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UPDATED ABSTRACT

Background: Methods based on real-time PCR may anticipate the diagnosis of invasive aspergillosis (IA) but are still limited by a lack of standardization. We validated the standardized MycAssay™ *Aspergillus* (Myconostica Ltd) for the diagnosis of IA in patients without hematological cancer, an emerging population at risk of IA.

Methods: We prospectively collected 322 samples (November 2009 - January 2011) from 175 patients with lower respiratory tract infection and the following IA predisposing conditions: solid cancer (16.7%), cirrhosis (17.3%), corticosteroid consumption (72.6%), HIV (15.5%), COPD (53%), solid organ transplantation (renal [1.2%], heart [3%], liver [4.8%]), or none (16.7%). Specimens were obtained when clinically indicated and were collected in the microbiology laboratory. Specimens were processed for microbiological culture; *Aspergillus* DNA was extracted and amplified by means of MycXtra® and MycAssay™.

Results: According to the EORTC and Bulpa's criteria (for patients with COPD), patients had probable/possible IA (n=15/3), or no IA. The 15 patients with probable IA had solid cancer (n=3), cirrhosis (n=3), COPD (n=6), HIV (n=2), or other (n=3). *Aspergillus* spp. was isolated from 65 samples (31 patients). The median time from sample culture until fungal growth visualization was 3 days. PCR results (~4 h sample to result) were negative (n=254), positive (n=54), or indeterminate (n=14). Sensitivity and specificity of the assay for the diagnosis of IA (first sample/any sample) were (86.7/93) and (87.6/82.4), respectively. Positive and negative predictive values were significantly influenced by the patients pre-test probability of infection.

Conclusions: MycAssay™ *Aspergillus* showed high sensitivity for the diagnosis of IA in patients without hematological cancer, which increased when multiple samples were used. PCR significantly reduced the time to diagnosis compared to fungal culture.

INTRODUCTION AND PURPOSE

Patients with non-hematological malignancy are emerging as populations at risk of invasive aspergillosis.

Invasive aspergillosis in these patients has a high mortality, probably due to the low index of suspicion and the consequent delay in diagnosis.

Methods based on real-time PCR may anticipate the diagnosis of invasive aspergillosis but are still limited by a lack of standardization.

