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Running head: Diagnosis of Invasive Pulmonary Aspergillosis in BAL

Key words: BAL, Diagnosis, Aspergillus PCR, Lateral-Flow Device Test, Aspergillus, Galactomannan, Beta-D-Glucan, Culture, Aspergillosis, Haematological Malignancy, Solid Organ Transplantation.
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Abstract:

Objectives: Galactomannan (GM) detection in bronchoalveolar-lavage (BAL) fluid is currently considered the gold standard test for diagnosis of invasive pulmonary aspergillosis (IPA). Limitations, however, are varying turnaround time and availability. We compared the performance of GM with that of conventional culture, *Aspergillus* Lateral-Flow-Device (LFD) test, Beta-D-Glucan (BDG) and an *Aspergillus* polymerase chain reaction (PCR) assay by using BAL samples from immunocompromised patients.

Methods: A total of 78 BAL samples from 78 patients at risk for IPA (74 samples from Graz, 4 from Mannheim) collected between December 2012 and May 2013 at two University hospitals in Austria and Germany were included. Three patients had proven IPA, 14 probable, 17 possible and 44 patients no IPA. Diagnostic accuracies of the different methods for probable/proven IPA were evaluated.

Results: Diagnostic odds ratios were highest for GM, PCR and LFD tests. Sensitivities for four methods (except culture) were between 70 and 88%. Combined GM (cut-off >1.0 ODI) with LFD increased the sensitivity to 94%, while combined GM (>1.0) with PCR resulted in 100% sensitivity (specificity for probable/proven IPA 95-98%). The performance of conventional culture was limited by low sensitivity, while that of BDG was limited by low specificity.

Conclusions: We evaluated established and novel diagnostic methods for IPA and found that *Aspergillus* PCR, LFD test and GM were the most useful methods for diagnosis of the disease by using BAL samples. In particular, combination of GM and PCR or, if PCR is not available, the LFD test, allows sensitive and specific detection of IPA.
**Introduction:**

Invasive fungal infections (IFI) remain an important cause of morbidity and mortality among immunocompromised patients. Invasive pulmonary aspergillosis (IPA) is caused by *Aspergillus fumigatus* as well as other *Aspergillus* species. Being an abundant component of inhaled air, they represent one of the leading causes of IFI related morbidity and mortality (1-5). Due to the crude mortality of 80-90% in absence of adequate treatment, timely diagnosis and early start of antifungal therapy are key factors in the successful treatment of IPA, as delayed antifungal therapy has a negative impact on survival of these patients (6). Various studies have shown that early diagnosis and initiation of antifungal therapy may improve IFI survival to above 80% (7-9). Diagnosis of IFI, however, remains difficult as clinical signs and symptoms as well as radiological findings are often unspecific and conventional culture methods display low sensitivity (8). In a large autopsy-based retrospective analysis, only 25% of patients with IFI confirmed by autopsy were identified pre-mortem by culture-based methods, underlining the difficulty in diagnosing IFI by conventional methods and emphasizing the need for novel serological and molecular markers (10, 11). In recent years, antigen testing has therefore become one of the cornerstones of IFI diagnostics (12, 13). Galactomannan (GM) is a polysaccharide component of the cell wall of *Aspergillus* spp. that is released into bloodstream by growing hyphae and germinating spores/conidia. Diagnostic performance of GM testing in bronchoalveolar lavage (BAL) specimens is promising as it has higher sensitivity compared to serum testing (14). The test, however, has several limitations. As false positive results may occur, factors such as co-medication (e.g. beta-lactam antibacterials), underlying diseases, host factors (e.g. renal failure), diagnostic imaging, clinical signs, and former medication of the patient must be taken into account for the correct interpretation of GM levels (15-17). Furthermore, some studies have shown that sensitivity of GM decreases significantly in case of administration of antifungal prophylaxis/empirical
therapy whereas other reports have shown its usefulness for diagnosing breakthrough IFI (13, 18-20).

Another target antigen for IFI diagnosis is Beta-D-Glucan (BDG), a cell wall component of most pathogenic fungi (e.g. Aspergillus spp., Candida spp.), except Mucorales and Cryptococci (21-27). Since 2008, the serum BDG test has also been a diagnostic criterion for IFI in the revised European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) guidelines (28). However, limited data currently exist on performance of BDG testing in BAL (29).

