotoxin antibody
- Urine mycotoxin - ELISA
- Organic acids test
- NK cell function (diff than total)  
Lytic units, <7 abn  
Quest
- NK cell total (freq normal)

## 7

- 2,4,5 ~ Aspergillus
- 4,5 ~ Colonization
- 6 ~ Aspergillus & Candida
- 9 ~ Fusarium

## 8

- VCStest.com \*calibrate screen first!\* Technically pass/fail but the higher the score the better.
- The maximum biotoxin score is 18 in each eye and 36 for both eyes.
- Columns A, B, - green is good, red means lack of ability to see the contrast (nutritional, glyphosate, SIBO)

- Columns C, D - blue is good (mold/biotoxin illness, insect venom, cyanobacteria, dinoflagellates-esp Pfiesteria and Ciguatera, Lyme+coinfections, parasites, tobacco use\*, some VOCs)
- Column E - green is good (biotoxin, metals, tobacco use\*, alcohol, lead, VOCs, age, low socioeconomic status)
- Columns D, E - worse with Herx, detox

9 

- 2,4,5 ~ Aspergillus
- 4,5 ~ Colonization
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10 

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11 

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12 

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13 

- 19, 20, 21 ~ Incr oxalate metabolites - yeast, mold, food
- 

14 

- 58 ~ Glutathione status
- 59 ~ Methylation ability
- 

## 15 Diagnostics ~ Tier 2

- Tier 2 purpose - how bad is it?
- CBC
  - Fe-def anemia
  - ↓WBC~relative↓lymph↑neutr↑eos
- CMP
  - ↑ALT, AST, GGT(↓GSH), ↑bilirubin, ↓GFR, ↓albumin, creatinine>1.0
- Vit D (25-OH)↓
- Vit D (1,25)↑ (↓Intracellular GSH?)
- hs-CRP↑
- RBC glutathione↓
- Urinalysis
  - +blood, yellow-brown/yellow-green(bili), (-)leuko, ↑urobilinogen

## 16 Diagnostics ~ Tier 2

- IgE mold + IgG/M/A candida
  - \*not useful for mycotoxins
- IgG/IgA total & subclasses
  - IgG subclass III def
    - potent pro-inflam aby
    - first to respond to viral infxn
    - gliotoxin (disulfide bonds)
- Lymphocyte Subset Panel
  - T- and B-cell total↓
- IL-6↑ → IL-10↑, TNF↑
- ANA↑
- BDG (Fungitell) Quant\*↑
- Galactomannan\*↑

## 17 Diagnostics ~ Tier 2

- Food allergy  
IgG/IgA (yeasts, peanuts, coffee, mushrooms, corn, potatoes, grains)
- Kidney health  
microalbumin/creatinine ratio >30  
combo w creatinine >1 = concern
- ADH <1
- Copeptin 4-14
- EKG  
2nd-degree AV block, atrial brady, supraventricular extrasystole, ventricular extrasystole
- Genetic mold canary  
HLA DR/DQ  
DRB1, DQB1, DRB3-5  
Detoxification snps

## 18 Diagnostics ~ Tier 3

- Tier 3 purpose ~ clarification
- Shoemaker labs (next section)
- SIBO breath test
- Ferritin & clotting ~ mixed results
- Venous blood gasses (no tourniquet!)
- MCAS labs  
Mayo ~ Urine 24-hr  
MUST be chilled to certain temp asap  
-Methylhistamine or MIA  
-PGD-2  
-17-Beta-PGF-2-alpha  
Diff to get right, pre-arrange w lab  
Not all have this capacity  
First r/o mastocytosis w tryptase

## 19 Diagnostics ~ Differentials

- B12 & MMA (B-12 def)

- Lyme & co-infections\*
- Lung CA/Mesothelioma
- GI ~ UC/Crohn's
- Liver/kidney CA
- Other autoimmune dzs
  - Celiac
  - SLE
  - Scleroderma
  - Sjogren's
- MCAS
- Alzheimer's, Parkinson's
- Glaucoma ~ eye exam
- Alcoholism

## 20 **STORY | Not An Alcoholic**

- Man late 50s
- Dr suspects alcoholism
- Hi GGT, AST/ALT on the rise
- BS on the rise, chol dropping 115
- Onset RUQ pain
- Episodes N/V
- Obese, pre-diabetic
- Worsening lethargy
- No alcohol ingestion, verified by wife
- Wife says lazy after kids moved out & got his man-cave
- Close door & use window air conditioner "always hot"
- Cool sanctuary, often falls asleep at his desk
- Window air conditioner was full of mold
- Mycotoxins mimicked alcohol
-

