Utility of Aspergillus Antigen Detection in Specimens Other than Serum Specimens

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The detection of circulating galactomannan in serum is an important tool for the early diagnosis of invasive aspergillosis. A commercial enzyme-linked immunosorbent assay (Platelia Aspergillus; BioRad) was shown to be both highly sensitive and specific for detection of galactomannan in serum samples. Despite the fact that this assay is validated for serum samples, specimens of other body fluids are increasingly used for detection of galactomannan, including urine, bronchoalveolar lavage fluid, and cerebrospinal fluid. Review of the literature shows that galactomannan can be detected in each of these samples from patients with invasive aspergillosis with higher sensitivity than is the case with culture, as well as early in the course of infection. However, the evidence thus far is based on case reports—predominantly retrospective studies—that often include heterogeneous patient populations and limited numbers of cases of proven infection. Clearly, well-designed prospective studies with systematic sampling and use of consensus case definitions are needed to compare the performance of antigen detection in samples other than serum specimens with that in serum specimens.

Invasive aspergillosis is the most common opportunistic invasive mycosis [1]. This disease is mainly caused by Aspergillus fumigatus [2]. Invasive aspergillosis predominantly affects patients with neutropenia or whose neutrophils are functionally compromised. The incidence of invasive aspergillosis has increased because of the increased number of patients undergoing hematopoietic stem cell transplantation or receiving courses of corticosteroid therapy for a prolonged time [3]. In addition, invasive aspergillosis may affect solid organ transplant recipients [4]. Despite the development and registration of new antifungal drugs, such as caspofungin and voriconazole, the overall survival rate remains low (30%–50%) [5, 6]. This is partly because of the difficulty in establishing the diagnosis in an early stage of the infection. Early diagnosis and subsequent early initiation of therapy improves outcome [5, 7, 8]. However, clinical signs and symptoms are generally nonspecific, and characteristic lesions are frequently absent from chest radiographs of neutropenic patients. The use of high-resolution CT with high-risk patients could result in earlier diagnosis, but characteristic lesions, such as the halo sign and the crescent sign, are not specific for Aspergillus species. The reference standard for diagnosis, histopathologic examination and subsequent culture of the tissue samples, is often not done because the patient’s status prohibits invasive procedures (e.g., the patient has thrombocytopenia or poor clinical condition). Furthermore, cultures for fungi and cytopathological examination of respiratory specimens often yield negative results and lack sensitivity for detecting the fungus in an early stage of the infection [9]. Because of the limitations of the aforementioned diagnostic methods, a nonculture method, based on the detection of the Aspergillus antigen galactomannan, has been developed.

Galactomannan is a cell wall polysaccharide that is released by Aspergillus species during growth. A commercially available sandwich ELISA (Platelia Aspergillus; BioRad) detects galactomannan by use of a rat mono-
clonal antibody (EB-A2), which is used as both detector and captor. This antibody reacts with the β(1→5)-linked galactofuranosyl residues that constitute the side chains of galactomannan [10]. The test has been validated for serum specimens only and has a detection limit of ~1 ng/mL. Reported sensitivity and specificity range from 50% to 92.6% and from 94% to 99.6%, respectively, in patients with hematologic malignancy [11–15]. Reported positive and negative predictive values for patients with proven invasive aspergillosis range from 85% to 93% and 95% to 98.7%, respectively [11–15]. False-positive results have been reported in adults and range from 5.7% to 14% when serum samples are used, but this may be overestimated because of diagnostic uncertainty [11, 12, 16, 17]. Rates of false-positive results are higher among pediatric patients and neonates and may be as high as 83% [13, 17, 18]. Reasons for false reactivity remain largely unknown, although recently, piperacillin-tazobactam was shown to cause cross-reactivity in adults [19, 20], and cross-reacting epitopes from Bifidobacterium species were proposed as a cause in neonates [21]. Issues regarding causes of false reactivity were recently reviewed elsewhere [22].

