Evaluation of MycAssay™ Aspergillus for Diagnosis of Invasive Pulmonary Aspergillosis in Patients without Hematological Cancer

Jesus Guinea1,2,3,4*, Camilo Padilla1,2, Pilar Escribano1,2,3, Patricia Munoz1,2,3, Belen Padilla1,2,
Paloma Gijon1,2, Emilio Bouza1,2,3,4

1 Clinical Microbiology and Infectious Diseases Department, Hospital General Universitario Gregorio Maranon, Universidad Complutense de Madrid, Madrid, Spain, 2 Instituto de Investigación Sanitaria del Hospital Gregorio Maranon, Madrid, Spain, 3 CIBER de Enfermedades Respiratorias (CIBERES CD06/06/0058), Palma de Mallorca, Spain, 4 Medicine Department, School of Medicine, Universidad Complutense de Madrid, Madrid, Spain

Abstract
Methods based on real-time polymerase chain reaction (PCR) can speed up the diagnosis of invasive aspergillosis but are limited by a lack of standardization. We evaluated the commercially available MycAssay™ Aspergillus test for the diagnosis of invasive aspergillosis in patients without hematological cancer. We prospectively collected 322 lower respiratory tract samples (November 2009–January 2011) from 175 patients with lower respiratory tract infection and the following predisposing conditions: solid cancer (16.8%), cirrhosis (16.8%), corticosteroid therapy (71.7%), HIV infection (15.6%), chronic obstructive pulmonary disease (COPD, 52.6%), solid organ transplantation (kidney [1.2%], heart [3%], liver [4.6%]), or none (3.5%). Specimens were obtained when clinically indicated and analyzed in the microbiology laboratory. Aspergillus DNA was extracted and amplified by means of MycXtra® and MycAssay™ Aspergillus. Aspergillus spp. was isolated from 65 samples (31 patients). According to the European Organization for Research and Treatment of Cancer and Bulpa’s criteria (for patients with COPD), 15 had probable invasive aspergillosis. MycAssay™ Aspergillus results were negative (n = 254), positive (n = 54), or indeterminate (n = 14). The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic odds ratio of the MycAssay™ (first sample/any sample) were 86.7/93, 87.6/82.4, 34.1/34.1, 92.2/100, and 48/68.75. The differences between the proportion of samples with positive PCR determinations (63%) and the proportion of samples with Aspergillus spp. isolation (75%) did not reach statistical significance (P = 0.112). The median time from sample culture to visualization of fungal growth was 3 days, compared with ~4 hours for MycAssay™ Aspergillus PCR. MycAssay™ Aspergillus showed high sensitivity for the diagnosis of invasive aspergillosis in patients without hematological cancer. Sensitivity increased when multiple samples were used. Compared with fungal culture, PCR significantly reduced the time to diagnosis.

Citation: Guinea J, Padilla C, Escribano P, Munoz P, Padilla B, et al. (2013) Evaluation of MycAssay™ Aspergillus for Diagnosis of Invasive Pulmonary Aspergillosis in Patients without Hematological Cancer. PLoS ONE 8(4): e61545. doi:10.1371/journal.pone.0061545

Editor: David R. Andes, University of Wisconsin Medical School, United States of America
Received December 21, 2012; Accepted March 11, 2013; Published April 19, 2013

Copyright: © 2013 Guinea et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was partially financed by grants from the Fondo de Investigación Sanitaria (FIS) CP09/00055 and PI09/1257 (Instituto de Salud Carlos III) and by grants from Myconostica. Jesús Guinea (MS09/00055) and Pilar Escricbano (CD09/00230) are supported by the FIS. The MycXtra® and MycAssay™ Aspergillus kits used to perform the study were kindly supplied by Izasa (Barcelona, Spain). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors declare that the MycXtra® and MycAssay™ Aspergillus kits were kindly supplied from Izasa (Barcelona, Spain). This does not alter the authors’ adherence to all the PLOS ONE policies on sharing data and materials.

* E-mail: jguineaeortega@yahoo.es

Introduction
Invasive aspergillosis is an opportunistic infection affecting patients with various degrees of immunosuppression. Patients with deep and prolonged neutropenia have traditionally had the highest risk of invasive aspergillosis [1,2,3,4,5]. Other at-risk groups include solid organ transplant recipients, patients with chronic obstructive pulmonary disease (COPD), patients receiving corticosteroids or other immunosuppressive agents, and patients with liver cirrhosis [6,7].

