Development and Performance of Myco*MEIA*-*Aspergillus*: A New Urine Diagnostic for Aspergillosis

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ABSTRACT

Background: Despite availability of urine-based diagnostics for several fungal infections, past efforts to develop a urine diagnostic for aspergillosis failed.

Methods: An enzyme immunoassay (EIA) was developed using antibodies specific for galactofuranose (gal*f*) – containing glycans. Urines were collected from 310 people with suspected and confirmed invasive aspergillosis (IA), and from people with pulmonary aspergillosis (PA) complicating lung transplant and COVID. Serial urines were obtained from people at high risk due to heme malignancies. Samples were tested with Myco*MEIA*, blinded to clinical diagnoses, which were adjudicated using consensus criteria. Cut-offs for the EIA were determined in a validation cohort with ROC curves. Performance was calculated as per-case sensitivity and specificity. To characterize antigen in urine, mass spectrometry (MS) and transmission electron microscopy (TEM) were performed.

Results: MS and TEM demonstrated that mAb476 recognizes fungal extracellular vesicles (EVs) in *Aspergillus* culture filtrate and in urine from people with IA. ROC curves generated AUC of 0.97, sensitivity 90.5% (95% CI 70-99) and specificity of 91.9% (95% CI 92-99). Per-subject performance in 48 people with proven or probable IA (cases) and 50 controls at the index cut-off of 0.6 yielded sensitivity of 91.2% (95% CI 76–98) and specificity of 89.2% (95% CI 82–94). For screening, 131 urines were analyzed from 20 people with heme malignancies. 10/11 cases and no controls had at least one positive assay, yielding 91% sensitivity and 100% specificity. Positive Myco*MEIA* pre-dated clinical diagnosis in 6/10 patients, at mean 24.2 (range 2–62) days. The assay was 72% (95% CI 43–87) sensitive and 91% (95% CI 72–99) specific in 54 people with PA complicating lung transplant or COVID.

Conclusions: EVs carry gal*f*-specific antigens in urine, enabling Ab recognition in an EIA format. Myco*MEIA* showed good sensitivity and specificity for IA and PA complicating lung transplant or injury.

Myco*MEIA* - *Aspergillus*

- Uses mAbs specific to galactofuranose – containing glycans
- Used in a 96-well plate enzyme immunoassay format with preprocessing of urine through a resin in microfuge to eliminate an inhibitor
- Interpreted as a sample OD index = [OD sample / mean OD threshold control] multiplied by a factor based on the threshold control value

CONCLUSIONS

- From 310 people with suspected invasive fungal disease, per subject sensitivity for IA was 91.2% (95% CI 76–98%) and specificity was 89.2% (95% CI 82–94%), with semi-quantitative performance aligned with certainty of IA.
- From 54 people with airway aspergillosis complicating COVID-19 and lung transplant, sensitivity was 72% (95% CI 43–87%) and specificity was 91% (95% CI 72–99%).
- Cross-reactivity was observed in a limited number of people who had infection with Histoplasma, Fusarium, and Blastomycetes, consistent with galf-specificity.
- Myco*MEIA* – *Aspergillus* showed early positivity relative to conventional diagnoses in a limited cohort of people who were tested longitudinally. Positive urine tests pre-dated other findings by a mean of 24 days; with a prevalence of 10%, having a negative urine Myco*MEIA* and a negative serum GM EIA provides >95% negative predictive value that the patient does not have IA, supporting a screening algorithm.
- In several subjects tested, antigen is present in urine on EVs that are recognized by the parent mAb476.
- Myco*MEIA* – *Aspergillus* is CE marked and undergoing FDA studies currently. PearlDx plans to make the test commercially available in late 2023.

ACKNOWLEGEMENTS

- Myco*MEIA* – *Aspergillus* was developed with support provided by State of Maryland TEDCO, U.S. National Science Foundation, National Institutes of Health and the Cystic Fibrosis Foundation

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Poster #02624

33rd ECCMID EUROPEAN CONGRESS OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

Copenhagen, Denmark
15–18 April 2023

PERFORMANCE

Figure 1. Frequency histogram of Myco*MEIA* – *Aspergillus* index results of 900 urine samples tested from 310 patients

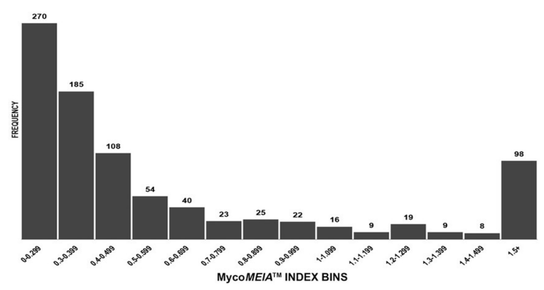


Table 1. Demographics, clinical risks, and adjudicated diagnoses of 310 patients with suspected IFI

