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REVIEW

Update on the contribution of galactomannan for the diagnosis of invasive aspergillosis

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Abstract The diagnosis of invasive fungal infections (IFI) remains a challenge, particularly for diseases caused by filamentous fungi such as *Aspergillus* species. Unfortunately, many patients affected by these conditions are not identified before autopsy. Therefore, there is a need for new diagnostic methods for IFI. Galactomannan is a soluble antigen released during hyphal growth in tissues. A commercially available sandwich ELISA assay that detects galactomannan has been used in Europe for many years and is now approved for use in the USA. The test has an excellent negative predictive value in the detection of invasive aspergillosis (IA) in high-risk patients. In addition, it is more sensitive than culture and allows IA to be diagnosed before clinical manifestations occur. However, false-negative and false-positive results in certain populations are the main limitations to its use. The purpose of this review is to summarize the current knowledge about galactomannan testing in patients at risk for IA.

Keywords Aspergillosis · Bone marrow transplantation · Galactomannan · Invasive fungal infections · Solid organ transplantation

Introduction

Invasive fungal infections (IFI) represent a major threat to immunocompromised patients, particularly hematopoietic stem-cell transplantation (HSCT) recipients. The diagnosis of invasive aspergillosis (IA) is particularly challenging, especially because of infrequent recovery of *Aspergillus* species in culture [1]. Therefore, there has been an intense search for better diagnostic methods for IA. If not the most promising, galactomannan is certainly the most studied among these new diagnostic tests. However, galactomannan testing performance varies widely according to the population of patients studied [2]. This review aims to summarize the current knowledge regarding the use of galactomannan as a diagnostic tool for IA.

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Physiology of galactomannan production

Galactomannan is a heat-stable heteropolysaccharide released from the cell wall during hyphae growing in tissues [3–5]. The molecule has a non-immunogenic mannan core with immunoreactive side chains containing galactofuranosyl units [6, 7]. In vitro studies have revealed that the quantity of galactomannan released can vary according to the *Aspergillus* species involved [8–10]. Lower levels are usually observed with *A. fumigatus*, while higher galactomannan concentrations are seen with *A. terreus*, *A. niger*, and *A. nidulans*. The clinical significance of this finding, however, remains to be determined. The amount of galactomannan released by *A. fumigatus* and *A. flavus* appears to be similar for strains from galactomannan testing-positive and -negative patients [8, 11]. This finding underscores the multi factorial pathogenesis for detection of biological markers such as galactomannan in IA. In addition to the *Aspergilli*, other fungi can also generate galactomannan during growth (Table 1) especially *Penicillium* species. Therefore, a positive galactomannan test must be interpreted with caution in areas where infections caused by *Penicillium marneffei* are prevalent. The magnitude of galactomannan release by other fungi is considerably lower in comparison to that seen with *Aspergillus* and *Penicillium* [10].

Table 1 Non-*Aspergillus* fungi causing false-positive galactomannan ELISA reactions

Fungus	References
Acremonium species	[9]
Alternaria alternata	[9]
Botrytis tulipae	[25]
Cladosporium cladosporioides	[25]
Cladosporium herbarum	[9]
Cryptococcus neoformans	[86, 87]
Fusarium oxysporum (but not *Fusarium solani*)	[9, 25]
Geotricum capitatum	[86]
Paecilomyces variotii	[10]
Penicillium chrysogenum	[9, 10]
Penicillium digitatum	[10, 25]
Penicillium marneffei	[88]
Rhodotorula rubra	[9, 10]
Trichophyton interdigitalis	[25]
Trichophyton rubrum	[25]
Wallemia sebi	[25]
Wangiella (Exophiala) dermatitidis	[9]

Most of these fungi rarely infect humans

Hope et al. [12] used an in vitro model of the human alveolus to investigate the relationship among galactomannan and the pathogenesis of IA. A close temporal relationship was observed between hyphae penetration of the endothelial cell layer and an increase in galactomannan levels, as soon as 14–16 h after the inoculation of *Aspergillus*. Moreover, by using a neutropenic rabbit model of IA, the antigen was detected earlier in the bronchoalveolar (BAL) fluid than in the endothelial compartment [12]. Considering that galactomannan is predominantly released by *Aspergillus* hyphae during growth and to a much lesser extent by conidia, its detection in the BAL fluid might be a better evidence for IA than culture or polymerase chain reaction (PCR) detection of *Aspergillus* [13, 14]. A positive PCR or culture for *Aspergillus* in the BAL can just imply the presence of conidia in the environment and colonization of the respiratory tract [15–17]. However, false-positive results for galactomannan testing may also occur in patients only colonized with *Aspergillus* when BAL is tested [3], which can be improved when high-resolution computed tomography (CT) scan is also performed [18, 19].