The number of patients without hematological malignancies who are affected by invasive aspergillosis is increasing. Mortality in this group is high, probably because of the low index of suspicion of the infection and the consequent delay in diagnosis [8,9,10,11]. As a favorable outcome depends on prompt and appropriate antifungal therapy, rapid diagnosis is increasingly important.

Detection of galactomannan in serum has proven useful for the diagnosis of invasive aspergillosis in neutropenic patients; unfortunately, its sensitivity is below 50% in patients without neutropenia [12,13,14,15,16]. Detection of galactomannan in bronchoalveolar lavage (BAL) samples is more sensitive than detection in serum samples, although BAL samples are not always available [13]. The isolation of Aspergillus in lower respiratory tract samples from non-neutropenic patients is often the first microbiological evidence of invasive pulmonary aspergillosis. However, as culture is slow, detection of Aspergillus in clinical samples is delayed.

Methods based on real-time polymerase chain reaction (PCR) can speed up the diagnosis of invasive aspergillosis but are limited by a lack of standardization [17,18]. MycAssay™ Aspergillus is a...
recently marketed real-time PCR technique for detection of *Aspergillus* DNA in lower respiratory tract samples. This assay has been studied mostly in BAL samples from patients with hematological malignancies or those admitted to intensive care units [19].

In the present study, we evaluated the MycAssay™ *Aspergillus* test in respiratory samples, including BAL, spontaneous sputum, and bronchial aspirate, for the diagnosis of invasive aspergillosis in patients without hematological cancer.

**Materials and Methods**

**Patients and clinical samples**

From November 2009 to January 2011, we recruited 175 patients with one or more lower respiratory samples submitted to the microbiology laboratory. Most of the patients (96.5%) had clinical suspicion of lower respiratory tract infection and at least one invasive pulmonary aspergillosis host factor, excluding hematological cancer. A total of 322 samples were collected. Samples with indeterminate results were retested, and the second result was chosen. Samples showing a confirmatory indeterminate PCR result were excluded from the analysis (n = 14; 4.3%). The number of samples studied/collected was as follows: spontaneous sputum (n = 142/145), bronchial aspirate (n = 104/111), BAL (n = 61/65), and protected brush catheter (n = 1/1).

Two patients had a single sample, each with an indeterminate result, and were excluded from the analysis. The remaining 173 patients were classified as having or not having invasive pulmonary aspergillosis or other mold infection according to the revised criteria of the European Organization for Research and Treatment of Cancer (EORTC) [20,21] or Bulpa’s criteria (exclusively for patients with COPD) [20,21]. Colonization was defined as the isolation of *Aspergillus* spp. in lower respiratory samples in patients not meeting the EORTC or Bulpa’s criteria.

Cirrhosis was included as a host factor, since invasive aspergillosis has been found in critically ill patients with cirrhosis and no other predisposing conditions [8]. The predisposing conditions for invasive aspergillosis were active solid cancer (16.8%), cirrhosis (16.8%), corticosteroid consumption (71.7%), HIV infection (15.6%), COPD (32.6%), solid organ transplantation (kidney [1.2%], heart [3%], liver [4.6%]), neutropenia, (4.6%), or none (3.5%). A high proportion of the patients (90%) were consuming antibiotics when the sample was collected.

All samples were obtained only when clinically indicated, and no additional samples were requested for the study. The samples were prospectively collected and the patients’ charts were retrospectively reviewed. Clinicians were blinded to the PCR result, which was not included as a microbiological diagnostic criterion.

**Sample processing, genomic *Aspergillus* DNA extraction, and amplification using MycAssay™ *Aspergillus***

Samples were split for fungal culture and DNA extraction. All samples were processed for *Aspergillus* DNA detection, and most (n = 298/308; 97%) were cultured on both bacteria and fungal media. BAL samples (1 mL) were centrifuged at 3,000 g for 10 minutes. The concentrated pellets obtained were processed for fungal culture and streaked on culture media plates. Sputum and bronchial aspirate were converted to fluid by the addition of acetylcysteine (Pharmazam, Spain) and added to the agar plates using a sterile loop (10 μL). The fungal culture media were Sabouraud-dextrose agar with chloramphenicol and brain-heart infusion agar with antibacterial agents. The bacterial culture media were sheep blood agar and chocolate agar. filamentous fungal isolates were identified according to standard morphological procedures.