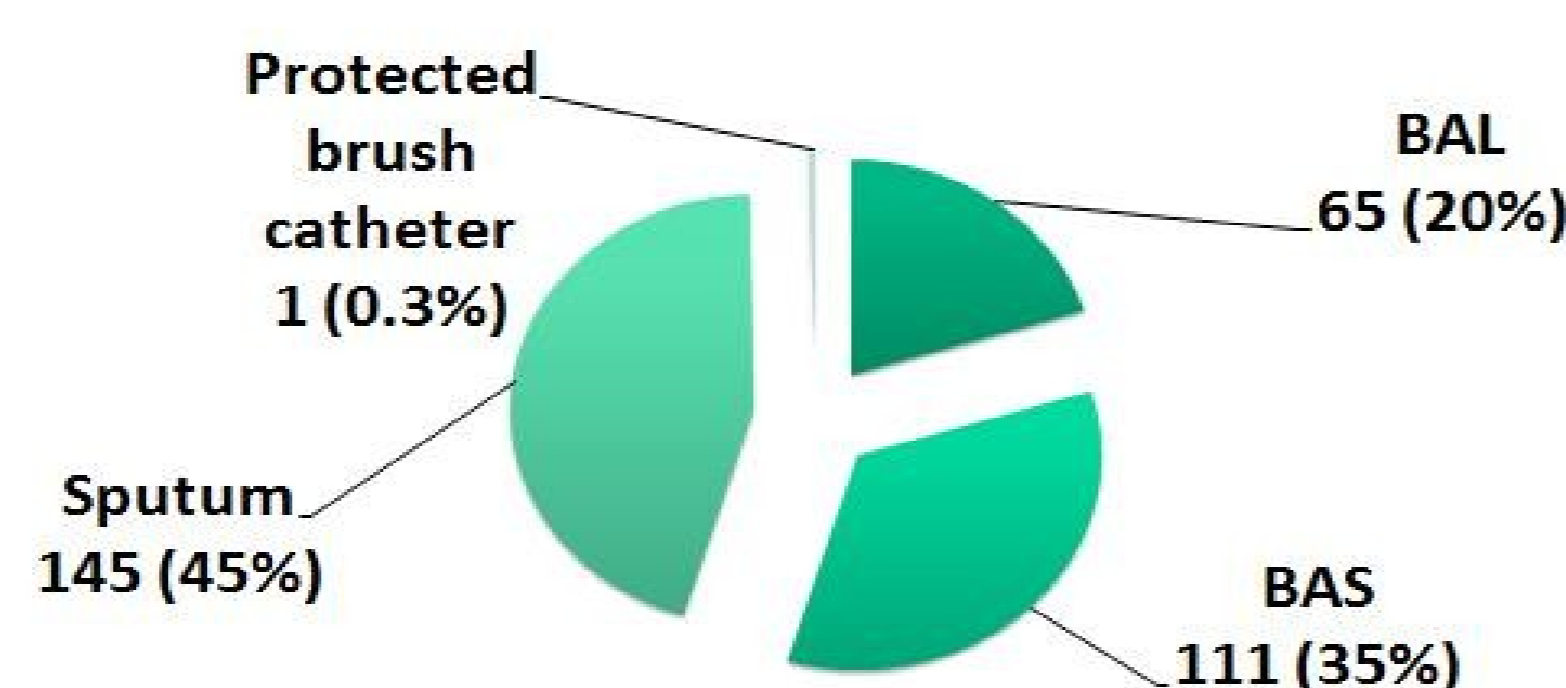
We validated the standardized MycAssay™ *Aspergillus* (Myconostica Ltd) in respiratory samples from patients without hematological cancer for the diagnosis of invasive aspergillosis.

METHODS

From November 2009 to January 2011, we prospectively collected 322 respiratory samples (Figure 1) from 175 patients admitted to Gregorio Marañón University Hospital in Madrid, Spain.

All specimens were obtained when clinically indicated and were selected in the microbiology laboratory.

Figure 1. Distribution of the 322 respiratory samples from the 175 patients included in the study.



The 175 patients were recruited because of clinical suspicion of lower respiratory tract infection and the presence of the following invasive aspergillosis predisposing conditions (excluding hematological cancer): solid cancer (16.7%), cirrhosis (17.3%), corticosteroid consumption (72.6%), HIV (15.5%), COPD (53%), solid organ transplantation (renal [1.2%], heart [3%], liver [4.8%]), or none (16.7%).

All specimens were processed for *Aspergillus* DNA detection. Specimens were cultured both in bacteria and fungal media, if enough sample volume was available (90% of samples). Fungal culture media included Sabouraud-dextrose agar with chloramphenicol, and brain-heart infusion agar with antibacterial agents.

Mucous samples, such as sputum and bronchoaspirate, were made fluid by the addition of BBL™ MycoPrep™ Reagent prior to DNA extraction. *Aspergillus* DNA was extracted and purified from the respiratory samples by means of the MycXtra® DNA extraction kit.

Purified *Aspergillus* DNA was amplified using the MycAssay™ *Aspergillus* kit in a Cepheid SmartCycler® platform. MycAssay™ was performed according to the manufacturer's instructions.

Patients were classified as having or not having invasive aspergillosis according to the revised EORTC criteria (de Paw et al., CID 2008) or Bulpa's criteria (Bulpa et al., Eur Respir J 2007) (exclusively for patients with COPD). Only patients with proven or probable invasive aspergillosis were considered true infections.