Detection of galactomannan in serum and other body fluids

A commercially available sandwich enzyme immunoassay (ELISA) test (Platelia™ *Aspergillus*, BioRad, France) detects galactomannan by use of EB-A2 rat monoclonal antibody. This test recognizes the $1 \rightarrow 5$-β-D-galactoruranose side chains of the galactomannan molecule [5, 20]. The test has been validated for serum specimens only and has a detection limit of approximately 1 ng/ml, which is 10–15 times lower than the limit of the latex agglutination test used previously for galactomannan detection (Pastorex *Aspergillus*, Sanofi Diagnostics Pasteur) [5, 21–23]. The results can be obtained in 3 h, which is a great advantage in comparison to standard culture methods. Platelia™ galactomannan has been in use in Europe for more than 10 years, and recently it was approved for use in the United States.

Since galactomannan is a water-soluble carbohydrate, it can be detected in several body fluids in addition to serum [3]. Using a cut-off of 1.0–1.5, most studies found the sensitivity of galactomannan testing in BAL to range from 85 to 100% [19, 23, 24]. While the antigen can be detected in the urine [4, 25–28], little is known about its pharmacokinetics and clearance by the kidney [29]. The effect of renal failure or dialysis on the clearance of galactomannan is unclear. Moreover, there is limited data regarding the correlation between urinary galactomannan and disease progression, in addition to the finding of false-positive results for galactomannan testing in the urine [3, 5, 21]. Galactomannan can also be detected in the CSF [30–32], but very little information is available regarding CSF testing.

Galactomannan testing can lead to an early diagnosis of IA

Circulating galactomannan can be detected at a median of 5–8 days before clinical manifestation of aspergillosis [4, 33–37]. Galactomannan detection also precedes the demonstration of abnormalities in high-resolution CT scan and the initiation of antifungal therapy by a median of 7.2 and 12.5 days, respectively [37]. For instance, a patient with *Aspergillus* meningitis had a positive galactomannan CSF test 45 days before a culture became positive [31].

Combining galactomannan with additional tests to diagnose IA

Francesconi et al. [38] recently compared the diagnostic yields of galactomannan detection by quantitative real-time PCR (qPCR) with quantitative cultures in BAL fluid by using a neutropenic rabbit model of IA. The optimal cut-off for galactomannan was 0.75, with an associated sensitivity and specificity of 100% in untreated controls. This was determined by receiver operating characteristic (ROC) curves, a plot of the true positive rate against the false positive rate for the different possible cutpoints of a diagnostic test. The optimal cut-off for qPCR was a crossover of 36 cycles (sensitivity 80%, specificity 100%). Quantitative culture of BAL had a sensitivity of 46% and a specificity of 100%. A reduction in sensitivity occurred for all methods when animals received antifungal drugs. This study reached two interesting conclusions: (i) both galactomannan and qPCR were more sensitive than culture; and (ii) for patients who receive drugs that could potentially decrease galactomannan sensitivity (see false-negative results, below), combining galactomannan and qPCR might be a better strategy then single testing to diagnose IA. However, these findings will have to be confirmed in studies involving humans.

Standard definitions for IFI were published in 2002 by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC/IFIG) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (NIAID/MSG) [39]. These definitions were intended for use in the context of clinical and/or epidemiological research, not for clinical decision-making. Clinically IFI were classified into three categories based on the level of certainty in diagnosis: proven (unequivocal) IFI, probable IFI and possible IFI. This classification depends on three key elements, which are host factors, clinical features, and mycological results. These criteria have just been revised and a draft is available at the *Aspergillus* website (www.aspergillus.org.uk). It seems that the most important change is the inclusion of the Platelia™ galactomannan test as one of the components of the microbiology criteria for IA. Due to lack of standardization, PCR was not included in this updated version of the consensus.

