Bronchoalveolar Lavage Lateral-Flow Device Test for Invasive Pulmonary Aspergillosis in Solid Organ Transplant Patients: A Semiprospective Multicenter Study

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Background. Invasive pulmonary aspergillosis (IPA) remains an important cause of morbidity and mortality among patients undergoing solid organ transplantation (SOT). Because of the crude mortality of 80% to 90% in the absence of adequate treatment, timely diagnosis and early intervention with antifungal drugs are key factors in the successful treatment of IPA. Diagnosis, however, remains difficult. Therefore, new diagnostic tests are urgently needed. The Lateral-Flow Device (LFD) test is a rapid (15 min) single-sample point-of-care test that is based on the detection of an Aspergillus extracellular glycoprotein antigen by monoclonal antibody JF5.

Methods. This semiprospective multicenter study evaluated the LFD test for IPA diagnosis (established by galactomannan and culture results) by using bronchoalveolar lavage (BAL) samples from patients after SOT. Participating centers were the three Austrian Medical Universities of Innsbruck, Vienna, and Graz.

Results. Forty-seven BAL samples from 47 SOT patients were included (26 patients had undergone lung transplantation, 13 liver, 6 kidney, and 2 heart transplantation; 11 probable or proven IPA, 11 possible IPA, 25 no IPA) at the three Austrian Medical Universities of Innsbruck, Vienna, and Graz. Sensitivity and specificity, positive and negative predictive values, as well as diagnostic odds ratio of BAL LFD tests for probable IPA were 91%, 83%, 63%, 97%, and 50% (95% confidence interval, 5.4%-467%), respectively.

Conclusion. To conclude, the LFD test of BAL specimens is performed easily and provides accurate and rapidly available results in patients after SOT. Therefore, this new point-of-care test may be a promising diagnostic approach for detecting IPA using BAL specimens from SOT patients.

Keywords: Lateral-Flow Device test, BAL, Galactomannan, Aspergillosis, Solid organ transplantation.

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Invasive pulmonary aspergillosis (IPA) remains an important cause of morbidity and mortality among patients undergoing solid organ transplantation (SOT) (1–4). The disease is second only to candidiasis as a cause of invasive fungal infections (IFIs) in SOT patients and is the most important mold infection (1, 4–6). In some subsets of patients (e.g., lung transplant recipients), IPA is even more frequent than invasive candidiasis (7–9). Because of the crude mortality of 80% to 90% in the absence of adequate treatment, timely diagnosis and early initiation of antifungal therapy are key factors in the successful treatment of IPA. Various studies have shown that early detection and prompt administration of antifungal drugs may improve IPA survival to greater than 80% (10, 11). Diagnosis of IPA, however, remains difficult because clinical signs and symptoms as well as radiologic findings are often unspecific and conventional culture methods lack sensitivity. Consequently, antigen testing has become a routine method for diagnosing the disease (12, 13). Galactomannan (GM) is a polysaccharide component of the cell wall of Aspergillus species that is released into the circulation by growing hyphae and germinating spores. The diagnostic performance of GM testing in bronchoalveolar lavage (BAL) specimens seems to be very promising (14, 15), but the test has several limitations. Because false-positive results are known to occur, factors such as comedications (e.g., β-lactam antibiotics), 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 (16–18). Furthermore, studies have shown that the sensitivity of GM decreases significantly in case of administration of antifungal prophylaxis or therapy, although other reports have shown its usefulness for diagnosing breakthrough IFI (19, 20).

One of the major limitations of the GM test is that the time to results varies between centers (between less than a day and several days), depending on the number of specimens to be tested and the distance or duration of transport between the clinic and the diagnostic laboratory. 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 monoclonal antibody (mAb) IF5, can be performed easily in every laboratory using BAL or serum specimens and has a time to result of approximately 15 min (21) (Fig. 1).