Circulating galactomannan may be detected at a median of 5–8 days (range, 1–27 days) before clinical signs and symptoms of invasive aspergillosis become evident [11–13, 23]. Furthermore, the concentration of circulating galactomannan corresponds with the fungal tissue burden [17, 24] and may therefore be used to monitor the patient’s response to antifungal treatment [12, 23]. Other methods to detect galactomannan include EIAs [16, 25–27], RIAs [28, 29], and latex agglutination tests [30, 31]. Only the latex agglutination test (Pastorex Aspergillus; BioRad) is commercially available and has a higher detection limit than the Platelia ELISA (15 ng/mL vs. 1 ng/mL).

Because galactomannan is a water-soluble carbohydrate, it can be detected in samples of other fluids obtained from patients with invasive aspergillosis, including urine, CSF, pleural fluid, and bronchoalveolar lavage (BAL) fluid [30, 32–34]. Although the Platelia ELISA is not validated for detection of galactomannan in these fluids, there is an increased tendency to use samples of these fluids, in addition to serum, for diagnosis of invasive aspergillosis. In addition, galactomannan can be detected in tissue specimens [35, 36]. The detection of galactomannan in specimens other than serum specimens may provide additional evidence for invasive aspergillosis via a non-invasive method and may help to exclude false-positive or false-negative test results obtained using serum samples.

The literature was systematically reviewed to describe the utility of galactomannan antigen detection in specimens other than serum for the diagnosis of invasive aspergillosis. PubMed (National Center for Biotechnology Information, National Institutes of Health) was used to search for English-language articles published since 1966 with use of the terms “galactomannan detection,” “antigen,” “Aspergillus,” “invasive aspergillosis,” “diagnosis,” “ELISA,” “latex agglutination,” “culture,” “tissue,” “urine,” “bronchoalveolar lavage fluid,” and “cerebrospinal fluid.”

GALACTOMANNAN DETECTION IN URINE SPECIMENS

Because urine specimen collection does not require invasive procedures, it appears to be a logical specimen to use for antigen detection. Larger volumes could be examined and examinations could be more frequent, thereby improving the sensitivity of the ELISA. Galactomannan can be detected in the urine specimens of patients with invasive pulmonary aspergillosis, indicating that at least a fraction of the circulating galactomannan is cleared renally [16, 26, 30, 37, 38]. When galactomannan is concentrated in the urine, testing of urine samples would yield positive results for patients with low levels of circulating galactomannan and therefore would be an early indicator of infection.

However, little is known about the pharmacokinetics of galactomannan and clearance by the kidney. When urine blots for neutropenic patients were probed with the anti-galactomannan EB-A1 antibody, diffuse staining was seen at molecular masses of ≥45 kDa, and a weak band was seen at 21 kDa [38]. In addition, the molecular mass of galactomannan in the urine of rabbits with invasive aspergillosis was also 18–21 kDa [37, 39]. The galactomannan-containing antigen in serum, as described by Lehmann and Reiss [40], had a mass of >125 kDa. It was suggested that an increased size resulted from complexing of the polysaccharide to protein in vivo [41]. Within 24 h after intravenous injection, 35% of galactomannan was excreted into the urine of immunocompetent rabbits [39]. The detection thresholds in serum and urine samples have been reported to be about the same: 1 ng/mL [16, 42, 43]. Several authors have suggested that urine is a better specimen for galactomannan detection than is serum [26, 30, 37]. With use of EIA and RIA, galactomannan could be found in urine from 7 of 13 patients with invasive aspergillosis but in only 2 serum samples from 12 patients with invasive aspergillosis [37]. Ansorg et al. [30] used the latex agglutination test, and the sensitivity was increased by concentrating the urine and lengthening the reaction time to up to 10 min. This yielded a sensitivity, specificity, positive predictive value, and negative predictive value of antigenuria for autopsy-proven aspergillosis and clinically suspected Aspergillus infection of 57%, 53%, 31%, and 77%, respectively [30]. With use of the same monoclonal antibody as used in the Platelia ELISA, urine samples were far more sensitive than serum samples for galactomannan detection in one prospective study [26]. In contrast, the superiority of serum in comparison to urine has been described in other reports [16, 42, 44]. Salonen et al. [42] compared the sensitivity of the ELISA...
for the detection of galactomannan in serum, urine, and BAL samples collected from 105 patients with hematologic disorders who received empirical amphotericin B to treat suspected fungal infection. Antigen was detected in urine from only 2 of 5 patients with proven aspergillosis. Concentration of the urine 10-fold yielded only 1 additional urine sample with a galactomannan-positive result. Styven et al. [16] reported a sensitivity of only 71% when urine samples were used, whereas serum samples had a sensitivity of 100%. However, the value of antigen detection in urine by one of the commercially available tests remains unclear because of the limited number of patients with proven invasive aspergillosis and the predominantly retrospective study design of most published studies. Differences in test results could also be explained by the different pretreatment procedures used for the urine samples (table 1).