Galactomannan test performance in different populations

Neutropenic patients

Studies evaluating the role of galactomannan assay in the diagnosis of IA have largely been conducted with patients undergoing cancer chemotherapy or HSCT recipients [20, 33, 34, 40–46]. Maertens et al. [33] prospectively evaluated the

use of Platelia™ galactommanan test as a tool for preemptive therapy in adult neutropenic patients with hematological disorders. Treatment episodes ($n = 362$) were classified according to the EORTC/MSG case definitions for IFI and were validated by necropsy in a large number of fatalities. Serum was collected twice weekly until discharge, and a positive result was considered in the presence of two tests with an optical density (OD) index of 1.0 or more. Overall, the test sensitivity was 89.7% and the specificity 98.1%. The positive and negative predictive values were also very high (87.5% and 98.4%, respectively).

Herbrecht et al. [47] investigated 3,294 serum samples from cancer patients and concluded that routine antigen testing is not useful in patients with febrile neutropenia, but no clinical or radiological signs suggestive of a pulmonary infection. Positive predictive value for IA in these patients was only 7.1%, in comparison to 95.2% in patients with suspected pulmonary infection. Importantly, there are so far no data comparing different sensitivities and specificities of galactomannan assay in patients with leukemia with other host groups [48].

Pazos et al. [37] evaluated the usefulness of serum galactomannan and $1 \rightarrow 3$-β-D-glucan detection in the serum for the diagnosis and therapeutic monitoring of IA in neutropenic adult patients at increased risk for IA. Using a cut-off of 1.5, galactomannan sensitivity, specificity, and positive and negative predictive values for IA were 87.5%, 89.6%, 70%, and 96.3%, respectively. Glucan detection assay tended to become positive earlier than the galactomannan test. Although the use of both methods did not improve the sensitivity of each test, the combination of tests was useful in confirming the existence of IA, since both markers were positive in patients with IA. Discrepancies in positivity between tests were helpful in identifying false-positive results by each test.

Hematopoietic stem-cell transplant (HSCT) recipients

The use of serum galactomannan testing as a surveillance tool performed weekly in HSCT recipients has been associated with an overall positive predictive value of only 10% [47]. The specificity of the galactomannan assay was lower in adult allo-HSCT recipients (93.4%) than in adult auto-HSCT recipients (99.4%) ($P < 0.001$). Accordingly, the positive predicted value was lower in adult allo-HSCT recipients (42.9%) than in adult non-allogeneic patients (92.1%; $P < 0.001$). The negative predicted value for adult allo-HSCT recipients and adult auto-HSCT recipients were 94.2% and 82.7%, respectively ($P = 0.001$). This study used a cut-off point of 1.5. Reducing the cut-off to 0.7 increased the overall sensitivity by 24%, while specificity was reduced by only 5.5%.

Solid-organ transplant (SOT) recipients

The performance characteristics of the galactomannan test are less well studied in SOT recipients. Fortun et al. [43] reported that the sensitivity of the test in the serum was 55.6% in liver transplant recipients, with an associated high specificity (93.9%). Another study in liver transplant recipients included only one patient with IA therefore sensitivity was not determined [44]. Husain et al. [45] showed that the serum galactomannan test had a specificity of 93% but a low sensitivity (30%) for the diagnosis of IA in lung transplant recipients. None of the cases of *Aspergillus* tracheobronchitis were detected by galactomannan testing in this study. Therefore, monitoring following SOT is not recommended for patients with non-hematological malignancies or disorders treated with less intense immunosuppression.

Summary of galactomannan testing accuracy

In a recent meta-analysis [49], 139 studies in English and Spanish language were reviewed regarding galactomannan serum assay for surveillance of IA in high-risk patients. A considerable heterogeneity was found among the studies. The median sensitivity for proven cases of IA was 77% (specificity 81%); for proven or probable cases, median sensitivity and specificity were 69% and 93%, respectively. While the sensitivity and specificity of the test for patients with hematological malignancy was 70% and 92%, for recipients of

SOT the sensitivity and specificity were 22% and 84%, respectively. These results confirmed that galactomannan testing is more useful in patients who have hematological malignancy or who have undergone HSCT than in SOT recipients.

