Clinical application of *Asp*ID PCR alone and in combination with *Aspergillus* lateral flow device (*Asp*LFD) in bronchoalveolar lavage (BAL) fluid of patients (pt) with classic risk factors for invasive pulmonary aspergillosis (IPA)

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Introduction

- Several novel diagnostic modalities have been developed to improve the accuracy of diagnosis of IPA
- Polymerase chain reaction (PCR) methods have been criticized in the past for false-positive reactions and lack of standardized testing platforms
- Recent studies have shown increased sensitivity with newer PCR techniques
- A newly-developed multiplex *Aspergillus* qRT-PCR assay, *Asp*ID (OLM Diagnostics, Newcastle-Upon-Tyne, UK) simultaneously detects a pan-*Aspergillus* target and an *A. terreus* target
- A pilot study using *Asp*ID on BAL samples demonstrated high sensitivity but only moderate specificity for non-terreus *Aspergillus* species
- We evaluated the test characteristics of *Asp*ID both alone and in combination with an *Aspergillus* lateral flow device (*Asp*LFD) on BAL fluid of patients at high risk for IPA

Methods

BAL repository

- Leftover BAL samples collected from 11/2015 to 2/2018 were stored at -70°C
- Charts were reviewed to identify patients at high risk for IPA
  - Recent history of neutropenia (<500 neutrophils/mm^3 for >10 days)
  - Allogeneic stem cell transplant
  - Prolonged high-dose corticosteroid use
  - T-cell immunosuppressive agents in the prior 90 days
  - Severe primary immunodeficiency
- IPA was defined as proven, probable, or possible by EORTC/MSG criteria
- Patients with proven or probable IPA (cases) were matched 1:2 with high risk patients without IPA (controls)
- Patients with possible IPA were excluded
- *Asp*ID and *Asp*LFD were performed on all samples

*Asp*LFD

- AspLFD procedure:
  - Samples thawed, vortexed, centrifuged for 1min at 14,000rpm; 100ul supernatant applied to the LFD
  - Results read after 15 minutes independently by 2 blinded investigators as negative or positive graded as +, ++, +++
  - Results were invalid if control line was not positive after 2 attempts

*Asp*ID

- DNA extraction was performed using Qiagen DNeasy Blood and Tissue Kit with a total DNA elution volume of 50 µL
- PCR was performed per manufacturer instructions at the DNA Sequencing Core Laboratory at the University of Michigan

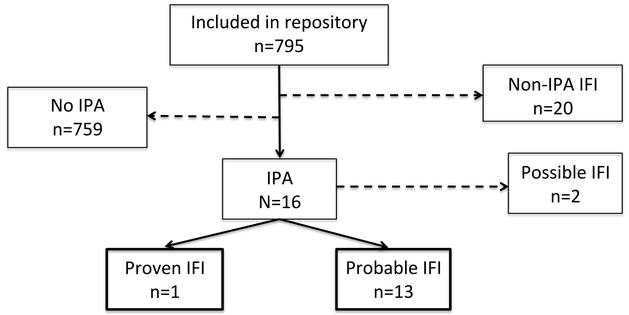
*Asp*ID interpretation

*Asp*ID: detection channels			
FAM	**HEX**	**ROX**	**Result**
+	-	+	*Aspergillus* **POSITIVE** sample, non-*A.terreus*. **IEC PASS. Valid result.**
+	+	+	*A. terreus* **POSITIVE** sample. **IEC PASS. Valid result.**
-	-	+	*Aspergillus* **NEGATIVE** sample. **IEC PASS. Valid result.**
+	+/-	-	*Aspergillus* **POSITIVE** sample. IEC outcompeted by high *Aspergillus* load. **Valid result.**
-	-	-	Invalid

FAM is a pan-*Aspergillus* target, HEX is an A. terreus specific target, and ROX is an internal extraction control (IEC)
Interpretation chart taken from *Asp*ID instruction manual