One of the major limitations of both the GM and the BDG test is that time to results varies between centres (ranging from less than a day to several days), depending on the number of specimens to be tested, and the distance/duration of transport between the clinical setting and the laboratory where the test is performed. These limitations are overcome by the Lateral-Flow Device (LFD) test, a new point-of-care test for IPA diagnosis developed at the University of Exeter, United Kingdom. This single-sample test, based on the detection of Aspergillus antigen by mAb JF5, can be performed easily in every laboratory using BAL or serum specimens and has a time to result of approximately 15 minutes. Recent studies have shown the immense potential of this test in human BAL and serum samples (30-32), but have been limited by the small sample sizes, especially BALs (30, 33). More extensive clinical studies are therefore needed to evaluate this new point-of-care device among different patient cohorts.

Even though sensitivity may decrease under antifungal prophylaxis, PCR has been shown to be a very promising method for detection of fungal infection by using BAL fluid from immunocompromised patients (34-37), especially when combined with the GM test (40). Despite major concerns such as the lack of external standardization, the variety of different methods employed and the recognised need for larger studies, PCR diagnostics using
BAL seems to be a promising approach for the diagnosis of IFI. Harmonization efforts are ongoing to enable inclusion of PCR into the next revision of the EORTC classification (35, 38).

In this study we compared the diagnostic performances for IPA of the GM test, conventional culture, *Aspergillus* LFD test, BDG test and *Aspergillus* PCR assay as single and combined diagnostic tools when used with BAL samples from immunocompromised patients.
Methods:

This part prospective, part retrospective study comprises 78 routine BAL samples from 78 adult immunocompromised patients (74 samples from Graz, 4 from Mannheim) that were tested in clinical routine for GM as well as conventional mycological culture between July 2012 and May 2013 at the two University Hospitals of Graz, Austria, and Mannheim, Germany. IPA was graded in accordance with the revised criteria by the European Organization for Research and Treatment of Cancer Invasive Fungal Infections Cooperative Group (EORTC) and the Mycoses Study Group of the National Institute of Allergy and Infectious Disease (MSG) (39, 40).

In 67 samples (63 from Graz, 4 from Mannheim) LFD, BDG as well as PCR testing was performed in addition to GM testing and mycological culture. In 11 additional samples (all from Graz) there was insufficient BAL material for PCR testing, therefore only LFD and BDG testing was performed in addition to GM testing and mycological culture. In contrast to BAL, serum GM and BDG levels were available in approximately two thirds of patients only and so these results were excluded from this study.

Conventional mycological culture was routinely and prospectively performed in Graz (Microbiology Laboratory, Department of Internal Medicine) and Mannheim (Institute of Medical Microbiology and Hygiene, Mannheim University Hospital). BAL GM concentrations were prospectively determined in clinical routine by the Platelia EIA (Bio-Rad Laboratories) in Graz (Institute of Hygiene, Microbiology and Environmental Medicine) and Mannheim (Institute of Medical Microbiology and Hygiene, Mannheim University Hospital), respectively. For GM we used the optical density index (ODI) cut-offs of 0.5 and 1.0 to evaluate the diagnostic performance of the test.

LFD testing was prospectively performed at the Microbiology Laboratory, Department of Internal Medicine, Medical University of Graz, and the Scientific Laboratory, Department of Internal Medicine, Medical University of Graz.
of Hematology and Oncology, Mannheim University Hospital, respectively. The LFD test is based on the detection of an *Aspergillus* antigen by mAb JF5. The target antigen is an extracellular glycoprotein that is exclusively secreted during active growth of the fungus and represents a surrogate marker of *Aspergillus* infection (30). Monoclonal antibody JF5 has been incorporated into an immuno-chromatographic assay (a point-of-care diagnostic tool), which is easy to use. The rapidity of the test using BAL samples – time to result is only 15 minutes – is particularly remarkable. The JF5 LFD results in qualitative data based on the test-line intensity ranging from strong positive (++++) to weak positive (+) or negative (-). As the test is read by naked eye the test interpretation depends on subjective evaluation. Wiederhold and colleagues have shown, however, that results are reproducible between different laboratories and studies (41). Regardless of the test-line intensity, however, all positive test results in BAL samples indicate germination of spores and development of potentially pathogenic hyphae in the lungs (30). Testing was performed according to the manufacturers’ instructions. For BAL testing, 100 μl of BAL sample were applied to the LFD, without having received pre-treatment (30). Results were read after 15 minutes and interpreted in line with previous publications (30).