Samples for DNA extraction were stored frozen at −20°C until batch analysis. Thick mucous samples, such as sputum and bronchial aspirate, were converted to fluid by the addition of BBL™ MycoPrep™ Reagent (Becton Dickinson, Shannon, Ireland). These samples and BAL samples (1 mL) were then centrifuged at 3,000 g for 20 minutes. The pellets obtained were processed for *Aspergillus* DNA extraction using the manual MycXtra® fungal DNA extraction kit (Myconostica, now a Lab21 company, Cambridge, UK), which includes a mechanical disruption step. BAL supernatants were stored for galactomannan determination.

**Medical history**

Cirrhosis was included as a host factor, since invasive aspergillosis has been found in critically ill patients with cirrhosis and no other predisposing conditions [8]. The predisposing conditions for invasive aspergillosis were active solid cancer (16.8%), cirrhosis (16.8%), corticosteroid consumption (71.7%), HIV infection (15.6%), COPD (32.6%), solid organ transplantation (kidney [1.2%], heart [3%], liver [4.6%]), neutropenia, (4.6%), or none (3.5%). A high proportion of the patients (90%) were consuming antibiotics when the sample was collected.

All samples were obtained only when clinically indicated, and no additional samples were requested for the study. The samples were prospectively collected and the patients’ charts were retrospectively reviewed. Clinicians were blinded to the PCR result, which was not included as a microbiological diagnostic criterion.

**Data analysis**

None of the patients had proven invasive pulmonary aspergillosis; only probable invasive aspergillosis was considered to be a true infection. We calculated the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio for a positive result (LR+), likelihood ratio for a negative result (LR−), and diagnostic odds ratio (DOR) of the PCR for the diagnosis of invasive pulmonary aspergillosis. As multiple respiratory samples were studied in several patients, the diagnostic values of the PCR were calculated based on the results of the first sample submitted to the microbiology laboratory and on the results of any sample from the same patient. PCR and fungal culture were described and compared using the chi-square test and a standard binomial method for calculation of 95% confidence intervals.

**Ethics statement**

This study was approved by the local ethics committee (Comité Ético de Investigación Clínica (CEIC-A1)). The participants provided their written informed consent to participate in the study. All patient data were anonymized after collection.

**Results**

The 173 patients studied were mostly male (76.3%) and had a mean age of 60.8±19.5 (2–96) years. Patients were classified as having probable invasive aspergillosis (n = 15; 8.7%), possible invasive aspergillosis (n = 3; 1.7%), *Aspergillus* colonization (clinically non-significant *Aspergillus* isolation [n = 21; 12.2%], pulmonary scedosporiosis [n = 1; 0.6%]), or non-invasive mold infection.
The 15 patients with probable invasive aspergillosis were summarized in Table 1. At the time of sample collection, all patients had fever that did not respond to broad-spectrum antibiotics, and 80% required admission to the intensive care unit. A high proportion (40%) had COPD as the underlying predisposing condition. In 14 out of the 15 cases, the lung was the only organ infected. Serum galactomannan determination was only positive in 5 (35.7%) out of the 14 patients in whom it was applied.

Aspergillus spp. were isolated in 63/308 (22%) samples from 31 patients. The species distribution was as follows: A. fumigatus (n = 45), A. niger (n = 10), A. terreus (n = 7), A. flavus (n = 5), and other (n = 3) spp. Aspergillus spp. was isolated in one or more clinical samples from the 15 patients with probable invasive aspergillosis. MycAssay™ Aspergillus was positive in 54/254 (17.5%) samples, and in at least one sample from 14 of the 15 patients with invasive aspergillosis. Cp values of positive PCR determinations were lower in samples from patients with probable aspergillosis than in samples from patients with clinically non-significant Aspergillus (50.18±3.3 vs. 33±2.66; P = 0.001). This finding indicated a higher load of Aspergillus spp. in patients with invasive aspergillosis than in those without.

As expected, the proportion of samples with Aspergillus isolation or with positive PCR results was higher in patients with invasive aspergillosis (Table 2). Concordance between fungal culture and PCR was high in all samples and in samples from patients with or without invasive aspergillosis (83.9%, 86.5%, and 83.5%, respectively). Discrepancies were mostly found in samples yielding Aspergillus without DNA amplification (approximately 10% of samples). Interestingly, most discrepancies were found in samples from patients without invasive aspergillosis (PCR-negative/culture-positive results, 86%; PCR-positive/culture-negative results, 94.4%). The samples with PCR-positive/culture-negative results showed higher Cp values than the samples in which culture and PCR were concordant. The analysis of samples from patients with invasive aspergillosis indicated that the proportion of samples in which the PCR result was positive (63%) did not differ from the proportion of samples in which Aspergillus spp. was isolated (75%) (P = 0.615). The results of the comparison of PCR and fungal culture with BAL, sputum, and bronchial aspirate samples are shown in Table 3. The performance of PCR and fungal culture did not differ with the type of sample studied (P>0.05).