Variable	N	%
Gender (M, %)	194	62.6%
Age, median (range)	58	(6–88)
Race		
Caucasian	223	71.9%
Black / African American	53	17.1%
Asian	13	4.2%
Latinx	8	2.6%
Not specified, Other	13	4.2%
Ethnicity, Hispanic (%)	13	4.2%
Clinical Risk for Aspergillosis		
Hematologic malignancy (no BMT)[1]	117	37.74%
BMT[2]	83	26.77%
Malignancy / tumor	28	9.03%
Solid Organ Transplant, lung	23	7.42%
Solid Organ Transplant, other[3]	19	6.13%
Autoimmune[4]	11	3.55%
HIV/AIDS	7	2.26%
Other[5]	15	4.84%
None	10	3.23%
Diagnosis		
Proven IA	6	1.94%
Probable IA	55	17.74%
Possible IFI	108	34.84%
Airway Aspergillosis (Proven / Probable)[6]	21	6.77%
Fungal sinusitis	3	0.97%
Other IFI[7]	46	14.84%
No Fungal infection[8]	45	14.52%
Other infections[9]	13	4.19%
Mixed infections[10]	16	5.16%

1 . CLL (3); CML (1); CMML(1); Aplastic anemia (1);
2. Allogeneic (23); autologous (2);
3. Kidney (3), Liver (4), liver and kidney (3), heart (2)
4. SLE (2); antisynthetase syndrome (1); vasculitis (1); polyglandular autoimmune syndrome (1),
5. Cystic fibrosis (5); COPD (2); severe influenza (1); elderly (1); diabetic nephropathy (1); lymphoma, distant (1); malnutrition (1); end-stage liver disease (1);
6. CF Class 4 (2); CF post lung-transplant probable IA (1);
7. Candidemia (2), blastomycosis (1); cryptococcosis (1); mucormycosis (1); histoplasmosis (5), PCP (3);
8. . Diffuse alveolar pathologies (2); lung cancer (1); CF (1);
9. Bacterial pneumonia (6); mycobacterial pneumonia (2); Rhinovirus infection (1);
10. probable IA + mycobacterial pneumonia (2); possible histoplasmosis + probable PCP (1); possible IA + probable PCP (1); possible IA + candidemia (1); probable IA + bacterial endocarditis (1); probable IA + probable PCP (1)

Figure 3. Results of A. Validation cohort containing 21 cases and 161 controls and B. ROC curve (AUC 0.99, 95% CI 0.98–1.0)