An important drawback of testing urine specimens is the occurrence of false-positive results, which varies between 8% and 47% [16, 30, 44]. The Platelia ELISA resulted in fewer false-positive results (8%) than did the latex agglutination test (42%) [16, 44]. Several explanations for false-positive results have been suggested. Airborne fungi contaminating the urine may cross-react with the EB-A2 monoclonal antibody [30]. Urinary antigen may reflect growth of a contaminating Aspergillus strain in urine or result from Aspergillus infection of the kidney [37]. Finally, the use of immunosuppressive agents, especially cyclophosphamide, has been associated with false reactivity in urine specimens from rats [46]. Specificity may be improved by the examination of at least 2 consecutive urine samples, by use of a higher cutoff, and by applying a longer reaction time instead of concentrating the urine [27, 30, 47].

False-negative urine reactivity has also been reported and may be associated with false-negative serum reactivity [37, 39, 48]. Alternatively, the presence of antibodies against Aspergillus that bind to circulating antigen may cause smaller amounts of galactomannan to be cleared by the kidney, resulting in lower sensitivity [39].

Little is known regarding the correlation between galactomannan detection in urine and disease progression. Anigenemia preceded antigenuria in all patients (n = 7) with proven or suspected invasive aspergillosis, as determined by the Platelia ELISA [16]. In that study, galactomannan was detected in urine samples obtained from 4 patients shortly before death. Urine samples from 2 other patients yielded positive results on the day that these patients died. In contrast, when the less sensitive latex agglutination test was used, antigenuria preceded antigenemia in 9 of 12 patients either with proven invasive aspergillosis or who were serologically suspected of exposure to Aspergillus species [30]. In an animal model, increasing urine galactomannan concentrations corresponded with disease progression [37]. Urinary galactomannan levels roughly paralleled the extent of disease in one prospective study that used the latex agglutination test [37]. In that study, only 1 of 5 patients who were apparently cured of their aspergillosis had galactomannan detected in urine samples, as opposed to 6 of 8 patients who died [37]. This might suggest a correlation between the presence of antigenuria and fungal burden.

**GALACTOMANNAN DETECTION IN BAL FLUID SPECIMENS**

The lungs are affected in most cases of invasive aspergillosis, reflecting the primary site of entry of Aspergillus conidia. Because galactomannan is predominantly released by Aspergillus hyphae during growth and to a much lesser extent by conidia, detection of galactomannan in BAL fluid provides better evidence for Aspergillus infection than do culture [49, 50] or PCR that do not discriminate between contaminating conidia and hyphae [51–53]. However, BAL samples obtained from patients colonized with Aspergillus, such as patients with late-stage AIDS and lung transplant recipients, show ELISA reactivity in the absence of invasive disease. Several cutoff values have been described for galactomannan detection by the Platelia ELISA in BAL fluid, ranging from 0.17 to 1 ng/mL [42, 43].