The sensitivity, specificity, PPV, NPV, LR+, LR−, and DOR of the MycAssay™ Aspergillus and fungal culture for the diagnosis of invasive pulmonary aspergillosis performed on lower respiratory samples of patients are shown in Tables 4 and 5. Sensitivity was higher when multiple samples per patient were studied; however, analysis of multiple samples did not affect specificity to any extent. In order to study how the diagnostic value of the PCR could be affected in different situations, patients were divided into the following groups: patients with COPD (n = 91), patients with infection not improving with antibiotics (n = 28), and patients in the intensive care unit with pneumonia (n = 35). Sensitivity and specificity remained unaffected by this stratification. MycAssay™ Aspergillus results were available approximately 4 hours after sample reception. In contrast, the number of days to visualization of fungal growth in the microbiological culture plates was as follows: mean, n = 4.3±4.1; median, n = 3; and mode, n = 2.

In 14 out of the 15 patients with probable invasive pulmonary aspergillosis, the samples were taken before the initiation of antifungal treatment, and no additional samples were studied during antifungal treatment. Serial samples from the remaining patient (no. 15, Table 1) were studied; of the five samples taken during antifungal treatment, three were positive for MycAssay and two were negative. Interestingly, after the samples became negative for MycAssay, the patient’s clinical condition improved. Of the three patients with possible invasive aspergillosis, one received voriconazole and improved, and two did not receive antifungal treatment and died. The result of MycAssay™ Aspergillus was negative for the samples from the three patients with possible invasive aspergillosis.

Discussion

The spectrum of patients at risk of invasive pulmonary aspergillosis has expanded in recent years because of an increase in the number of patients with predisposing conditions other than hematological disorders. This expansion has been illustrated in studies conducted at large tertiary hospitals where case collection was not restricted to the hematology ward and intensive care units and where a large number of autopsies are performed [6,8,23].

Invasive aspergillosis in patients with non-hematological malignancies is characterized by high mortality (60–100%) [6,10,11], which probably reflects the limitations of current diagnostic tools based on radiology or microbiology and the low index of clinical suspicion.

Diagnosis of invasive aspergillosis is based on a combination of compatible clinical findings in patients with risk factors, together with histopathological evidence of invasion, radiological findings, isolation of Aspergillus spp in lower respiratory tract samples, or detection of circulating biomarkers in fluids. Histopathology is necessary to obtain a definitive diagnosis, although lung biopsies are rarely obtained [24]. Compared with culture and molecular diagnostic testing, the accuracy of histomorphological diagnosis is at best 80% [25]. The radiological findings that are common in patients with neutropenia and invasive aspergillosis are infrequent in non-neutropenic patients [8,26]. Culture of lower respiratory tract samples from patients with clinical suspicion is still widely used, although it is slow and limited by low sensitivity and specificity [27]. Furthermore, the detection of circulating galactomanann in serum samples from non-neutropenic patients has a low sensitivity for the diagnosis of invasive aspergillosis [6,14,16].

In this scenario, the development of fast, sensitive, and specific diagnostic procedures to detect Aspergillus in respiratory tract samples from patients without hematological malignancies is attractive. One of the most encouraging new procedures is DNA Aspergillus detection by real-time PCR assays. In our study, PCR results were available as quickly as 4 hours after sample collection, which is a must faster turnaround than the 3 days required to observe growth of Aspergillus in culture. However, the time from sample culture and detection of fungal growth could be reduced by inspecting plates every day, even at the weekend. Furthermore, simultaneous amplification of DNA on respiratory samples and fungal culture will allow us to identify isolates to species level and perform antifungal susceptibility testing on isolates.

Detection of Aspergillus DNA in BAL samples is encouraging and confirms a diagnosis of invasive aspergillosis in more patients than conventional procedures [28]. It has been studied mostly in patients with hematological disorders [29,30,31]. A recent meta-analysis showed a sensitivity and specificity of 0.91 and 0.92, respectively, but highlighted the lack of standardization [32]. Nevertheless, the role of PCR in respiratory tract samples from non-hematological patients requires further evaluation. BAL samples were collected in 4 out of the 15 patients with probable invasive aspergillosis. In all cases, the BAL galactomannan concentrations were above 0.5 ng/ml.