After performing culture, GM and LFD all 78 samples were frozen and stored at -70°C for retrospective BDG and PCR testing.

BDG testing was performed retrospectively in all 78 samples at the Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, using the commercial available Fungitell® assay (Cape Cod Diagnostics) with an adopted protocol suitable for use on a routine BCS XP® coagulation analyser, as described previously (42). For BDG we used the cut-offs 80 pg/mL and 200 pg/mL to evaluate the diagnostic performance of the test.
A nested *Aspergillus* PCR assay was performed retrospectively in 67 samples at the Scientific Lab of the University Hospital of Mannheim according to the protocol of Skladny et al., as described previously (43).

Our study was conducted in accordance with the Declaration of Helsinki, 1996, Good Clinical Practice, and applicable local regulatory requirements and law. The study protocol was approved by the local ethics committee, Medical University Graz, Austria (EC-number 25-221 ex 12/13) and registered at ClinicalTrials.Gov (Identifier: NCT02058316).

Statistical analysis was performed using SPSS, version 20 (SPSS Inc., Chicago, IL, USA). Negative predictive value (NPV), positive predictive value (PPV), sensitivity and specificity were calculated where applicable. The different diagnostic methods were compared using the diagnostic odds ratio (DOR) method. All DOR values were displayed with 95% confidence intervals (95% CI). Specificity, PPV, NPV and DOR were calculated three times, (a) for probable/proven IPA versus no IPA (exclusion of possible IPA cases) (b) for probable/proven IPA versus possible/no IPA, and (c) for possible/probable/proven IPA versus no IPA. This was necessary as cases with possible IPA might in fact have had IPA or not, while IPA can almost always be excluded in patients that do not fulfil the criteria at all.

Receiver operating characteristics (ROC) curve analysis was performed for BDG and GM levels. Area under the curve (AUC) values were displayed including 95% confidence intervals (CI).
Results:

A total of 78 patients (54 males, 24 females, median age 58 years, range 24-77 years) were included in the study. The majority (47/78, 60%) of patients had underlying hematologic malignancies, 11/78 had undergone solid organ transplantation, 12/78 had underlying chronic lung diseases (including high grade COPD and pulmonary sarcoidosis), 4/78 had sepsis treated in intensive care unit (ICU), 2/78 patients had AIDS, 2/78 had chronic autoimmune diseases (under immunosuppressive therapies). Demographic data and underlying diseases of patients with/without PCR results are depicted in Table 1.

In 67 samples all five methods (including PCR) were compared. Out of these 67 patients, three had proven IPA, seven probable IPA, 16 possible IPA and 41 no IPA. Sensitivity, specificity, PPV, NPV and DOR of all five tests as well as for three combinations (positive PCR and/or positive LFD, GM >1.0 ODI and/or positive PCR, GM >1.0 ODI and/or positive LFD) for probable/proven IPA vs. no IPA are shown in Table 2. Specificity, PPV, NPV and DOR of PCR (alone or in combination) for probable/proven IPA vs. possible/no IPA were as follows: PCR (96%, 78%, 95%, 60.7; 95%CI 8.6-429); PCR and/or BDG>80 pg/mL (63%, 30%, 97%, 15.4; 95%CI 1.8-130); PCR and/or LFD (81%, 45%, 98%, 37.7; 95%CI 4.3-329); PCR and/or GM>1.0 ODI (95%, 77%, 100%, 327; 95%CI 15.7-6809). Sensitivity, PPV, NPV and DOR of PCR (alone or in combination) for possible/probable/proven IPA vs. no IPA were as follows: PCR (35%, 100%, 71%, 45; 95%CI 2.5-817); PCR and/or BDG>80 pg/mL (77%, 67%, 84%, 10.3; 95%CI 3.2-33); PCR and/or LFD (69%, 90%, 83%, 44; 95%CI 8.5-228); PCR and/or GM>1.0 ODI (46%, 92%, 74%, 34.3; 95%CI 4.1-288).