We studied the sensitivity, specificity, positive predictive value (PPV) and negative predictive

RESULTS

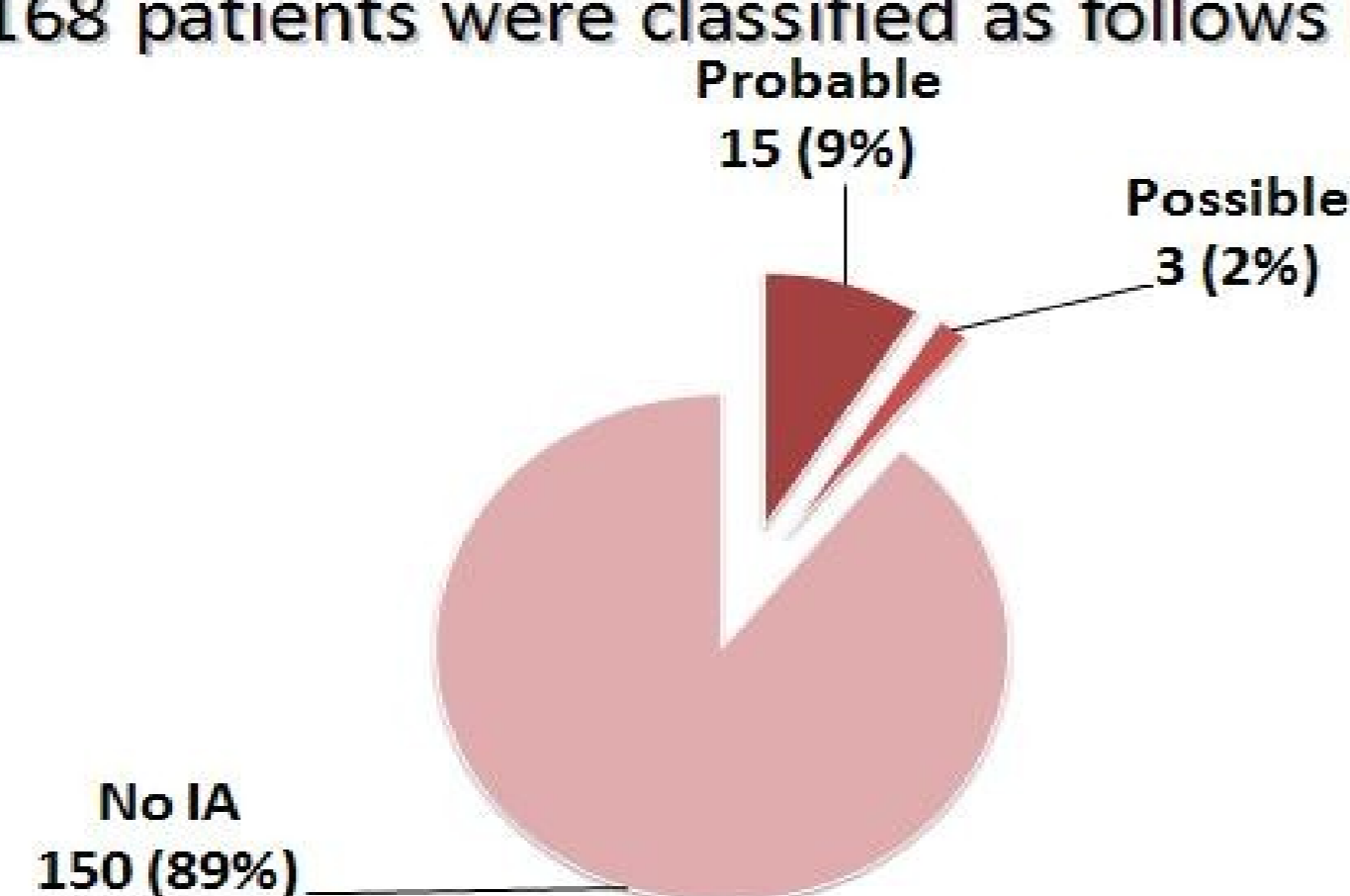
Aspergillus spp. was isolated in 65 samples from 31 different patients by culture. The MycAssay™ results for the 322 samples studied were as follows (Figure 2):

Figure 2. MycAssay™ *Aspergillus* results for the 322 samples studied.