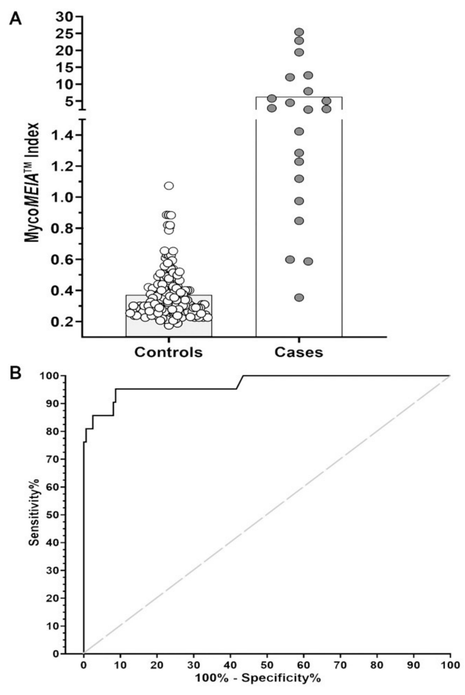


Figure 2. Distribution of results according to diagnosis

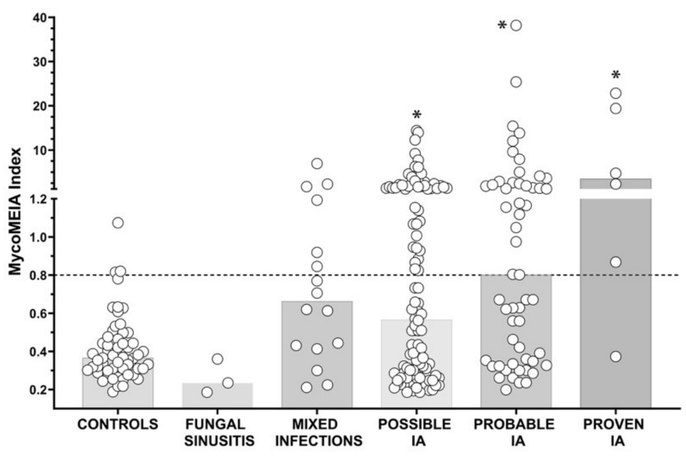
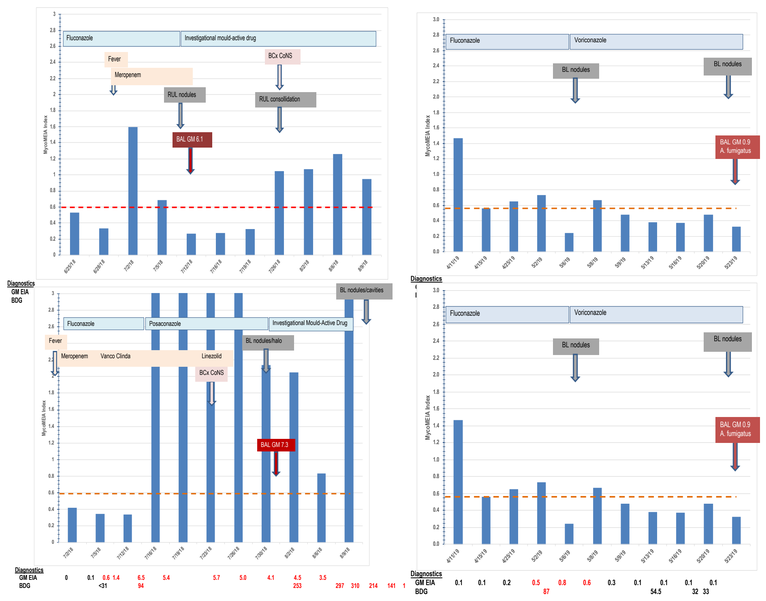


Table 2. Performance per sample and subject in people with different diagnoses

Pathology	MYCOMEIA+ / sample	%	MYCOMEIA+ / subject	%
Candidemia[1]	1 / 8	12.5%	1 / 3	33.3%
Histoplasmosis (Probable / Proven)	9 / 21	42.9%	9 / 19	47.4%
Blastomycosis	1 / 1	100%	1 / 1	100%
Pneumocystis pneumonia	2 / 6	33.3%	1 / 4	25%
Fungal sinusitis, NOS	0 / 4	0%	0 / 3	0%
Bacterial pneumonia	0 / 12	0%	0 / 5	0%
Mycobacterial pneumonia	0 / 2	0%	0 / 2	0%
Pseudomonas bacteremia	2 / 11	18.2%	1/2	50%
Viral pneumonia	1 / 12	8.3%	1 / 2	50%
Non-infectious: diffuse alveolar hemorrhage	0 / 1	0%	0 / 1	0%
Non-infectious: diffuse alveolar damage	0 / 1	0%	0 / 1	0%
Non-infectious: Lung cancer	1 / 1	100%	1 / 1	100%

1 Two people with C. glabrata negative; 1 person with *C. krusei* with low positive MycoMEIA and undefined pulmonary nodules on CT

Figure 6. Myco*MEIA* results (blue bars) in patients with AML and/or BMT with comparative clinical and diagnostic results show early positivity of urine results. Blood tests are shown relative to day of testing, below figures. Cut off for Myco*MEIA* is represented by dotted line.



URINARY ANTIGEN

Figure 4. Left panel shows mAb476 binding to extracellular fractions of patient (VU7) urine (top), processed by serial ultracentrifugation compared to (bottom) binding of human EV marker (CD63). IA patient has mAb476 binding, while controls do not. On right panel is mass spect results from 2 patient urines immunoprecipitated with mAb476, with GO ontology describing functionality. Results showed heterogeneity in urinary Aspergillus glycoproteins

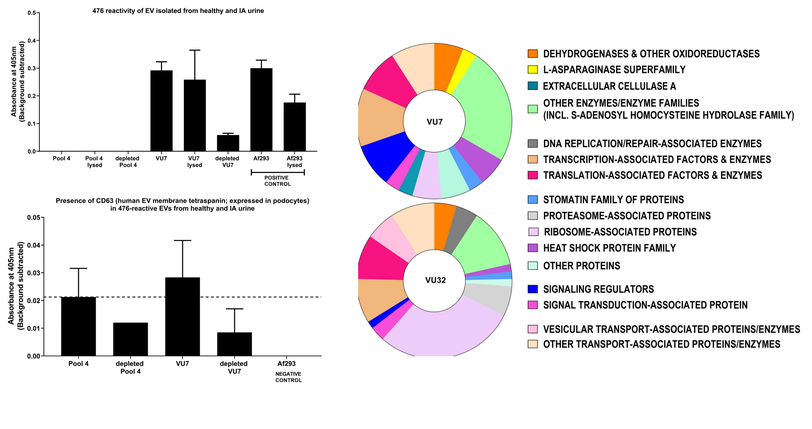


Figure 5. Transmission electron micrograph of IA patient (VU7) urine with gold-mAb476 shows mAb binding to small EVs and larger EVs (latter consistent with apoptotic bodies