In another 11 patients, four methods (except PCR) were compared. Seven of these 11 patients had probable IPA, one possible and three no IPA. Sensitivity, specificity, PPV, NPV and DOR of the GM, BDG, and LFD test, mycological culture and one combination (GM and DOR of the GM, BDG, and LFD test, mycological culture and one combination (GM and
>1.0 ODI and/or positive LFD) for probable/proven IPA in all 78 patients (including the above mentioned 11) are depicted in Table 3. Specificity, PPV, NPV and DOR for probable/proven IPA vs. possible/no IPA were as follows: GM >1.0 ODI (98%, 92%, 92%, 144; 95%CI 15.4-1345); GM >0.5 ODI (87%, 64%, 95%, 30.9; 95%CI 7.2-132); mycological culture (97%, 82%, 88%; 33.2; 95%CI 6.1-182); BDG >80 pg/mL (64%, 41%, 95%, 13.3; 95%CI 2.8-64); BDG >200 pg/mL (72%, 41%, 90%, 6.2; 95%CI 1.9-20); LFD (84%, 60%, 96%, 38.3; 95%CI 7.5-194); BDG >80 pg/mL and/or GM >1.0 ODI (64%, 42%, 98%, 28.4; 95%CI 3.5-229); BDG >80 pg/mL and/or LFD (56%, 38%, 94%, 9.4; 95%CI 2.4-44.9); GM >1.0 ODI and/or LFD (82%, 59%, 98%, 73; 95%CI 8.7-608). Sensitivity, PPV, NPV and DOR for possible/probable/proven IPA vs. no IPA were as follows: GM >1.0 ODI (35%, 92%, 66%, 26.6; 95%CI 3.3-217); GM >0.5 ODI (59%, 91%, 75%, 30; 95%CI 6.2-145); mycological culture (26%, 82%, 63%; 7.6; 95%CI 1.5-38); BDG >80 pg/mL (74%, 68%, 78%, 7.4; 95%CI 2.7-20); BDG >200 pg/mL (65%, 76%, 76%, 9.7; 95%CI 3.3-28); LFD (68%, 92%, 79%, 44; 95%CI 9.215); BDG >80 pg/mL and/or GM >1.0 ODI (76%, 68%, 80%, 8.7; 95%CI 3.1-24.4); BDG >80 pg/mL and/or LFD (82%, 67%, 83%, 10; 95%CI 3.4-29.6); GM >1.0 ODI and/or LFD (76%, 96%, 84%, 140; 95%CI 16.5-1182). Diagnostic odds ratios were highest for GM, PCR and the LFD test. Combination of GM (>1.0 ODI) with LFD increased the sensitivity to 90% (94% [16/17] when including the 11 samples where PCR was not performed) while combination of GM (>1.0 ODI) with PCR resulted in a 100% sensitivity (specificity for probable/proven vs. possible/no IPA 95%, specificity after exclusion of possible IPA 98%).

ROC curve analysis revealed AUC values of 0.899 (95%CI 0.776-1.0) for GM and 0.858 (95%CI 0.750-0.967) for BDG for differentiation between probable/proven and no IPA, AUC values of 0.883 (95%CI 0.773-1.0) for GM and 0.833 (95%CI 0.721-0.945) for BDG for differentiation between probable/proven and possible/no IPA, and AUC values of 0.890
for GM and 0.749 (95% CI 0.625-0.853) for BDG for differentiation between possible/probable/proven and no IPA.

One invasive mould infection other than aspergillosis was observed among patients included. In this patient with invasive fusariosis only culture and BDG (398 pg/mL) resulted positive.

When analyzing the subgroup of patients with hemato-oncological malignancies (n=47, one proven, 5 probable, 9 possible, 32 no IPA), sensitivity for probable/proven IPA was highest for BAL GM>0.5 ODI (5/6 [83%]; GM>1.0 ODI 4/6; LFD 4/6; PCR 3/4; BDG >80 pg/mL 4/6; BDG>200 pg/mL 2/6; mycological culture 2/6). In patients with possible IPA and those without IPA positive results were observed as follows: BAL GM >0.5 ODI in 2/9 possible IPA and 1/32 no IPA cases, GM>1.0 in 1/32 no IPA case, BAL BDG >80 pg/mL in 5/9 possible IPA and 9/32 no IPA cases, BAL BDG >200 pg/mL in 5/9 possible IPA and 5/32 no IPA cases, LFD in 2/9 possible IPA cases.

