Transplant type, number (%)

- 2

2. Not exclusive of underlying disease. Includes 2 cases with combined kidney and liver transplants

NOTES:

Age, mean (range), years

Factor Finding

4. Not exclusive of infectious diagnoses. Includes 1 case with probable cryptococcosis and proven histoplasmosis

3. Defined according to modified MSG / EORTC criteria (methods)

Time between presentation and urine sample, median (range), days

5 8 (4 – 51)

Conclusion:

Urine testing using this rapid and easy dipstick assay may provide weight glycans as predominant antigens found in urine.

Purpose:

- Identification of Aspergillus galactofuranosidase (GalFr) assays as potential non-mammalian Eukaryotes and some Prokaryotic pathogens
- A. fumigatus cell wall contains Gal-containing polysaccharides and glycoconjugates, including
- O-glycans
- N-Glycans
- Fungal-type Galactomannan (GM) which is the (Antigenic target of Platelia™ EB-A2)

Mouse IgM mab476 developed against A. fumigatus conidia binds Gal-bearing antigens

- MAV476 binds in immunosassays to
- Antigen precipitated via Ethanol (‘EP’) from culture supernatant of A. fumigatus & related molds
- Chemically purified ‘GM’ prepared from EP
- Recognizes Gal-bearing antigens in infected animal serum, BAL, fung homogenate
- Rapidly localizes to bladder in infected mice
- Proper control urine testing was shown using a lateral flow (LFMD) format with urines from infected guinea pig and human IA patient
- Discovery of small MW inhibitor in urine

Optimized prototype dipstick assay using mab476 recognizes Gal-antigens in urine

- Urine sample: healthy volunteer urine spiked with EP antigen at different concentrations (Panel A), or urine samples collected from patients upon clinical suspicion of IA (Panel B)
- Processed through de-salting column to remove small MW inhibitor of antigen-antibody interaction
- Dipstick exposed to urine samples; visual read in 10-20 minutes after sample application
- Results showed good in vitro reproducibility
- Limit of Detection: –200 ng/mL EP spiked in urine
- Tested urine samples from suspected/confirmed IA patients (n=76) from 2 JHU clinical studies
- EORTC/MSG definitions: proven/probable IA = case; no IA, no invasive fungal infection (IFI) or other IFI = control

Prototype urinary dipstick assay has excellent performance characteristics

- Overall cohort (n=78)
  - Proven/Probable IA (N=30) 24 (80) 2 80 (61.4-92.3) 92 (74.9-99)
  - Controls (N=25) 2 (8) 1 8 (6.1-92.3) 95 (69.8-99.7)
- Proven/Probable/Possible IA (N=53) 37 (69.2) 2 69.2 (57-80.8) 99 (74.8-99.7)
- Overall cohort using extended clinical diagnoses with serum β-Gal-Glucan values
  - Proven/Probable (N=34) 27 (79.4) 2 79.4 (62.1-93.1) 92.9 (68.1-98.8)
  - Controls (N=14) 1 (7.1) 1 71 (62.1-93.1) 92.9 (68.1-98.8)
- Sub-Group: hematological malignancy / solid tumor / BMT (N=50)
  - Proven/Probable IA (N=19) 17 (89.5) 2 89.5 (66.7-98.7) 90.9 (58.7-99.8)
  - Controls (N=11) 1 (9.1) 1 91 (62.1-93.1) 92.9 (68.1-98.8)

NOTES:

1. Standardized proteins: A. niger, A. flavus
2. Cross-reactivity to PMF and other IFI
3. Standardization to EORTC/MSG criteria including
4. Include both IA/IFI and other IFI
5. Use standard urine samples
6. Use standard urine samples

Serum GM EIA indices were comparable to semi-quantitative urinary urine values

- Serum GM EIA index values are clinically measured using the commercial assay Platelia™ in subjects on suspicion of IA
- Occasionally GM EIA indices are estimated in bronchoalveolar lavage (BAL) fluid in addition to or instead of in sera
- GM EIA indices date-matched to urine samples were plotted against the urinary dipstick assay results on a visual grade ( –/+/++) based on Test line intensity
- Highest visual grade (++) significantly correlated with subjects with high GM indices (Kruskal Wallis, Dunn’s post test with multiple comparison correction)

SUMMARY

- ASSAY
  - Using assays VUT (high antigen signal in EIA with mab476) & VUT (low antigen signal), both raw and desalted (ds) samples
  - Urines were spin-concentrated using Amicon devices
  - Reducing SDS PAGE, followed by Western blots probed by mAb476-reactive bands from VU7 were enriched via immunoprecipitation with antibody-bound magnetic beads
  - Mass spectrometry identified small molecular moieties in patient urine (VU7 and VU32), but not in healthy controls

- Immunoprecipitation and analyzed via MS/MS

- Analysis of sera or urine samples spiked with GalFr activity

- Urine sample: healthy volunteer urine spiked with EP antigen at different concentrations (Panel A), or urine samples collected from patients upon clinical suspicion of IA (Panel B)

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