Results

Patient selection



Demographics

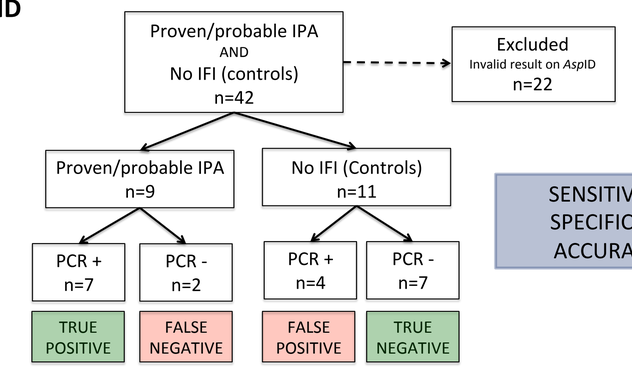
Features	CASE (IPA) n=14	CONTROL (NO IPA) n=28
Mean Age	52.7 ± 17.5	53.6 ± 15.6
Female	6	8
Classic Risk Factors for IPA		
Neutropenia	3	0
High-dose steroids	4	11
T cell depleting agent	9	17
Allo-HCT	0	2

*Asp*ID was performed on all 42 clinical samples (cases & controls); valid in 20.
*Asp*LFD was performed on the 20 samples with a valid *Asp*ID result; 19 were valid.

Results of *Asp*ID and *Asp*LFD

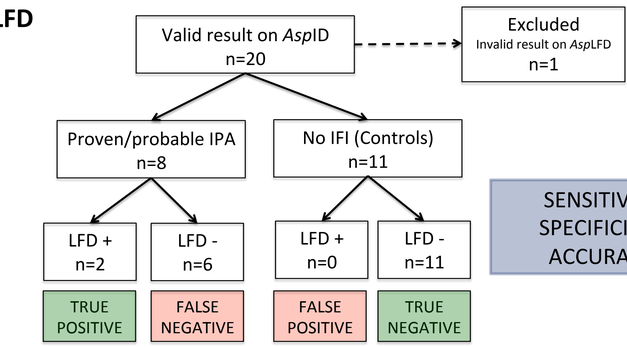
Age/Sex	IFI status	Host factors	*Asp*ID	*Asp*LFD
42M	Proven IPA	Steroids (Cushing's disease)	+	-
32F	Probable IPA	Steroids, T cell depletion	+	-
22M	Probable IPA	Neutropenia (AML)	+	-
42M	Probable IPA	T cell depletion (lung Tx)	+	-
23F	Probable IPA	T cell depletion (lung Tx)	+	-
60M	Probable IPA	Neutropenia (AML)	-	Invalid
63F	Probable IPA	T cell depletion	-	-
60M	Probable IPA	T cell depletion	+	+
69F	Probable IPA	Neutropenia, steroids, T cell depletion	+	+
68M	No IFI	Neutropenia (AML)	-	-
46M	No IFI	Steroids, T cell depletion	-	-
42F	No IFI	T cell depletion	-	-
60M	No IFI	T cell depletion (lung Tx)	-	-
56F	No IFI	Steroids, T cell depletion	+	-
60F	No IFI	T cell depletion (lung Tx)	-	-
41F	No IFI	Steroids	+	-
63M	No IFI	Steroids, T cell depletion (lung Tx)	+	+
58M	No IFI	T cell depletion (lung Tx)	-	-
87M	No IFI	Steroids	-	-
40M	No IFI	T cell depletion (lung Tx)	+	-

*Asp*ID



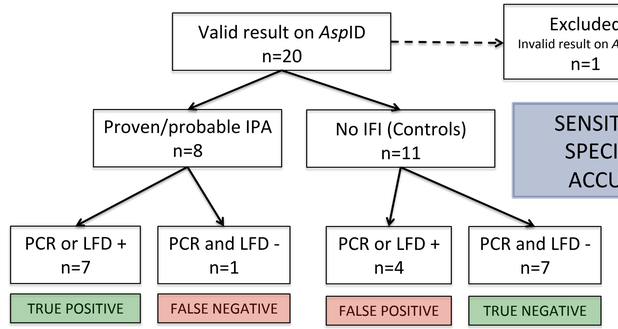
SENSITIVITY 78%
SPECIFICITY 64%
ACCURACY 70%

*Asp*LFD



SENSITIVITY 25%
SPECIFICITY 100%
ACCURACY 68%

Union analysis



SENSITIVITY 87.5%
SPECIFICITY 64%
ACCURACY 74%

Conclusions

- *Asp*ID had higher sensitivity than *Asp*LFD and *Asp*LFD had higher specificity than *Asp*ID
- Using both tests in combination did not improve the ability to diagnose IPA in patients at high risk of IPA
- Our study was limited by a small sample size; larger studies are needed to determine the application of these assays

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