Discussion:

We evaluated established and novel diagnostic methods for IPA and found that GM, *Aspergillus* PCR, and the LFD test had the highest diagnostic potential in BAL samples. With respect to combinations, GM (with a cut-off 1.0 ODI) and/or positive PCR or combination of
GM (> 1.0 ODI) and/or a positive LFD test exhibited high sensitivities (over 90%) as well as highest DORs. Performance of conventional culture was limited by low sensitivity, while that of BDG was limited by low specificity.

BAL GM is the current gold standard for diagnosing IPA, has been reported to have an positive impact on the early initiation of appropriate antifungal therapy, and may therefore have an impact on patient’s survival (2, 44, 45). However, recommendations for optimal GM cut-off ODIs from BAL specimens vary widely between 0.5 and 3.0 (44-48). In this study, not surprisingly, sensitivity decreased with a higher cut-off value (from 82% at 0.5 ODI to 71% at 1.0 ODI) while specificity increased. Overall, DOR showed that 1.0 ODI might be as useful as a cut-off of 0.5 ODI, also AUCs in ROC curve analysis were nearly identical for both cut-off values.

In our cohort, *Aspergillus* PCR from BAL was a promising single test method with a sensitivity of 70%, a near to perfect specificity and convincing DORs. While in this study a well evaluated PCR protocol was used (35, 36, 46, 49, 50), it has to be emphasized that the results of our study are not transferable to other PCR assays, as the performances of the multiple PCR methods currently in use differ, and also lack external standardization. Another drawback of PCR testing in general is the amount of specimen needed to perform valid testing (about 2 mL), which is markedly more material when compared to GM (600 µL including serial retesting), BDG (about 200 µL incl. serial retesting) and the LFD test (100 µL). For this reason we were not able to perform PCR for all of the samples.

Another promising method is the *Aspergillus* LFD test with a sensitivity of 88% and specificity between 84% and 95%. Recent single-center studies, including one from our center, have shown the potential of this test in diagnosing IPA using human BAL and serum samples (30-33, 51, 52). The high NPV (95% to 96% in this study) may be particularly valuable. The test may therefore be a valuable tool for enabling immediate treatment.
decisions, as the time to result is 15 minutes only. Due to the high NPV, LFD testing may not only facilitate early diagnosis, but also may prevent overtreatment which has become frequent (53).

In a recent study, combination of GM and PCR from blood samples has been reported to be effective in directing treatment and in reducing the use of empirical antifungal treatment (54). With respect to combination tests of BAL samples, GM with a cut-off 1.0 ODI and/or positive PCR (sensitivity 100%) exhibited the highest DOR followed by the combination of GM > 1.0 ODI and/or a positive LFD test result (sensitivity 94%).

Mycological culture of BAL fluid is indispensable as antifungal susceptibility testing is currently available from culture only. Culture may also grow other moulds like Mucorales which do not result in positive GM or BDG levels or LFD test results. It has to be emphasized, however, that the low sensitivity of conventional culture is the major drawback. Lass-Flörl and colleagues have previously reported a sensitivity as low as 34% of conventional mycologic culture for IFI (21/61 samples positive) (8). In this study sensitivity of mycological culture was found to be around 50% and therefore represents also the major drawback of this method.

In contrast to culture, BAL BDG sensitivity was high (for the 80 pg/mL cut-off), but specificity was the major limitation of this test. In our collective, specificity varied between 64% and 84% depending on the cut-off level employed. A meta-analysis conducted by Karageorgopoulos et al. showed an overall sensitivity of serum BDG assays for IFI diagnosis of approximately 77% [95% confidence interval (CI), 67%-84%] and a specificity of about 85% (95% CI, 80%-90%). PPV ranged from 59% to 96% and NPV reached 95% in some studies (55). Specificity in BAL samples seems therefore to be lower when compared to serum samples, which might be also a result of frequent Candida colonization of the upper and lower respiratory tract in critically ill patients. Nevertheless, we found a NPV between...
94% and 95% for the 80 pg/mL cut-off even though our collective did not include Pneumocystis infections, where NPVs of 99% and more have been reported previously. A negative BDG result in BAL might therefore still have clinical value.

In conclusion, GM, Aspergillus PCR, and the LFD test had the highest diagnostic potential for IPA in BAL samples. With regard to combinations, GM and PCR or GM and LFD seem to be most promising. Studies with larger sample sizes are needed to further evaluate these diagnostic approaches.