• \* \* \*

## 21 **Mycotoxin Discussion**

## 22 **The Path of the Mycotoxin**

- In WDB exposure ~  
Inspiration  
Absorption - sinus mucosa to lung alveoli  
Carried via blood  
Liver & Kidney  
Kidney - filtration  
Liver - bound to bile and delivered to lumen  
Left-over absorbed into lipid-rich tissue for later mgmt
- Why test urine?  
Filtrate of blood
- Why test serum?  
Blood & serum antibody reactions
- Note 1: Ingested mycotoxins may remain unbound in lumen
- Note 2: No data on mycotoxin secretion in sweat

## 23 **Mycotoxin Detection Methods**

- The question is not which - it's if, when and how
- Methods currently in use in US ~  
Urine ELISA (enzyme-linked immunosorbent assay)  
Urine LC-MS (liquid chromatography with mass spectrometry)  
Serum antibody (IgE, IgG)
- Considerations ~  
Which mycotoxins are tested?  
Pt's immune status  
Pt's liver & kidney health  
Pt's supplementation  
Cost, coverage  
Compare to previous test  
Practitioner comfort  
Lab - certifications, ongoing independent validations, and willingness to share validation results

- PMID 32121036

## 24 **Mycotoxin Controversy**

- Mostly driven by insurance companies
- Source ~
  - Food - studies are mixed
  - Fat stores
  - Lifestyle - ie: smoking, coffee, alcohol
  - Certainly not WDBs
- Except, levels have been shown to decrease with removal from WDB
- Inducible ~  
Independent case study  
Urinary mycotoxins ↑10 fold, 4-6 hrs after sauna tx  
Courtesy Dr. Joseph Brewer
- PMID: 28240164

## 25 **Urine Mycotoxin Split Sample**

- "Members of three fungal genera, *Aspergillus*, *Fusarium*, and *Penicillium*, are the major mycotoxin producers [in food].
- While over 300 mycotoxins have been identified, six (aflatoxins, trichothecenes, zearalenone, fumonisins, ochratoxins, and patulin) are regularly found in food, posing unpredictable and ongoing food safety problems worldwide.
- In addition to concerns over adverse effects from direct consumption of mycotoxin-contaminated foods and feeds, there is also public health concern over the potential ingestion of animal-derived food products, such as meat, milk, or eggs, containing residues or metabolites of mycotoxins."

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## 27 **Mycotoxin Study**

- Sample ~  
Urine, sputum, tissue biopsy (lung/liver/brain)
- Mycotoxins tested ~  
Aflatoxin, Ochratoxin, Trichothecenes, Gliotoxin
- Findings ~  
Normal controls ~ no detectable mycotoxins in tissues or fluids  
WDB pts ~ detectable mycotoxins, varying degrees in tissues and fluids
- Why urine?  
Adequate and reliable method to detect mycotoxins (though may underreport trichothecenes)  
Least invasive  
Lowest cost
- Study limitation - author's possible conflict of interest
- PMID: 19468319

## 28 **Urine Mycotoxin Split Sample**

- "This study was conducted to investigate mycotoxin exposure in 260 rural residents (age 18-66 years; mean age 36.9 years, average BMI was 23.0kg/m<sup>2</sup>) in Nanjing, China. All participants were healthy and free from chronic diseases.
- Paired plasma and first morning urine samples were analyzed for 26 mycotoxin biomarkers, including 12 parent mycotoxins and 14 mycotoxin metabolites, by an ultra-high-performance liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) method.
- Individuals with previous medical records indicating liver, kidney or other metabolic problems were excluded from this study. "

## 29 **Urine Mycotoxin Split Sample**

- "In the plasma samples, [95 out of 260] 36.5% of samples were found to contain mycotoxins.
- OTA was the most prevalent one (incidence of 27.7%) and its concentration ranged from 0.312 to 9.18mg/L.

- AFB1-lysine, FB1, DON, ZEN and ZAN were also detected in plasma with incidences of 19.6%, 2.7%, 2.3%, 6.5% and 1.2%, respectively."

### 30 **Urine Mycotoxin Split Sample**

- "In the urine samples, one or more mycotoxins were detected in 144 out of 260 (55.4%) participants.
- DON-15-GlcA (incidence of 43.8%), a urinary metabolite of DON, was the most abundant mycotoxin in urine samples; its concentration ranged from 0.828 to 37.7mg/L (0.694e37.3mg/g Cr).
- AFM1, OTA, FB1, T-2, DON, DON-3-GlcA, ZEN and ZAN were detected in 10.4%, 1.2%, 3.1%, 2.3%, 10.0%, 15.8%, 6.9% and 7.7% of the urine samples, respectively.