Balloy et al. [50] compared mice treated with corticosteroids or chemotherapy to demonstrate that the pathogenesis of invasive pulmonary aspergillosis varies according to the type of immunosuppresion. In the presence of corticosteroid-induced immunosuppresion, a rapid and extensive influx of neutrophils occurred into the affected lungs, resulting in small numbers of conidia and very low levels of galactomannan in the lungs, kidneys and brain. On the other hand, the BAL fluid of mice with chemotherapy-induced cytopenia showed no influx of polymorphonuclear cells, with consequential invasion by large numbers of hyphae and high levels of galactomannan. A significant difference in mortality between these groups was also observed (higher survival for corticosteroid-treated mice; $P < 0.001$). This suggests that differences in patient selection might account for the disparities seen for susceptibility for the galactomannan test for the diagnosis of IA.

False-negative results of galactomannan testing

Galactomannan assay sensitivity for IA has varied markedly among studies, from as low as approximately 30% to as high as 100% [34, 45, 47, 51]. In addition to issues related to the host (as discussed previously), the main reasons for a false-negative result are exposure to antifungal agents and high cut-off values.

Previous exposure to antifungal drugs

The use of antifungal agents may lower antigen levels by decreasing the fungal load [13, 19, 38, 52–54], making the test less useful in patients receiving antifungal prophylaxis. For instance, exposure to mold-active antifungal agents reduced the sensitivity of galactomannan testing to 20% compared with 80% in those not receiving these drugs [53, 55]. However, the sensitivity of the test in BAL fluid was reduced by only 8% in a study where neutropenic rabbits were treated with antifungal drugs [38]. In the same study, triazoles and echinocandins differed markedly in their effects on detection of galactomannan in the BAL fluid. While a significant decline in BAL galactommanan occurred in rabbits treated with ravuconazole, rabbits receiving micafungin demonstrated persistently elevated levels of BAL galactomannan. The authors suggest that this finding is probably related to the effect of the echinocandin in disrupting the cell wall and the inability of the drug to clear histological detectable organism from lung tissue, thus causing the release of the antigen into the surrounding epithelial lining fluid of the alveoli.

Cut-off-related issues of galactomannan testing

There is considerable variability in the literature regarding the cut-off values used for serum galactomannan testing. Although the manufacturer recommended a cut-off of 1.5, most institutions used a lower cut-off varying from 1.0 to 0.5. The approved cut-off in the USA is 0.5. Defining an appropriate cut-off is probably the most important variable influencing the sensitivity of the galactomannan test considering that lower cut-off values result in higher sensitivity and lower specificity of the assay.

Verweij et al. [56] recently have shown results of a study aimed to define the optimal cut-off value for serum galactomannan testing. The study included 203 patients from two European institutions. Using ROC analysis the authors showed that the cut-off of 0.5 had the highest sensitivity (97.4%), but still maintaining a good specificity (90.5%) for diagnosing IA. The positive and negative predictive value of the test for UA was 66.1% and 99.4%, respectively. Therefore, 0.5 is now considered the cut-off accepted in both Europe and the USA for serum galactomannan testing.

Inappropriate diagnostic criteria for IA

Galactomannan test sensitivity depends on the criteria used to diagnose IA. The assay sensitivity is usually lower when more rigorous diagnostic

criteria like the EORTC/MSG standards such as mentioned before are used to diagnose IA [49]. In addition, when these criteria are used to support the diagnosis of IA, sensitivity is generally much higher for proven cases than for probable or possible cases of IA [47].

Inadequate frequency of galactomannan testing

Considering that galactomannanemia is transient, inadequate sampling strategies could possibly compromise the test sensitivity. Although twice-weekly determination of galactomannan levels has been recommended for patients at risk for IA, the optimal sampling strategy for screening is unknown. Racil and colleges [57] measured galactomannan levels four times a day in oncology patients and found variations in OD index ranging from 0.17 to 1.15 (mean 0.6). As suggested by Hope and Denning [58], levels should be determined immediately in patients where the diagnosis of IA is likely to occur.