The lack of standardization of the Aspergillus PCR to date has hampered its introduction in the defining criteria for probable aspergillosis [20]. MycAssay™ Aspergillus is a commercially
| Patient | Admission to ICU | Sample Obtained in the ICU | Main predisposing condition | Organ affected | TAC findings | Serum GM>0.5 | BAL GM | MycAssay result on first sample | MycAssay result on any sample | Aspergillus species isolated in any of the clinical samples | Clinical outcome | Antifungal treatment |
|----------|-----------------|-----------------------------|-----------------------------|----------------|-------------|-------------|--------|-----------------------------|----------------------------|---------------------------------|----------------|---------------------|
| 1        | Yes             | Yes                         | COPD<sup>a</sup>            | Lung           | ND          | Negative    | No BAL sample | Positive                   | Positive                   | A. terreus                      | Poor            | Voriconazole        |
| 2        | Yes             | Yes                         | COPD                        | Lung           | Bilateral and multifocal nodules | Negative    | No BAL sample | Positive                   | Positive                   | A. flavus                      | Poor            | Voriconazole+caspofungin |
| 3        | Yes             | Yes                         | Solid tumor                 | Lung           | Bilateral and multifocal nodular consolidation | Positive   | No BAL sample | Positive                   | Positive                   | A. fumigatus                      | Poor            | Voriconazole        |
| 4        | Yes             | Yes                         | HIV                         | Lung           | ND          | Positive    | No BAL sample | Positive                   | Positive                   | A. nidulans+A. niger               | Poor            | Voriconazole+caspofungin |
| 5        | No              | Yes                         | Tracheal prosthesis         | Trachea        | ND          | Negative    | No BAL sample | Positive                   | Positive                   | A. niger+A. terreus                        | Favorable       | Voriconazole        |
| 6        | Yes             | No                          | COPD                        | Lung           | ND          | Negative    | No BAL sample | Positive                   | Positive                   | A. fumigatus+A. terreus                     | Poor            | Caspofungin        |
| 7        | No              | No                          | Solid tumor                 | Lung           | ND          | Negative    | No BAL sample | Positive                   | Positive                   | A. fumigatus                      | Poor            | Voriconazole        |
| 8        | Yes             | Yes                         | Corticosteroids             | Lung           | ND          | Negative    | 4 ng/ml      | Positive                   | Positive                   | A. fumigatus                      | Favorable       | Voriconazole        |
| 9        | No              | No                          | Corticosteroids+Liver cirrhosis | Lung       | Bilateral diffuse infiltrates | Negative    | No BAL sample | Positive                   | Positive                   | A. fumigatus                      | Poor            | Voriconazole        |
| 10       | Yes             | Yes                         | Corticosteroids+Liver cirrhosis | Lung       | ND          | Positive    | No BAL sample | Positive                   | Positive                   | A. niger                          | Poor            | Voriconazole+amphotericin B |
| 11       | Yes             | Yes                         | Corticosteroids+Liver cirrhosis | Lung       | ND          | Positive    | 4 ng/ml       | Positive                   | Positive                   | A. fumigatus                      | Poor            | Voriconazole        |
| 12       | Yes             | No                          | Corticosteroids+Liver cirrhosis | Lung       | ND          | ND<sup>d</sup>    | No BAL sample | Positive                   | Positive                   | A. fumigatus                      | Poor            | Caspofungin        |
| 13       | Yes             | Yes                         | COPD+solid tumor            | Lung           | Multiple lung infiltrates and cavitations | Positive   | 2.765      | Positive                   | Positive                   | A. fumigatus                      | Poor            | Voriconazole        |
| 14       | Yes             | Yes                         | Corticosteroids             | Lung           | Bilateral lung infiltrates | Negative    | 0.63       | Negative                   | Negative                   | A. fumigatus                      | Poor            | Voriconazole        |
| 15       | Yes             | Yes                         | HIV                         | Lung           | Bilateral lung infiltrates | Negative    | No BAL sample | Negative                   | Positive                   | A. fumigatus                      | Favorable       | Voriconazole+amphotericin B |