### 31 **Does Gender Matter?**

- "42.7% were female and 57.3% were male. There was no significant difference in age and BMI between males and females ( $p > 0.05$ ).
- The incidence and concentration of mycotoxins in males and females were slightly different.
- Compared to females, males presented higher levels of plasma FB1, plasma DON, urinary T-2, urinary DON-3-GlcA, urinary DON-15-GlcA, and urinary ZEN,
- but lower levels of plasma AFB1-lysine, plasma ZEN, urinary OTA, urinary DON and urinary ZAN.
- However, the differences of the mean mycotoxin concentrations between male and female were not significant ( $p > 0.05$ )."

### 32 **Urine ELISA Mycotoxin**

- Established use for 15 years
- Indirect measure
- The idea - due to the body's ability to modify mycotoxins, antigen detection vs molecular matching will catch more metabolites and give a better view of body burden
- Strengths ~  
 Detect both the mycotoxin in pure form and metabolites of mycotoxins due to common antigens on most modified forms  
 Levels correlate to symptoms in majority of my patients ("bell-curve")

- Challenges ~
  - Not controlled for creatinine
  - Antigen selection by lab
  - Non-specific reactions (aka background noise) w poss false-positives
  - Varying accuracy for pts w issues detoxing and excreting
  - Doesn't help answer the question of whether currently being exposed
  - Unknown degree of contamination via ingestion

### 33 **Urine LC-Mass Spect Mycotoxin**

- Gold standard for small molecules
- Direct measure
- The idea - molecular identification as direct detection of the presence in the urine
- Strengths ~
  - Controlled for creatinine
  - Specific metabolites of mycotoxins can be tested and reported as an individual finding, then grouped for a bigger picture
  - Levels correlate to symptoms in majority of my patients ("bell-curve")
- Challenges ~
  - Some of the molecules are similar in structure, peak together, leading to possible cross-reporting
  - May miss metabolites if not specifically identified as a structure to monitor
  - Extraction method to prep sample varies
  - Varying accuracy for pts w issues detoxing and excreting
  - Doesn't help answer the question of whether currently being exposed
  - Unknown degree of contamination via ingestion

### 34 **Serum Mycotoxin Antibody**

- New kid on the block commercially
- Indirect measure
- The idea - the mere presence of a mycotoxin not as important as knowing what the body thinks about it

- Strengths ~
  - Not an excretion test
  - Detection of metabolites of mycotoxins that share common antigens
  - IgE helps to answer whether it's a current exposure
- Challenges ~
  - Antigen selection by lab
  - May miss metabolites if antigens have been modified
  - Immune status of the pt
  - IgG remains positive for up to 6 months
  - Unknown degree of contamination via ingestion

### 35 **Urine Mycotoxin Split Sample**

- “Three extraction methods, namely salting-out liquid–liquid extraction (SALLE), miniQuEChERS (quick, easy, cheap, effective, rugged, and safe), and dispersive liquid–liquid microextraction (DLLME), were evaluated and compared based on analytical parameters for the quantitative LC-MS/MS measurement of 11 mycotoxins (AFB1, AFB2, AFG1, AFG2, OTA, ZEA, BEA, EN A, EN B, EN A1 and EN B1) in human urine.
- DLLME was selected as the most appropriate methodology, as it produced better validation results for recovery (79–113%), reproducibility (RSDs < 12%), and repeatability (RSDs < 15%)

### 36 **Random Urine Adequate?**

- 
- Comparison study, in-house
- LC-MS method
- Creatinine controlled
- 3 variations ~
  - First-morning, 6 hour, 24 hour
- Results ~
  - Positives remained positive
  - Negatives remained negative
  - Values not necessarily the same, varied by mycotoxin

### 37 **Split Sample - Mass Spect vs ELISA**

### 38 **Split Sample - Mass Spect vs ELISA**

### 39 **Questions Raised**

- Controlling for creatinine ~  
Does this explain the differences in OTA results?  
To what degree does creatine supp affect creatinine clearance?
- Sweating/exercise ~  
Was twin 2 more detoxed bc of exercise?  
Are the results of mass spect falsely lower bc of "detox bolus" the night before when worked out?...OR...
- Glutathione administration ~  
Falsely lower the mass spect results?
- Is the bigger issue "normal ranges"? How are these determined?  
ie: OTA 1.10 (1.8-2.0) vs 9.19 (1.2-5.0)  
ie: OTA 1.51 (1.8-2.0) vs 5.27 (1.2-5.0)
- Is there such a thing as a test with all zeros?  
(answer - YES!)