Severity of aspergillosis

The sensitivity of the galactomannan testing is lower in patients with non- or minimally invasive manifestations of aspergillosis (e.g., aspergilloma or tracheobronchitis) [59]. Therefore, galactomannan detection is not a useful test for patients suffering from chronic cavitary pulmonary aspergillosis (CCPA) or allergic bronchopulmonary aspergillosis (ABPA). The utility of the test in patients with post-operative aspergillosis has not been formally studied [60].

Volume of sampling

The volume of sampling for galactomannan assay was recently investigated using a new method in which 750 µl of serum was compared with the recommended 300 µl, filtered through a 50-kDa Microcon filter [8]. The thus achieved concentration of the sample resulted in galactomannan reactivity in samples that tested negative with the conventional pre-treatment method. This resulted in earlier detection of circulating antigen as well as detection of galactomannan in patients with IA who were falsely negative using the standard pre-treatment method [8]. These preliminary findings might further increase the diagnostic value of galactomannan testing, but additional studies are needed to validate this approach.

Long-term storage

Pereira et al. [46] observed that 20% of serum samples retested after 4 years of storage showed lower reactivity to the ELISA galactomannan test. The influence of storage on the performance of galactomannan testing in patients with IA was also suggested in a recent review [51], although no detailed information was provided.

False-positive results for galactomannan testing

In general, detection of galactomannan can be considered a very specific test to diagnose *Aspergillus* infections. Maertens showed the frequency of a false-positive result in the normal population to be only 2% [61]. The main factors associated with false-positive results are discussed below.

Use of antibiotics

The drugs originated from fungi such as antibiotics might be associated with false-positive test results, including ampicillin-sulbactam, piperacillin-tazobactam, and amoxicillin-clavulanic acid [62–67]. In many cases, a positive galactomannan result can be obtained by testing directly the antibiotic batch. In a study testing 15 antibiotics [68], ampicillin expressed the highest level of galactomannan (OD index of 0.54), followed by piperacillin-tazobactam (index 0.235). Other antibiotics—including ampicillin-sulbactam, ampicillin, penicillin G, ceftriaxone, cefepime, imipenem, clarithromycin, ciprofloxacin, vancomycin, gentamicin, trimethoprim-sulfamethoxazole, and ornidazole—showed galactomannan levels ranging from 0.011 to 0.188. The timing of collection of the sample may influence the test results, with reactivity being less likely to occur in samples collected at trough levels or prior to the

administration of the antibiotic dose [69, 70]. However, it was demonstrated that a false-positive galactomannan test can persist for over 10 days after the administration of a β-lactam antibiotic [67, 71]. The kinetics of antigenemia appeared to vary according to the treatment duration and the level of antigen detected in the antibiotic batch [67].

Pediatric patients and neonates

Very high rates of false-positive results for galactomannan testing have been observed for children [41, 72–74]. In one study, rates as high as 83% occurred [74], which might be related to cross-reactivity with *Bifidobacterium bifidum*, found in large inocula in the guts of breast- and formula-fed infants [75]. In addition, galactomannan is heat-resistant, and therefore not eliminated from food by sterilization. The presence of a damaged gut endothelium might increase the absorption of dietary galactomannan [76]. Passage of dietary galactomannan into the blood from the intestinal tract could also explain false-positive results in allo-HSCT recipients, as these results occur mainly during the first month after transplantation, when chemotherapy-induced mucosal lesions are maximal [47].

Bacteremia

False-positive results for galactomannan testing have also been shown in bacteremic patients [10, 37]. The mechanism behind this finding is not really understood, and some cases might be in fact related to the use of antibiotics. No specific microorganism has been consistently associated with a false-positive galactomannan reaction. Moreover, bacteria recovered from those patients showed no reactivity by the galactomannan sandwich ELISA [10].