Galactomannan could be detected by the latex agglutination test in 43% of 30 BAL samples obtained from 42 patients with pulmonary aspergillosis, of whom 38 had invasive pulmonary aspergillosis [54]. By use of the Platelia ELISA, galactomannan was detected in BAL fluid from 2 of 3 [42] and from 7 of 10 [55] patients with proven invasive aspergillosis. In the former study, cultures for fungi yielded negative results for all BAL samples that tested positive for galactomannan, although microscopic examination showed hyphae in 2 different samples from a single patient [42].

Some investigators have differentiated between BAL and bronchial lavage [8, 54, 56]. Evidence of Aspergillus in bronchial lavage fluid could be demonstrated more frequently by antigen detection than by culture. It was hypothesized that in the bronchi there may be more Aspergillus hyphae, with subsequently more galactomannan release, than in the alveoli [49, 54]. In contrast, for BAL fluid, culture more often yielded positive results than did antigen detection. Possible reasons for this finding are that very small conidia are predominantly found in the alveoli or that the released galactomannan is diluted by the lavage to a level below the lower detection threshold of the ELISA [57].

In contrast to culture of BAL fluid, antigen detection in BAL specimens, when combined with high-resolution CT, could lead to earlier diagnosis and, subsequently, earlier treatment, resulting in better survival rates [58, 59]. A prospective study involving patients with hematologic disorders who had invasive pulmonary aspergillosis reported positive predictive and negative predictive values of 100% when galactomannan detection
Table 1. Galactomannan detection in urine samples obtained from patients with invasive aspergillosis.

<table>
<thead>
<tr>
<th>Study, classification of disease</th>
<th>No. of patients</th>
<th>Study design</th>
<th>Underlying condition(s)</th>
<th>Pretreatment of the urine sample</th>
<th>Galactomannan detection method</th>
<th>No. of patients positive for galactomannan</th>
<th>No. of patients who were cured/ no. who died</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dupont et al., 1987 [37]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proven</td>
<td>13</td>
<td>NR</td>
<td>Hematologic malignancies, other hematologic diseases, breast cancer, inflammatory diseases, BMT</td>
<td>Dialysis</td>
<td>RIA/ELISA</td>
<td>6/7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5/8</td>
</tr>
<tr>
<td>Suspected</td>
<td>8</td>
<td>NR</td>
<td>Hematologic malignancy, aplastic anemia, CGD</td>
<td>Dialysis</td>
<td>RIA/ELISA</td>
<td>0/0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>NR</td>
</tr>
<tr>
<td>Haynes et al., 1990 [38]: proven or probable</td>
<td>3</td>
<td>Prospective</td>
<td>Hematologic malignancy, BMT</td>
<td>Filtration</td>
<td>Immunoblot</td>
<td>2</td>
<td>NR</td>
</tr>
<tr>
<td>Rogers et al., 1990 [26]: proven</td>
<td>8</td>
<td>Prospective</td>
<td>Hematologic malignancy, inborn errors of metabolism, solid tumor, aplastic anemia, congenital immunodeficiency</td>
<td>Dialysis</td>
<td>Inhibition ELISA</td>
<td>44%</td>
<td>NR</td>
</tr>
<tr>
<td>Ansorg et al., 1994 [30]</td>
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<tr>
<td>Proven</td>
<td>4</td>
<td>Retrospective</td>
<td>Hematologic malignancy, BMT</td>
<td>Centrifugation</td>
<td>Pastorex</td>
<td>3</td>
<td>4/4</td>
</tr>
<tr>
<td>Probable</td>
<td>3</td>
<td>Retrospective</td>
<td>Hematologic malignancy, BMT</td>
<td>Centrifugation</td>
<td>Pastorex</td>
<td>2</td>
<td>1/2</td>
</tr>
<tr>
<td>Suspected</td>
<td>9</td>
<td>Retrospective</td>
<td>Hematologic malignancy, BMT</td>
<td>Centrifugation</td>
<td>Pastorex</td>
<td>9</td>
<td>7/2</td>
</tr>
<tr>
<td>Stynever et al., 1995 [16]</td>
<td></td>
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</tr>
<tr>
<td>Proven</td>
<td>3</td>
<td>Retrospective</td>
<td>Hematologic malignancy</td>
<td>Centrifugation</td>
<td>Platelia</td>
<td>2</td>
<td>0/3</td>
</tr>
<tr>
<td>Probable</td>
<td>2</td>
<td>Retrospective</td>
<td>Hematologic malignancy, aplastic anemia</td>
<td>Centrifugation</td>
<td>Platelia</td>
<td>2</td>
<td>0/2</td>
</tr>
<tr>
<td>Possible</td>
<td>2</td>
<td>Retrospective</td>
<td>Hematologic malignancy, aplastic anemia</td>
<td>Centrifugation</td>
<td>Platelia</td>
<td>1</td>
<td>0/2</td>
</tr>
<tr>
<td>Salonen et al., 2000 [42]</td>
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</tr>
<tr>
<td>Proven&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5</td>
<td>Prospective</td>
<td>Hematologic malignancy</td>
<td>Centrifugation</td>
<td>Platelia</td>
<td>2/2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0/5</td>
</tr>
<tr>
<td>Probable&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1</td>
<td>Prospective</td>
<td>Hematologic malignancy, BMT</td>
<td>Centrifugation</td>
<td>Platelia</td>
<td>0/0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0/1</td>
</tr>
<tr>
<td>Possible&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15</td>
<td>Prospective</td>
<td>Hematologic malignancy, SCT</td>
<td>Centrifugation</td>
<td>Platelia</td>
<td>5/10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9/6</td>
</tr>
</tbody>
</table>