Recent single-center studies, including one from our study group, have shown the enormous potential of the test in diagnosing IPA using human BAL and serum samples (21–26). Multicenter studies evaluating bigger sample sizes from well-defined patient collectives at risk for IPA are needed, however, before the test can be established in clinical routine. In this multicenter study, we evaluate the LFD test using BAL specimens for early diagnosis of IPA among SOT patients.

RESULTS

A total of 47 BAL samples from 47 patients (29 men, 18 women; median age, 51 years; range, 18–71 years) were included (17 samples from Innsbruck Medical University, 22 samples from Medical University of Vienna and 8 samples from Medical University of Graz). Twenty-six patients had undergone lung transplantation, 13 liver, 6 kidney, and 2 heart transplantation. In 18 patients, BAL samples were obtained in the early phase after transplantation (within 3 weeks after surgical procedure), whereas in 29 patients, samples were obtained between 5 weeks and 10 years after transplantation. According to the Treatment of Cancer Invasive Fungal Infections Cooperative Group and the Mycoses Study Group of the National Institute of Allergy and Infectious Disease (EORTC-MSG) criteria, 11 patients showed probable or proven IPA (6 from Vienna, 3 from Innsbruck, 2 from Graz) and 11 showed possible IPA. Twenty-five patients did not fulfill the IPA criteria. Mycologic evidence in cases of probable and proven cases of IPA was established by BAL GM tests (nine cases), fungal culture of BAL (nine cases) or sterile tissue (one case), microscopy (two cases), and histology (two cases). Demographic data, underlying diseases, and test results of patients with probable or proven IPA are displayed in Table 1.

Potential false-positive LFD test results were observed in three cases with possible IPA (two of the three were receiving systemic antifungal therapy at the time of sample collection, whereas one showed probable invasive infection caused by Penicillium species) and three cases without IPA. In these cases, which did not fulfill clinical IPA criteria (all had undergone lung transplantation), Aspergillus species were also cultured from BAL. In one of these three cases, BAL was also positive for GM. However, in another 10 patients with positive BAL cultures, but without IPA, the LFD test showed negative results.

Sensitivity and specificity of BAL LFD for probable or proven IPA were 91% and 83% (positive predictive value, 63%; negative predictive value [NPV], 97%), respectively. Diagnostic odds ratio was 50 (95% confidence interval [CI], 5.4–467). Results (per center, overall as well as for lung transplant recipients in particular) are depicted in Table 2.

The LFD test gave a false-negative result in one patient with probable IPA who had undergone kidney transplantation at the Medical University of Graz, Austria. The corresponding BAL sample had a GM value of 24.97 U/L, whereas the culture remained negative.

With regard to potential cross-reactivity with other fungi, the LFD test gave a positive result in a case of probable IFI caused by Penicillium species (possible IPA). The test resulted negative, however, in another patient with probable Penicillium infection and also in a patient with probable invasive Fusarium solani infection. The LFD test also resulted negative in 10 patients without IPA but growth of various Aspergillus species in BAL cultures (1 of 10 patients showed also a positive BAL GM result).

DISCUSSION

We performed a semiprospective multicenter study to evaluate the Aspergillus LFD test using BAL specimens for

**FIGURE 1.** LFD test result using BAL fluid showing strong positive (+++) test (7) line. Internal control line (C) indicates that the test has run correctly. LFD, Lateral-Flow-Device; BAL, bronchoalveolar lavage.
Aspergillus niger
BAL LFD result
Aspergillus fumigatus
Aspergillus fumigatus
Y
Aspergillus flavus
species infection. This finding confirms that
Aspergillus fumigatus
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Aspergillus fumigatus,
Probable
3
Aspergillus species,
Probable
Aspergillus fumigatus
Probable
None
Probable
species
Penicillium
Y
species without IPA (e.g., caused by colonization
28
Aspergillus fumigatus
species, an
Probable
Aspergillus terreus
Probable
Aspergillus fumigatus
Probable
Y
29
Exact value displayed if available, otherwise stated as positive table. The cutoff chosen in the study was 1.0.
Proven