<sup>a</sup> MycAssay in Patients without Hematological Cancer

<sup>b</sup> COPD: Chronic obstructive pulmonary disease

<sup>c</sup> ND: Not determined

<sup>d</sup> ND<sup>d</sup>: Not determined
available real-time PCR for the detection of the most clinically relevant Aspergillus species [22]. As a fully standardized CE-marked test with full manufacturing quality controls, MycAssay™ Aspergillus fulfils the requirements for standardization of Aspergillus PCR necessary to confirm a diagnosis of invasive aspergillosis. MycAssay™ Aspergillus was previously assayed on BAL samples [19], sputum samples [33,34], and tissue samples [35]. MycAssay can be performed in the clinical microbiology laboratory by personnel trained in molecular biology technology; the estimated cost of the test per determination in Spain is €25–30.

We evaluated MycAssay™ Aspergillus on lower respiratory tract samples (including BAL, spontaneous sputum, and bronchial aspirate) from patients with predisposing conditions other than hematological malignancy and clinical suspicion of invasive aspergillosis. We found that the three kinds of lower respiratory tract samples studied were suitable for detection of Aspergillus spp. DNA. Our gold standard was clinical in that we classified patients using the criteria proposed by the EORTC [20,21] or by Bulpa [20,21]. The sensitivity of the PCR was high and increased when several samples per patient were studied; specificity was not affected by including multiple samples per patient. Sensitivity was also high in patients with COPD, a predisposing condition in a large proportion of the patients studied. MycAssay™ Aspergillus showed a high NPV, thus suggesting that a PCR-negative result in any lower respiratory tract sample from a patient without hematological cancer could prove useful for ruling out invasive aspergillosis. In contrast, the PPV of the assay was low. An explanation for this finding is that MycAssay™ Aspergillus is able to detect Aspergillus DNA in samples from colonized patients or from patients with other forms of aspergillosis, including chronic

| Table 2. Comparison of fungal culture and MycAssay™ Aspergillus for all samples and for samples from patients with or without invasive aspergillosis. |
|-------------------------------------------------|
| Cultured samples | All patients (n = 173) | Patients with IPA* (n = 15) | Patients without IPA (n = 158) |
| Positive (% and 95% CI) | 298b | 37 | 261 |
| Samples with MycAssay determinations | 308 | 37 | 271 |
| Positive (% and 95% CI)* | 51 (16.5%, 13–21%) | 24 (64.9%; 49–81%) | 27 (10.3%, 7–14%) |
| Concordance between fungal culture and MycAssay™ |  |
| Culture +/MycAssay + (% and 95% CI) | 217 (72.8%, 68–78%) | 9 (24.3%, 10–39%) | 208 (79.7%, 75–85%) |
| Culture +/MycAssay − (% and 95% CI) | 33 (11.1%, 7–15%) | 23 (62.2%, 46–79%) | 10 (3.8%, 1–6%) |
| Culture −/MycAssay + (% and 95% CI) | 18 (6%, 3–9%) | 1 (2.7%, −3–8%) | 17 (6.5%, 3–10%) |
| Culture −/MycAssay − (% and 95% CI) | 30 (10.1%, 7–14%) | 4 (10.8%, 0–21%) | 26 (10%, 6–14%) |

The table shows the proportion of samples with positive results and the concordance between both procedures for all samples and for samples from patients with and without invasive aspergillosis.

*IPA, invasive pulmonary aspergillosis.

bFungal or bacterial culture was performed in 298 out of the 308 samples studied.

CI, confidence interval.

The analysis of concordance was performed in 298 samples after excluding the remaining 10 samples in which only PCR was performed.

doi:10.1371/journal.pone.0061545.t002

| Table 3. Comparison between fungal culture and MycAssay™ Aspergillus according to the type of lower respiratory tract sample studied. |
|-------------------------------------------------|
| Bronchoalveolar lavage | Sputum | Bronchial aspirate |
| Cultured samples* | 61 | 136 | 101 |
| Positive (% and 95% CI)b | 8 (13.1%, 4–22%) | 36 (26.5%, 19–34%) | 19 (18.8%, 11–27%) |
| Samples with MycAssay determinations | 61 | 143 | 104 |
| Positive (% and 95% CI) | 7 (11.5%, 3–20%) | 27 (18.9%, 11.24%) | 20 (19.2%, 12–28%) |
| Concordance between fungal culture and MycAssay™ |  |
| Culture −/MycAssay − (% and 95% CI) | 49 (80.3%, 70–91%) | 90 (66.2%, 58–74%) | 78 (77.2%, 69–86%) |
| Culture +/MycAssay + (% and 95% CI) | 3 (4.9%, −1–11%) | 14 (10.3%, 5–15%) | 16 (15.8%, 9–23%) |
| Culture −/MycAssay + (% and 95% CI) | 4 (6.6%, 0–13%) | 10 (7.4%, 3–12%) | 4 (4%, 0–8%) |
| Culture −/MycAssay − (% and 95% CI) | 5 (8.2%, 1–15%) | 22 (16.2%, 10–22%) | 3 (3%, 0–6%) |