Transparency Declaration:
The study was funded by an investigator initiated research grant from Pfizer (WI174981). LFD tests used in this study were provided by Dr. C. Thornton, University of Exeter. No other funding was obtained for this study.

Potential conflicts of interest:
M. Hoenigl received research grant from Merck and Pfizer; served on the speakers’ bureau of Pfizer, Gilead, Astellas and Merck and received travel grants from Astellas, Merck, Gilead and Pfizer.
D. Buchheidt is consultant to Gilead Sciences; received research grants from Gilead Sciences and Pfizer; served on the speakers’ bureau of Astellas, Gilead Sciences, Merck Sharp & Dohme/Merck, and Pfizer; and received travel grants from Astellas, Merck Sharp & Dohme/Merck, and Pfizer.

W. Buzina served on the speakers’ bureau of Merck and received travel grants from Pfizer.

M. Reinwald received travel grants from Astellas.

All other authors no conflict.

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References


Table 1: Demographic Data and Underlying Diseases of Patients with GM, LFD, BDG and Mycological Culture Results

<table>
<thead>
<tr>
<th></th>
<th>Patients with PCR Results (in addition to GM, BDG, LFD and Culture)</th>
<th>Patients without PCR Results (in addition to GM, BDG, LFD and Culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>67</td>
<td>11</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male/Female</td>
<td>45 (67%) / 22 (33%)</td>
<td>9 (82%) / 2 (18%)</td>
</tr>
<tr>
<td>Age in Years: Median (Range)</td>
<td>58 (24-77)</td>
<td>55 (34-67)</td>
</tr>
<tr>
<td>Primary Underlying Disease/Condition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematological Malignancy</td>
<td>43 (64%)</td>
<td>4 (36%)</td>
</tr>
<tr>
<td>AML</td>
<td>23 (34%)</td>
<td>2 (18%)</td>
</tr>
<tr>
<td>MDS</td>
<td>6 (9%)</td>
<td></td>
</tr>
<tr>
<td>NHL</td>
<td>7 (10%)</td>
<td>2 (18%)</td>
</tr>
<tr>
<td>Others</td>
<td>7 (10%)</td>
<td></td>
</tr>
<tr>
<td>Solid Organ Transplantation</td>
<td>4 (6%)</td>
<td>7 (64%)</td>
</tr>
<tr>
<td>Underlying Chronic Lung Disease</td>
<td>12 (18%)</td>
<td></td>
</tr>
<tr>
<td>ICU (Sepsis)</td>
<td>4 (6%)</td>
<td></td>
</tr>
<tr>
<td>AIDS</td>
<td>2 (3%)</td>
<td></td>
</tr>
<tr>
<td>Chronic Autoimmune Disease</td>
<td>2 (3%)</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** AIDS, acquired immune deficiency syndrome; AML, acute myeloid leukaemia; BDG, Beta-D-glucan; GM, Galactomannan; ICU, intensive care unit; LFD, Lateral-Flow-Device test; MDS, myelodysplastic syndrome; NHL, non-Hodgkin’s lymphoma; PCR, polymerase chain reaction.
Table 2: Diagnostic Performance of BAL GM, Culture, BDG, LFD and PCR for Probable and Proven IPA vs. no IPA in Patients with all Test Results available. Sensitivity, Specificity, PPV, NPV and DOR displayed.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity n=10</th>
<th>Specificity n=41</th>
<th>PPV</th>
<th>NPV</th>
<th>DOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM &gt;1.0 ODI</td>
<td>70%</td>
<td>98%</td>
<td>88%</td>
<td>93%</td>
<td>93.3; 95%CI 8.5-1030</td>
</tr>
<tr>
<td>GM &gt;0.5 ODI</td>
<td>80%</td>
<td>98%</td>
<td>89%</td>
<td>95%</td>
<td>160; 95%CI 12.9-1984</td>
</tr>
<tr>
<td>Mycological Culture</td>
<td>50%</td>
<td>95%</td>
<td>71%</td>
<td>89%</td>
<td>19.5; 95%CI 3.3-129</td>
</tr>
<tr>
<td>BDG &gt;80 pg/mL</td>
<td>80%</td>
<td>76%</td>
<td>44%</td>
<td>94%</td>
<td>12.4; 95%CI 2.3-68</td>
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<tr>
<td>BDG &gt;200 pg/mL</td>
<td>60%</td>
<td>88%</td>
<td>55%</td>
<td>90%</td>
<td>10.8; 95%CI 2.2-52</td>
</tr>
<tr>
<td>LFD</td>
<td>80%</td>
<td>95%</td>
<td>80%</td>
<td>95%</td>
<td>78; 95%CI 9.5-639</td>
</tr>
<tr>
<td>PCR</td>
<td>70%</td>
<td>100%</td>
<td>100%</td>
<td>93%</td>
<td>161; 95%CI 7.5-3445</td>
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<tr>
<td>PCR and/or BDG &gt;80 pg/mL</td>
<td>90%</td>
<td>76%</td>
<td>47%</td>
<td>97%</td>
<td>27.9; 95%CI 3.1-248</td>
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<tr>
<td>PCR and/or LFD</td>
<td>90%</td>
<td>95%</td>
<td>82%</td>
<td>98%</td>
<td>176; 95%CI 14.2-2154</td>
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<tr>
<td>GM &gt;1.0 ODI and/or PCR</td>
<td>100%</td>
<td>98%</td>
<td>91%</td>
<td>100%</td>
<td>567; 95%CI 21.5-14946</td>
</tr>
<tr>
<td>GM &gt;1.0 ODI and/or LFD</td>
<td>90%</td>
<td>93%</td>
<td>75%</td>
<td>97%</td>
<td>114; 95%CI 10.6-1228</td>
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</tbody>
</table>