Dialysis

El Saleeby et al. [77] reported that a paradoxical rise in serum galactomannan levels occurred in a patient with acute myeloblastic leukemia diagnosed with IA who was responding to antifungal therapy. Considering that the patient was on hemodialysis, the authors suggested that reduced renal clearance was responsible for the rise in galactomannan levels. The dialysate tested negative for galactomannan, demonstrating the inability of hemodialysis to clear larger molecules such as *Aspergillus* galactomannan from the serum. Kwak et al. [44] showed that liver transplant recipients with false-positive galactomannan tests were more likely to have required dialysis than those with true-negative tests ($P = 0.001$).

Auto-antibodies

False-positive galactomannan testing results are more likely to occur in patients undergoing liver transplantation for autoimmune liver disease than in patients with other liver conditions [44]. A false-positive test galactomannan test, most likely due to autoreactive antibodies or paraproteins, has also been reported in a patient with chronic graft-versus-host disease after allogeneic bone marrow transplantation [78].

Other factors

Patients undergoing lung transplantation for cystic fibrosis and chronic obstructive pulmonary disease (COPD) were more likely to have repeatedly false-positive galactomannan testing results [45]. Whether that resulted from surgery leading to *Aspergillus* translocation or represented cases of IA remains speculative. The importance of COPD as a predisposing condition to IA has received growing attention, mainly in patients treated with high-doses of corticosteroids and receiving multiple broad-spectrum antibiotics [79].

In one study, cotton swabs contaminated with viable *Aspergillus* spores resulted in a false-positive galactomannan result when a brain biopsy was tested [80] (Tables 2, 3).

Monitoring antifungal therapy with galactomannan testing

The concentration of circulating galactomannan corresponds with the fungal tissue burden [41, 53,

Table 2 Variables influencing galactomannan ELISA testing

Related to the patient	Age
	Underlying disease
	Previous use of antimicrobial drugs, including antifungals
	Auto-immunity
	Dialysis
Related to the *Aspergillus* infection	*Aspergillus* species
	Disease severity
Related to the method	Sample volume
	Cut-off
	Storage of sampling
	Frequency of testing
	Laboratory experience

Table 3 Factors associated with a false-negative and false-positive results with galactomannan ELISA testing

False-negative	False-positive
Non-neutropenic patient	Use of antibiotics
Previous exposure to antifungal drugs	Pediatrics and neonates
Cut-off too high	Infections caused by *Penicillium* species
Inappropriate diagnostic criteria	Dialysis
Low frequency of testing	Auto-antibodies
Disease of low severity	Contaminated cotton swabs
Low volume of sampling	Bacteremia
Long term storage	Airway colonization with *Aspergillus*

72], and may therefore be used to monitor the response to treatment [4, 20, 33, 35, 81, 82]. *Aspergillus* meningitis has also been effectively monitored with CSF galactomannan testing [30, 31]. The course of the galactomannan titer generally corresponds with the clinical response to antifungal therapy; declining levels have been found in patients responding to treatment while rising galactomannan antigenemia is usually associated with treatment failure. Although, in patients receiving salvage therapy with caspofungin the galactomannan titer corresponded with clinical response [83], a case was recently described in which a paradoxal rise of the galactomannan titer in the serum was observed [84]. Exposure of the *A. fumigatus* strain to caspofungin in vitro caused increased release of galactomannan in the culture supernatant compared with strains exposed to voriconazole [84]. This observation suggests that the course of the antigen titer in patients receiving caspofungin might not be in accordance with the clinical response. Chabot et al. also showed that exposure to caspofungin—and to a lesser extent voriconazole—acutely increased galactomannan release in *A. fumigatus*, which was not observed with amphotericin B [85].

Conclusions

There is a clear need for better diagnostic tools for patients with IA. Although not an ideal test, proper use of galactomannan testing can lead to an early diagnosis and possibly to a better outcome in IA. The excellent negative predictive value of galactomannan testing may facilitate the decision to withhold or not to start antifungal therapy in patients at risk for IA. Therefore, all high-risk patients at risk for IA should be tested for galactomannan. Combining multiple diagnostic tests for IA (i.e., galactomannan, qPCR, $1 \rightarrow 3$-β-D-glucan and/or culture) is a very attractive option. However, further studies are required to validate this approach. Development and improvement of new diagnostic tests for IA such as galactomannan testing could decrease the prohibitive costs of modern antifungal therapy for many institutions, especially in developing countries.

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