The table shows the proportions of samples with PCR− or fungal culture−positive results and the concordance between both procedures for bronchoalveolar lavage samples, sputum samples, and bronchial aspirate samples.

*Fungal or bacterial culture was performed in 298 of the 308 samples studied.

bCI, confidence interval

The analysis of concordance was performed in 298 samples after excluding the remaining 10 samples in which only PCR was performed.

doi:10.1371/journal.pone.0061545.t003
and allergic pulmonary aspergillosis [36,37] (Table 5). Another explanation may be the low prevalence of invasive pulmonary aspergillosis found in the study population.

The main limitation of our study is that we were not able to include histologically proven cases because no lung biopsies were collected or post-mortem examinations performed. This is a common limitation in studies evaluating the role of new diagnostic tools for the diagnosis of invasive aspergillosis. Therefore, the patients were diagnosed with invasive aspergillosis based on microbiological or radiological data. The fact that *Aspergillus* was isolated from lower respiratory tract samples in all patients could explain the high correlation found between fungal culture and PCR. In our patients, MycAssay™ Aspergillus did not allow us to diagnose new cases that could be missed with fungal culture. Another limitation is that we classified our patients using the EORTC criteria, which were specifically developed for patients with cancer. EORTC criteria were chosen in the absence of specific criteria for non-cancer patients.

We conclude that MycAssay™ Aspergillus performed on lower respiratory tract samples showed high sensitivity for the diagnosis of invasive aspergillosis in patients without hematological cancer.

Sensitivity increased when multiple samples were analyzed. Sensitivity was high for patients with COPD, which is an emerging risk population in some hospitals. MycAssay™ Aspergillus proved to be particularly useful for ruling out the diagnosis of invasive aspergillosis and significantly reduced time to diagnosis compared with conventional fungal culture. MycAssay™ Aspergillus should be used simultaneously with fungal culture of lower respiratory tract samples in order to provide additional data on antifungal susceptibility and ensure accurate identification of the isolates.

### Acknowledgments

We would like to thank Thomas O’Boyle for editing and proofreading the article. We appreciate the valuable suggestions of David W. Denning after his critical review of the manuscript. This study was presented in part at the 21st European Congress on Clinical Microbiology and Infectious Diseases (ECCMID) and 27th International Congress on Chemotherapy (ICC), Milan, Italy, 2011 (P-2095).

### Table 4. Sensitivity, specificity, LR+, LR−, and DOR of MycAssay™ Aspergillus for the diagnosis of invasive pulmonary aspergillosis performed on lower respiratory samples from patients without hematological cancer.

|                      | First sample | Any sample |
|----------------------|--------------|------------|
|                      | Fungal culture | MycAssay™ | Fungal culture | MycAssay™ |
| All patients (n = 173) |              |            |              |            |
| Sensitivity           | 86.7         | 86.7       | 100          | 93.3       |
| Specificity           | 83.5         | 88         | 81.6         | 82.9       |
| LR+                  | 5.3          | 7.2        | 5.4          | 5.5        |
| LR−                  | 0.16         | 0.15       | 0            | 0.08       |
| DOR                  | 33.13        | 48         | ∞            | 68.75      |
| COPD patients (n = 91) |              |            |              |            |
| Sensitivity           | 100          | 100        | 100          | 100        |
| Specificity           | 77.6         | 85.9       | 74.1         | 78.8       |
| LR+                  | 4.5          | 6.2        | 3.9          | 4.7        |
| LR−                  | 0            | 0          | 0            | 0          |
| DOR                  | ∞            | ∞          | ∞            | ∞          |

A sub-analysis including only patients with chronic obstructive pulmonary disease is shown.