40  **Split Sample - Baseline Twin 1**

41  **Split Sample - Mass Spect Methods**

42  **Split Sample - Mass Spect Methods**

43  **Split Sample - Mass Spect Methods**

44  **Building Test Results**

- 
- 
- ALL CLEAR currently!
- 
- But had moved 4 mo prior from place with Fusarium in HVAC.

45  **Split Sample - Baseline Twin 2**

46  **Split Sample - Mass Spect Methods**

47  **Split Sample - Mass Spect Methods**

48  **Split Sample - Mass Spect Methods**

49  **Building Test Results**

- 
- Fusarium in HVAC

- 
- Asp/Pen in flooring around bathroom shower

#### 50 **More Questions Raised**

- MPA ~  
Not only from Pen. Also from Asp. Reporting on both labs confusing linkage to only Pen.  
Why high on one but not the other?  
What is the population norm for MPA?
- Is second method catching more because of expanded metabolites?

#### 51 **Urine Mycotoxin Split Sample**

#### 52 **Questions Raised**

- To what degree does Candida overgrowth alter urine mycotoxin labs?
- Gliotoxin also formed from Candida
- Are Candida/Rhodotorula cause or protective effect of mold exposure?
- Which mycotoxins are detoxed with which natural remedies?

#### 53 **Mycotoxin Negative but Mold-Sick**

- Not testing all mycotoxins
- Confounding factors before sample~  
Random urine  
Exercise  
Sauna  
Glutathione admin  
Acute viral challenge
- Exposure duration too limited
- Intimate contact/parental toxification

#### 54 **Glutathione Pre-Assessment**

#### 55 **Urine Mycotoxin Pre-Testing Guidelines**

- 2 days minimum before test ~  
Avoid ingestion of mold/mycotoxin containing foods, bevs, supps, meds  
Avoid binders  
To provoke or not? If so, how?

- Do not fast more than physiological
- Morning of test ~
  - Collect first-morning's urine
  - Avoid food/water prior
  - Avoid exercise
  - Avoid intercourse
  - Avoid sauna and hot shower

56  **Serum Mycotoxin Antibody**

57  **Serum Mycotoxin - Urine Mycotoxin LC-MS**

58  **Serum Mycotoxin Antibody**

59  **Serum Mycotoxin Antibody**

60  **Serum Mycotoxin Antibody**

- "HPLC-MS/MS detection was used for the analysis of dried serum spots (DSS) and dried blood spots (DBS).
- Detection of aflatoxins (AFB1, AFB2, AFG1, AFG2, AFM1), trichothecenes (deoxynivalenol, DON; DON-3-glucuronic acid, DON-3-GlcA; T-2; HT-2; and HT-2-4-GlcA), fumonisin B1 (FB1), ochratoxins (OTA and its thermal degradation product 2'R-OTA; OT $\alpha$ ; 10- hydroxyochratoxin A, 10-OH-OTA), citrinin (CIT and its urinary metabolite dihydrocitrinone, DH-CIT), zearalenone and zearalanone (ZEN, ZAN), altenuene (ALT), alternariols (AOH; alternariol monomethyl ether, AME), enniatins (EnA, EnA1, EnB, EnB1) and beauvericin (Bea) was validated for two matrices, serum (DSS), and whole blood (DBS).

61  **Serum Mycotoxin Antibody**

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62  **Serum Mycotoxin Antibody**

- For most analytes, LOQs (limit of quantitation) in the lower pg/mL range and excellent recovery rate were achieved using matrix-matched calibration.
- Besides validation of the method, the analyte stability in DBS and DSS was also investigated. Stability is a main issue for some analytes when the dried samples are stored under common conditions at room temperature.
- This methodical study establishes a validated multi-mycotoxin approach for the detection of 27 mycotoxins and metabolites in dried blood/serum spots based on a fast sample preparation followed by sensitive HPLC-MS/MS analysis.

#### 63 Serum Mycotoxin Antibody

- The use of capillary blood from finger-pricks versus venous blood was evaluated. The analyte levels correlate indicating that the less invasive finger-prick sampling gives also reliable results.
- No significant hematocrit effect was observed.
- In this experiment, finger-prick samples typically consist of about 90  $\mu$ L blood. Therefore spots of 75, 100 and 125  $\mu$ L blood were prepared and analyzed. Similar to the hematocrit effect, no considerable influence was observed.