**NOTE.** BMT, bone marrow transplantation; CGD, chronic granulomatous disease; NR, not reported; Pastorex, latex agglutination test; Platelia, sandwich ELISA; SCT, stem cell transplantation

<sup>a</sup> RIA result/ELISA result.

<sup>b</sup> Unconcentrated urine/concentrated urine sample.

<sup>c</sup> Classification according to the European Organization for Research and Treatment of Cancer Mycoses Study Group consensus definitions [45].
The cutoff for CSF samples is probably lower than that for ELISA, RIA [66], and Western blotting, respectively [63, 67].

*A. fumigatus*, from patients with CNS aspergillosis caused by and findings in CSF and CT results are often not specific [63–65].

Culture of CSF seldom yields positive results [4, 62], and both chemistry and biopsies do not always result in a clear diagnosis [61]. Diagnosis of CNS aspergillosis is very difficult, and even brain biopsies do not always result in a clear diagnosis [61].

**Galactomannan detection in CSF samples obtained from patients with CNS aspergillosis.**

<table>
<thead>
<tr>
<th>Study</th>
<th>Classification according to</th>
<th>No. of patients</th>
<th>Study design</th>
<th>Underlying condition</th>
<th>Galactomannan detection method</th>
<th>No. of patients positive for galactomannan</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kami et al., 1999 [65]</td>
<td>Proven</td>
<td>5</td>
<td>NR</td>
<td>Hematologic malignancy</td>
<td>Pastorex and Platelia</td>
<td>4</td>
<td>NR</td>
</tr>
<tr>
<td>Verweij et al., 1999 [63]</td>
<td>Probable</td>
<td>1</td>
<td>Case report</td>
<td>Bilateral mastoidectomy</td>
<td>Platelia</td>
<td>1</td>
<td>Cured</td>
</tr>
<tr>
<td>Machetti et al., 2000 [68]</td>
<td>Probable</td>
<td>1</td>
<td>Case report</td>
<td>BMT</td>
<td>Platelia</td>
<td>1</td>
<td>Died</td>
</tr>
<tr>
<td>Nenoff et al., 2001 [69]</td>
<td>Proven</td>
<td>1</td>
<td>Case report</td>
<td>Diabetes mellitus type II</td>
<td>Pastorex</td>
<td>1</td>
<td>Died</td>
</tr>
<tr>
<td>Viscoli et al., 2002 [32]</td>
<td>Probable</td>
<td>5</td>
<td>Prospective</td>
<td>BMT</td>
<td>Platelia</td>
<td>5</td>
<td>Died (all)</td>
</tr>
<tr>
<td>Moling et al., 2002 [70]</td>
<td>Probable</td>
<td>1</td>
<td>Case report</td>
<td>Chronic alcohol abuse</td>
<td>Pastorex and Platelia</td>
<td>1</td>
<td>Cured</td>
</tr>
</tbody>
</table>