Before this investigation, only one study had evaluated
the performance of the LFD test using BAL samples from a
mixed patient cohort including 10 SOT patients. That single-
center retrospective study found that the LFD test accurately
detected all five cases of probable IPA with results that
corresponded with positive GM values (26). The test also
resulted positive in one patient with possible IPA but was
negative in all four cases of patients without IPA. In this
multicenter study, we confirmed the accuracy of the LFD test
for the detection of IPA in a cohort of 47 patients, with NPVs
of 86% or greater in all three participating centers. When
all cohorts are analyzed together, the performance of the test
for the exclusion of IPA in BAL samples from SOT patients
was near excellent, with an NPV of greater than 97%. The test
may therefore be a valuable tool for enabling immediate
treatment decisions because the time to result is 15 min only.
Because of the high NPV, the LFD test may not only facilitate
temporary diagnosis but also prevent overtreatment, which has
become frequent (27).
A previous study has reported that the sensitivity of the
LFD test may be reduced in the presence of antifungal therapy when testing serum samples, although this was not
the case for BAL samples (28). In contrast, reduced GM
sensitivity in case of antifungal prophylaxis or treatment has
been described also for BAL samples (29). Two cases with
possible IPA had negative GM but positive LFD results in this
study. This may therefore be explained by the theory that
antifungal therapy seems less influential on LFD results when
compared with GM. With regard to previously reported cross-
reactivity of the LFD test with fungi other than Aspergillus, we
report a positive result for a patient with possible IPA and
probable IFI caused by Penicillium species. However, the LFD
resulted negative with BAL from another patient with prob-
able Penicillium species infection. This finding confirms that
mAb JF5 may cross-react with certain Penicillium species, an
observation that was described previously (21). We also ob-
served positive LFD results in three patients who did not fulfill
clinical IPA criteria but showed growth of Aspergillus species
in BAL fluid. In contrast, the BAL cultures of 10 patients
without IPA showed growth of various Aspergillus species,
but negative LFD test results. This indicates that the presence
of Aspergillus species without IPA (e.g., caused by colonization
or contamination) may in some cases cause false-positive LFD
test results.

Strategies to reduce IPA in SOT recipients include
mould active antifungal prophylaxis. Whether antifungal

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Underlying disease</th>
<th>Study center</th>
<th>Patient’s age (y), sex</th>
<th>BAL GM value (95% CI)</th>
<th>BAL LFD result</th>
<th>Fungal growth in BAL culture</th>
<th>IPA according to EORTC 2008 criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>KTx 2013</td>
<td>Graz</td>
<td>50, male</td>
<td>24.92</td>
<td>–</td>
<td>None</td>
<td>Probable</td>
</tr>
<tr>
<td>2</td>
<td>LTx 2012</td>
<td>Graz</td>
<td>59, male</td>
<td>18.74</td>
<td>++</td>
<td>Aspergillus fumigatus</td>
<td>Probable</td>
</tr>
<tr>
<td>3</td>
<td>KTx 2005</td>
<td>Vienna</td>
<td>50, female</td>
<td>5.75</td>
<td>++</td>
<td>Aspergillus fumigatus</td>
<td>Probable</td>
</tr>
<tr>
<td>4</td>
<td>LuTx 2013</td>
<td>Vienna</td>
<td>58, male</td>
<td>7.94</td>
<td>+</td>
<td>Aspergillus niger</td>
<td>Probable</td>
</tr>
<tr>
<td>5</td>
<td>DLuTx 2013</td>
<td>Vienna</td>
<td>51, female</td>
<td>5.78</td>
<td>+</td>
<td>Aspergillus fumigatus, Aspergillus nidulans</td>
<td>Probable</td>
</tr>
<tr>
<td>6</td>
<td>LuTx 2012</td>
<td>Vienna</td>
<td>57, female</td>
<td>10.36</td>
<td>+</td>
<td>Aspergillus fumigatus</td>
<td>Probable</td>
</tr>
<tr>
<td>7</td>
<td>DLuTx 2010</td>
<td>Vienna</td>
<td>19, male</td>
<td>ND</td>
<td>+</td>
<td>Aspergillus fumigatus</td>
<td>Probable</td>
</tr>
<tr>
<td>8</td>
<td>DLuTx 2013</td>
<td>Vienna</td>
<td>55, male</td>
<td>7.86</td>
<td>+</td>
<td>Aspergillus flavus</td>
<td>Probable</td>
</tr>
<tr>
<td>9</td>
<td>LTx 2010</td>
<td>Innsbruck</td>
<td>49, male</td>
<td>ND</td>
<td>++</td>
<td>Aspergillus fumigatus</td>
<td>Proven</td>
</tr>
<tr>
<td>10</td>
<td>LTx 2011</td>
<td>Innsbruck</td>
<td>57, female</td>
<td>positive</td>
<td>++</td>
<td>Aspergillus terreus</td>
<td>Probable</td>
</tr>
<tr>
<td>11</td>
<td>LTx 2011</td>
<td>Innsbruck</td>
<td>52, male</td>
<td>ND</td>
<td>+</td>
<td>Aspergillus fumigatus</td>
<td>Probable</td>
</tr>
</tbody>
</table>