#### 64 Serum Mycotoxin Antibody

- "The results of this study revealed for the first time a high exposure of coffee consumers to 2'R-OTA, a compound formed from OTA during coffee roasting.
- Since little information is available regarding toxicity and possible carcinogenicity of this compound, further OTA monitoring in blood including 2'R-OTA is advisable."

#### 65 Mycotoxin Testing - Yes? No? How?

- Above all, be mindful of cost ~  
If you know it's mold, skip the test and tx  
And/or consider methods validated/ins coverage ie: Neuroquant
- ELISA Urine ~  
Immune deficient, able to detox, body burden, track tx
- LC-MS Urine ~  
Immune deficient, able to detox, body burden, track tx, liv/kidney creatinine clearance
- Serum antibody ~  
Excretion/detox-challenged, liv/kidney dz, IgE - is it a "now" issue?
- Be mindful of supplement use ~  
Binders, glutathione, creatine
- To provoke or not? Depends more on your pt than agent

- OR...maybe use a clinical questionnaire

## 66 Shoemaker Highlights

## 67 Shoemaker ~ Diagnostic Indicators

- Visual Contrast Test at VCStest.com  
Convergence disorder  
L/R eye visual processing in brain+eye m impairment
- TGF- $\beta$ 1 (transforming growth factor beta-1)  $\uparrow$   
Cytokine  
 $\uparrow$  impairs T-reg fxn  $\rightarrow$  immune overactivation/asthma  
Range <2380 pg/ml  
(Quest $\rightarrow$ Cambridge Biomedical)
- MMP-9 (matrix metalloproteinase 9)  $\uparrow$   
Tissue repair enzyme induces I/S stim  
Range ~ 85-332 ng/ml  
Drs. Patel/Farshchian - link to MCAS  
(Labcorp)

## 68 Shoemaker ~ Diagnostic Indicators

- ADH (vasopressin)  $\downarrow$   
Range ~ 1.0-13.3 pg/ml;  
Test in conjunction with blood osmolality ~ 280-300 mosmol
- VIP (vasoactive intestinal polypeptide)  $\downarrow$   
Neuro and cardio-reg hormone with receptors in the hypothalamus  
Range 23-63 pg/ml
- MSH (melanocyte stim hormone)  $\downarrow$   
Pituitary hormone w neurohormonal and anti-inflammatory actions  
Range 35-81 pg/ml

## 69 Colonization, Allergy, Infection

## 70 Culture Nares

- 2" depth
- Pediatric swab
- Off all nasal tx for 3 days

- +MRSA/+MARCoNS common
- Consider false neg if positive urine mycotoxins
- Specific lab (MARCoNS)
- 

## 71 Colonization

- Posterior Nasal Culture
  - Req posterior nasal wash
  - Fungus tenaciously adhered
  - Consider false neg if positive urine mycotoxins
  - PCR/DNA better yield
  - +MARCoNS
- Ophthalmology
  - Freq changing vision w sinus colonies (empirical exper)
- BDG (Fungitell) Quantitative (<60 pg/mL)
  - (1,3)-Beta-D-glucan
  - Invasive Fungal Infection (IFI)
  - Note: not present in Mucorales, Cryptococcus, Blastomyces
  - Note: false + if taking Beta-lactam antibiotic
- Galactomannan - (<0.5 index)
  - Less sensitive than BDG (81% vs 49%)
  - Charting tx of known Aspergillosis
  - BDG option if on Beta-lactams

## 72 Allergy

- Skin Testing
  - RAST limitations
  - Update ~ Intradermal Provocation Neutralization
  - Training req'd
  - Caution: ↑ potential of harm
  - Off all antihistamines\* incl supp

## 73 Infection

- Swab
  - Oral, vaginal/groin, perianal, intertrigo

- Tissue sample  
Skin, scalp, toenail
- Biopsy/culture  
Culture  
Tissue biopsy (sinus, lung) Varying yields  
Flexible laryngoscopy ~ sinus  
Transthoracic CT ~ fine needle aspirate lung nodules

#### 74 **Advances In Imaging**

#### 75 **Imaging**

- Sinus
  - X-ray
  - CT
- Chest
  - XRay  
(may not be abn in pulm fibrosis→CT)
  - High-res CT
  - Fiberoptic bronchoscopy

#### 76 **Imaging**

- Abdomen
  - Upper endoscopy
  - Colonoscopy
- Brain
  - MRI NeuroQuant
  - SPECT - volumetric

#### 77 **First 3 Rules of Toxic Exposure**

#### 78 **Review**

- Diagnostic Assessments
- Mycotoxin Discussion

- Shoemaker Panel Highlights
- Colonization, Allergy, Infection
- Advances In Imaging

79  **Thank You**

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