LR+, likelihood ratio for a positive result; LR−, likelihood ratio for a negative result; DOR, diagnostic odds ratio; COPD, chronic obstructive pulmonary disease.

doi:10.1371/journal.pone.0061545.t004

### Table 5. Positive predictive value (PPV) and negative predictive value (NPV) of the MycAssay™ Aspergillus and *Aspergillus* culture for the diagnosis of invasive aspergillosis in different clinical situations.

|                      | No. of patients | No. of patients with invasive aspergillosis (prevalence) | MycAssay™ Aspergillus/*Aspergillus* culture |
|----------------------|-----------------|----------------------------------------------------------|------------------------------------------|
|                      | No. of patients | PPV   | NPV   | PPV   | NPV   |
| All patients         | 173             | 15 (8.7%) | 34.1/34.1 | 99.2/100 |
| Patients with COPD   | 91              | 6 (6.6%)  | 25.0/21.4  | 100/100 |
| Patients in ICU with pneumonia | 35          | 12 (34%)  | 78.6/80   | 95.2/100 |
| Patients with infection not improving with antibiotics | 28            | 15 (53.6%) | 87.5/83.3 | 91.6/100 |

The analysis was performed based on the results of any of the studied samples.

doi:10.1371/journal.pone.0061545.t005
Author Contributions
Conceived and designed the experiments: JG CP EB. Performed the experiments: JG PE. Analyzed the data: JG CP PM BP PG. Contributed reagents/materials/analysis tools: JG CP PE EB. Wrote the paper: JG CP PE EB.

References
1. Kontoyiannis DP, Marr KA, Park BJ, Alexander BD, Anaissie EJ, et al. (2010) Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSMET) Database. Clin Infect Dis 50: 1091–1100.
2. Neofytos D, Horn D, Anaissie E, Steinbach W, Olyaei A, et al. (2009) Epidemiology and outcome of invasive fungal infection in adult hematopoietic stem cell transplant recipients: analysis of Multicenter Prospective Antifungal Therapy (PATH) registry. Clin Infect Dis 48: 265–273.
3. Neofytos D, Fishman JA, Horn D, Anaissie E, Chang CH, et al. (2010) Epidemiology and outcome of invasive fungal infections in solid organ transplant recipients. Transpl Infect Dis 12: 220–229.
4. Denning DW (2006) Aspergillus and aspergillosis—Progress on many fronts. Med Mycol 44 (S1):1–1.
5. Pagano L, Caira M, Candoni A, Offidani M, Martino B, et al. (2010) Invasive aspergillosis in critically ill patients without malignancy. Am J Respir Crit Care Med 180: 621–625.
6. Meersman W, Lagrou K, Maertens J, Van Wijngaerden E (2007) Invasive aspergillosis in non-hematological patients with clinical isolation of Aspergillus fumigatus. J Mol Diagn 8: 376–384.
7. Singh N, Huisan S (2009) Invasive aspergillosis in solid organ transplant recipients. Am J Transplant 9 Suppl 4: S180–191.
8. Meersman W, Vandecasteele SJ, Wilmer A, Verbeken E, Peetermans WE, et al. (2009) Accuracy of the diagnosis of invasive aspergillosis using PCR on bronchoalveolar lavage fluid. J Clin Microbiol 47: 3842–3848.
9. Denning DW, Park S, Furler C, Frazier MG, Kirwan M, et al. (2011) High-frequency triple antifungal resistance found in nonculturable Aspergillus fumigatus from lungs of patients with chronic fungal disease. Clin Infect Dis 52: 1123–1129.
10. Sun W, Wang K, Gao W, Su X, Qian Q, et al. (2011) Evaluation of Aspergillus PCR protocols for testing serum specimens. J Clin Microbiol 49: 3842–3848.
11. Guine J, Jensen J, Pelaez T, Gijon P, Alonso R, et al. (2008) Value of a single galactomannan determination (Platelia for the diagnosis of invasive aspergillosis in non-hematological patients with clinical isolation of Aspergillus spp. J Clin Microbiol 46(8):2579–2583.
12. White PL, Barton R, Guiver M, Linton CJ, Wilson S, et al. (2006) A consensus on fungal polymerase chain reaction diagnosis?: a United Kingdom-Ireland evaluation of polymerase chain reaction methods for detection of systemic fungal infections. J Mol Diagn 8: 376–384.
13. White PL, Barton R, Guiver M, Linton CJ, Wilson S, et al. (2006) A consensus on fungal polymerase chain reaction diagnostic?: a United Kingdom-Ireland evaluation of polymerase chain reaction methods for detection of systemic fungal infections. J Mol Diagn 8: 376–384.