| BAL, bronchoalveolar lavage; DLuTx, double lung transplantation; EORTC, European Organization for Research and Treatment of Cancer Invasive Fungal Infections Cooperative Group; GM, galactomannan; IPA, invasive pulmonary aspergillosis; KTx, kidney transplantation; LFD, Lateral-Flow Device; LTx, liver transplantation; LuTx, lung transplantation; ND, not done. |  

A previous study has reported that the sensitivity of the LFD test may be reduced in the presence of antifungal therapy when testing serum samples, although this was not

<table>
<thead>
<tr>
<th>Medical center/SOT</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>DOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innsbruck</td>
<td>100 (3/3)</td>
<td>79 (11/14)</td>
<td>50 (3/6)</td>
<td>100 (11/11)</td>
<td>29 (1.2–700)</td>
</tr>
<tr>
<td>Vienna</td>
<td>100 (6/6)</td>
<td>81 (13/16)</td>
<td>67 (6/9)</td>
<td>100 (13/19)</td>
<td>50 (2.2–1121)</td>
</tr>
<tr>
<td>Graz</td>
<td>50 (1/2)</td>
<td>100 (6/6)</td>
<td>100 (1/1)</td>
<td>86 (6/7)</td>
<td>13 (0.34–505)</td>
</tr>
<tr>
<td>Overall (all three centers)</td>
<td>91 (10/11)</td>
<td>83 (30/36)</td>
<td>63 (10/16)</td>
<td>97 (30/31)</td>
<td>50 (5.4–467)</td>
</tr>
<tr>
<td>LuTx</td>
<td>100 (5/5)</td>
<td>86 (18/21)</td>
<td>63 (5/8)</td>
<td>100 (18/18)</td>
<td>58 (2.6–1307)</td>
</tr>
</tbody>
</table>

Values are presented as percentages with absolute numbers and 95% CIs.
CI, confidence interval; DOR, diagnostic odds ratio; LuTx, lung transplantation; NPV, negative predictive value; PPV, positive predictive value; SOT, solid organ transplantation; LFD, Lateral-Flow-Device; IPA, invasive pulmonary aspergillosis.
As prophylaxis is indicated after SOT (e.g., lung transplantation) is, however, still a matter of debate (30–32). Results of some studies indicating that voriconazole prophylaxis may not reduce the incidence of IPA are limited by the fact that therapeutic drug monitoring (TDM) of plasma levels was not performed (33). Usefulness of voriconazole and posaconazole TDM has been proven by several studies as breakthrough IPA under mold-active prophylaxis is associated mostly with low azole trough levels (34–36). In the absence of prophylaxis or TDM, timely and accurate diagnosis of IPA remains the most important factor for improving survival. The LFD test may facilitate this process, allowing facilities that do not have access to other diagnostic tests, such as GM, to conduct IPA detection on site.