We excluded 7 patients with indeterminate results. According to the clinical gold standard, the remaining 168 patients were classified as follows (Figure 3 and Table 1):

Figure 3. Classification of the 168 patients studied as having probable or possible invasive aspergillosis, or noninvasive aspergillosis.



The sensitivity and specificity of the MycAssay™ *Aspergillus* determination for the diagnosis of invasive aspergillosis are shown in Table 2:

MycAssay™ <i>Aspergillus</i>	1 st sample		Any sample	
	Culture	MycAssay™	Culture	MycAssay™
Sensitivity	86.7	86.7	100	93
Specificity	83	87.6	81.7	82.4

Table 2. Sensitivity and specificity of the MycAssay™ *Aspergillus* for the diagnosis of invasive aspergillosis in the 168 non-hematological patients studied.

The sensitivity and specificity for patients with COPD (first sample/any sample) were 100/100 and 85.7/78.3.

Sensitivity and specificity remained unaffected by stratification of risk. However, similar to culture, the PPV and NPV for the MycAssay™ *Aspergillus* were dependent on the population pre-test probability of infection and reflected the 16 patients for which *Aspergillus* was detected without clinical suspicion of disease (Table 3).

Patient	Isolation of <i>Aspergillus</i> in any sample	Infection not responding to antibiotics	Admission to ICU	Sample obtained in the ICU	Risk Group	Involvement	Serum GM > 05
1	Yes	Yes	Yes	Yes	Solid tumor	Pulmonary	Positive
2	Yes	Yes	No	No	Solid tumor	Pulmonary	Negative
3	Yes	Yes	Yes	Yes	Liver cirrhosis	Pulmonary	Positive
4	Yes	Yes	Yes	Yes	COPD	Pulmonary	Negative
5	Yes	Yes	Yes	Yes	COPD	Pulmonary	Negative
6	Yes	Yes	Yes	Yes	COPD	Pulmonary	Negative
7	Yes	Yes	Yes	No	COPD	Pulmonary	Negative
8	Yes	Yes	Yes	Yes	COPD + solid tumor	Pulmonary	Positive
9	Yes	Yes	Yes	Yes	Corticosteroids	Pulmonary	Negative
10	Yes	Yes	Yes	Yes	Liver cirrhosis	Pulmonary	Positive
11	Yes	Yes	Yes	No	Liver cirrhosis	Pulmonary	Not done
12	Yes	Yes	No	No	COPD + autoimmune dis.	Pulmonary	Negative
13	Yes	Yes	No	Yes	Tracheal prosthesis	Tracheobronchitis	Negative
14	Yes	Yes	Yes	Yes	HIV	Pulmonary	Positive
15	Yes	Yes	Yes	Yes	HIV	Pulmonary	Negative

Table 1. Underlying conditions, organs affected, and microbiological findings of the 15 patients with probable invasive aspergillosis

MycAssay™ <i>Aspergillus</i>	PPV	NPV
All patients (n=168)	34.1	99.2
Patients with COPD (n=89)	25.0	100
Patients with infection not improving with antibiotics (n=28)	87.5	91.7
Patients requiring ICU admission (n=33)	78.6	94.7

Table 3. PPV and NPV of the MycAssay™ *Aspergillus* for the diagnosis of invasive aspergillosis in the situations with different pre-test probability of invasive aspergillosis.

MycAssay™ *Aspergillus* results are available approximately 4 hours after sample reception. In contrast, the number of days to the visualization of fungal growth was as follows: mean, n=4.3 ± 4.1; median, n=3; and mode, n=2.

CONCLUSIONS

MycAssay™ *Aspergillus* showed high sensitivity for the diagnosis of invasive aspergillosis in patients without hematological cancer. This sensitivity increased when multiple samples were used.

Sensitivity was high for patients with COPD, an emerging risk population, particularly in some hospitals.

MycAssay™ *Aspergillus* proved to be particularly useful when ruling out the diagnosis of invasive aspergillosis.

PCR significantly reduced the time to diagnosis compared to conventional fungal culture.

ACKNOWLEDGEMENTS

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