Abbreviations: 95%CI, 95% confidence interval; BDG, Beta-D-Glucan; DOR, diagnostic odds ratio; GM, Galactomannan; LFD, Lateral-Flow-Device test; NPV, negative predictive value; ODI, optical density index; PCR, polymerase chain reaction; PPV, positive predictive value.
Table 3: Diagnostic Performance of BAL GM, Culture, BDG, and LFD for Probable and Proven IPA vs. no IPA in all Patients included. Sensitivity, Specificity, PPV, NPV and DOR displayed.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>DOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM &gt;3.0 ODI</td>
<td>41%</td>
<td>100%</td>
<td>100%</td>
<td>81%</td>
<td>63.6; 95%CI 3.4-1203</td>
</tr>
<tr>
<td>GM &gt;1.0 ODI</td>
<td>71%</td>
<td>98%</td>
<td>92%</td>
<td>90%</td>
<td>103.2; 95%CI 11-970</td>
</tr>
<tr>
<td>GM &gt;0.5 ODI</td>
<td>82%</td>
<td>95%</td>
<td>88%</td>
<td>93%</td>
<td>98; 95%CI 14.8-648</td>
</tr>
<tr>
<td>Mycological Culture</td>
<td>53%</td>
<td>95%</td>
<td>82%</td>
<td>84%</td>
<td>23.6; 95%CI 4.3-130</td>
</tr>
<tr>
<td>BDG &gt;80 pg/mL</td>
<td>88%</td>
<td>73%</td>
<td>56%</td>
<td>94%</td>
<td>20; 95%CI 4-100</td>
</tr>
<tr>
<td>BDG &gt;200 pg/mL</td>
<td>71%</td>
<td>84%</td>
<td>63%</td>
<td>88%</td>
<td>12.7; 95%CI 3.4-47</td>
</tr>
<tr>
<td>LFD</td>
<td>88%</td>
<td>95%</td>
<td>88%</td>
<td>95%</td>
<td>158; 95%CI 20.3-1219</td>
</tr>
<tr>
<td>BDG &gt;80 pg/mL and/or GM &gt;1.0 ODI</td>
<td>94%</td>
<td>73%</td>
<td>57%</td>
<td>97%</td>
<td>42.7; 95%CI 5.3-358</td>
</tr>
<tr>
<td>GM &gt;1.0 ODI and/or LFD</td>
<td>88%</td>
<td>68%</td>
<td>51%</td>
<td>94%</td>
<td>16.1; 95%CI 3.2-80.1</td>
</tr>
</tbody>
</table>

Abbreviations: 95%CI, 95% confidence interval; BDG, Beta-D-Glucan; DOR, diagnostic odds ratio; GM, Galactomannan; LFD, Lateral-Flow-Device test; NPV, negative predictive value; ODI, optical density index; PPV, positive predictive value.