Our study has several limitations including the relatively small sample size with only 10 cases of probable IPA and a single case of proven IPA. Further possible assay variability across study sites cannot be ruled out because the test is read by the naked eye. In a recent study, Wiederhold and colleagues (28) demonstrated, however, that the LFD assay is reproducible between different laboratories and studies. Lastly, although the revised EORTC-MSG criteria were expanded to include SOT, in clinical practice, it is often difficult to apply the radiographic and mycologic criteria to lung transplant patients.

In conclusion, the LFD test of BAL specimens is performed easily and provides accurate and rapidly available results in patients after SOT. Therefore, this new point-of-care test may constitute a promising diagnostic approach for detecting IPA in BAL specimens from SOT patients.

MATERIALS AND METHODS

This semiprospective cohort study comprises BAL samples from SOT patients with clinical suspicion of IPA that were tested routinely for the presence of Aspergillus species between January 2010 and September 2013. Patients at the Medical University Hospital of Graz, Austria (n=8), and the Medical University Hospital of Vienna, Austria (n=22), were included prospectively between February 2013 and December 2013. Patients at the Medical University Hospital Innsbruck were included, in part, prospectively (n=6, January 2013 to May 2013). In Innsbruck, another 11 samples were tested from patients who had been included in the Innsbruck fungal infection biobank sample collection between 2010 and 2012. All samples had been tested directly after BAL collection or had been frozen at −70°C before the LFD test was performed. The LFD test was performed at the Microbiology Laboratory, Department of Internal Medicine, Medical University of Graz; the Division of Hygiene and Microbiology, Innsbruck Medical University; and the Division of Clinical Microbiology, Medical University of Vienna, Austria, depending on where the patient was included in the study.

The LFD test is based on the detection of an Aspergillus diagnostic 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 (22). Monoclonal antibody JF5 has been incorporated into an immunochromatographic assay (a “point-of-care” diagnostic tool), which is easy to use. Time to results of the test using BAL samples is 15 min. The JF5 LFD results in qualitative data based on the test-line intensity ranging from strong positive (+++) to weak positive (+) or negative (−). The test is read by the naked eye, and test interpretation depends on subjective evaluation. Regardless of the test-line intensity, all positive test results in BAL samples indicate the germination of spores and the development of potentially pathogenic hyphae in the lungs (22).

Testing was performed according to Dr. Thornton’s instructions. For BAL testing, 100 μL of neat BAL sample was applied to the LFD, with no pretreatment (22). Results were read after 15 min and interpreted in line with previous publications (22).

Test results were compared with routinely performed BAL GM test, direct microscopic, and culture results. A BAL GM cutoff of 1.0 optical density index was used (37, 38). Clinical data were collected. Invasive pulmonary aspergillosis was graded in accordance with the EORTC-MSG revised criteria (39, 40).

The 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) as well as the ethics committees of the Medical University of Vienna (EC number 1656/2013) and the Innsbruck Medical University (EC number UN 4926). The performance evaluation of a medical product was also reported to the Austrian Agency for Health and Food Safety (Protocol number INS-621000-0478) and registered at ClinicalTrials.Gov (Identifier NCT02058316).

Statistical analysis was performed using SPSS, version 20 (SPSS Inc., Chicago, IL). Negative predictive value, positive predictive value, sensitivity, and specificity were calculated when applicable, as well as diagnostic odds ratio, including 95% CI. A P value less than 0.05 was considered statistically significant.

REFERENCES