**NOTE.** BMT, bone marrow transplantation; EORTC-MSG, European Organization for Research and Treatment of Cancer Mycoses Study Group; NR, not reported; Pastorex, latex agglutination test; Platelia, sandwich ELISA.

Galactomannan was detected in CSF by the Platelia ELISA for the detection of galactomannan in CSF was higher with the Platelia ELISA (85%–100%) [59] than with the latex agglutination test (73%–83%) [58, 60], whereas the sensitivity of antigen detection in serum was much lower for both tests (47% and 41%–45% for the Platelia ELISA and the latex agglutination test, respectively). Combination of PCR and antigen detection could result in higher sensitivity and specificity, because each method refers to different fungal targets.

**Galactomannan detection in CSF specimens.**

The CNS is the most common secondary site of invasive aspergillosis and is affected in 10%–20% of all cases of invasive aspergillosis [4, 32]. The prognosis for invasive aspergillosis of the CNS is extremely poor in immunocompromised patients. Diagnosis of CNS aspergillosis is very difficult, and even brain biopsies do not always result in a clear diagnosis [61]. Culture of CSF seldom yields positive results [4, 62], and both chemistry findings in CSF and CT results are often not specific [63–65].

Galactomannan was detected in CSF specimens obtained from patients with CNS aspergillosis caused by *A. fumigatus*, *Aspergillus terreus*, and *Aspergillus flavus* by use of the Platelia ELISA, RIA [66], and Western blotting, respectively [63, 67]. The cutoff for CSF samples is probably lower than that for serum samples [63], which may be explained by a lower background reactivity of CSF. The CSF galactomannan indices of patients with probable CNS aspergillosis were statistically significant higher than were those of control patients with other neurological diseases [32]. The median CSF galactomannan index was 10.52 in patients, compared with 0.287 in control subjects [32]. A summary of reports that describe the use of the Platelia ELISA for the detection of galactomannan in CSF is shown in table 2. The sensitivity and specificity were 80% and 100%, respectively, in a study describing 5 patients with proven CNS aspergillosis [65]. Culture of CSF samples yielded negative results, but PCR yielded positive results for all patients. False-negative ELISA reactivity of CSF was reported in a patient with documented *Aspergillus* meningitis, although *A. fumigatus* was cultured from the CSF and PCR yielded positive results [70]. In contrast, others report galactomannan detection to be more successful than PCR [63].

There is evidence that galactomannan detection in CSF by the Platelia ELISA may result in earlier diagnosis of CNS aspergillosis [63, 70]. In 1 patient, galactomannan could be detected in the CSF sample 45 days before culture results were positive [63]. In another patient with *Aspergillus* meningitis, galactomannan was detected in the CSF sample 9 months before culture of CSF yielded positive results [70]. In addition to early diagnosis of the infection, the course of the antigen titer has been described to correspond with the clinical response to treatment [63, 68].

**Galactomannan detection in tissue specimens.**

Although the demonstration of *Aspergillus* in tissue is the reference standard for diagnosis of invasive aspergillosis, a definitive diagnosis is possible only after identification of the fungus cultured from that tissue, because the microscopic morphology of many other hyalohyphomycetes, such as *Scedosporium* species and *Fusarium* species, is often indistinguishable from that
of *Aspergillus* species. However, up to 70% of tissues showing septate hyphae yielded negative results by culture, thereby preventing a definitive diagnosis [71]. Other techniques, such as PCR, in situ hybridization, and immunohistochemical techniques, may prove to be useful tools when culture results are negative and rapid identification to species level is necessary [35]. Immunohistochemical tissue analyses have been described until now with 2 commercially available monoclonal antibodies against galactomannan (EB-A1 and Mab-WF-AF-1), as well as with the latex agglutination test and Platelia ELISA [35, 72–74]. A protocol concerning the processing of solid-tissue specimens for the Platelia test has been described on the Internet [75]. The monoclonal antibody EB-A1 showed good performance in the detection of *Aspergillus* hyphae in routinely processed tissue sections, providing generic identification [72]. This method was not superior to histological examination with conventional stains for demonstrating the presence of an infection [36]. Another report described the use of Mab-WF-AF-1 (DAKO) on paraffin-embedded sections obtained from pediatric patients with invasive aspergillosis [35]. The antibody was highly immunoreactive with cell wall, septa, and cytoplasm of *Aspergillus* species. This antibody is specific for *A. fumigatus*, *A. flavus*, and *Aspergillus niger*. In necrotic tissue specimens, immunohistochemical techniques may perform better than in situ hybridization. Degradation of nucleic acid could affect the performance of in situ hybridization, whereas this is less likely to affect immunohistochemical analysis because *Aspergillus* remnants in the cytoplasm of some phagocytic cells can be detected by this method [72, 76]. Finally, a case report described the use of the latex agglutination test in detecting galactomannan in a brain biopsy specimen [74]. This tissue was first grounded in sterile 0.9% NaCl and centrifuged, and finally the supernatant was used for galactomannan detection. The latex agglutination test yielded positive results, but a fungal infection could not be confirmed with microscopic evaluation and culture of the tissue. A cotton swab used to obtain the brain specimen was found to have caused the reactivity. Because wooden toothpicks also have been shown to cause reactivity [77], cellulose was hypothesized to be responsible for the false-reactivity [74].

**GALACTOMANNAN DETECTION IN OTHER SPECIMENS**

By use of an inhibition ELISA, galactomannan antigen levels exceeding 50 ng/mL were documented in cyst fluids from 2 patients with polycystic kidney disease [78]. Although culture of samples of these fluids was not done, culture of kidney epithelium yielded *Paecilomyces* and *Penicillium* species. It is possible that these fungi may have cross-reacted with the inhibition ELISA. Galactomannan could also be detected in a subphrenic abscess of a 4-year-old boy with chronic granulomatous disease who had invasive aspergillosis [48]. High levels of galactomannan (70 and 48 ng/mL) were detected in 2 fine-needle aspirations of the subphrenic abscess, and culture of both specimens yielded *A. fumigatus*. Neither circulating *Aspergillus* DNA nor galactomannan were detected, suggesting that encapsulation of the abscess prevented leakage of surrogate markers to the serum. Finally, galactomannan was detected in pus specimens from patients with fungal rhinosinusitis [79].

**CONCLUSIONS**

Review of the literature indicates that, at best, antigen detection in urine, BAL fluid, and CSF specimens is a promising diagnostic tool in addition to serum monitoring. The evidence thus far is based on case reports, predominantly retrospective studies, often heterogeneous patient populations, various assays, and limited numbers of cases of proven infection. Clearly, well-designed prospective studies with systematic sampling that make use of consensus case definitions are needed to compare the performance of antigen detection in samples other than serum samples with that in serum samples. These will also provide information concerning the earliest positive sample and temporal relationships with serum. In addition, more insight into the kinetics of galactomannan is needed to understand the release and clearance of galactomannan during infection in different patient groups. When these conditions are met, optimal, cost-effective strategies can be designed that incorporate analysis of various samples and high-resolution CT.

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