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Significance of galactomannan antigen for aspergillosis diagnosis: A review

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Abstract:

Invasive aspergillosis is one the major causes of death in hematopoietic and solid-transplant recipients. One of the most critical problems in medical mycology is the inability to consistently make a convincing and early diagnosis of invasive fungal infection. Conventional diagnosis methods such as culture-based approaches are limited by the insufficient accessibility sensitivity and the non-culture-based approaches based on the detection of specific fungal antigens, fungal metabolites, and fungal DNA are limited by the slow speed of analysis. Early diagnosis of invasive aspergillosis is the main complication in its treatment. Galactomannan is a polysaccharide present in the cell wall of *Aspergillus* species. This carbohydrate is released into the serum during the invasion *of Aspergillus*. Recently, a double-sandwich enzyme-linked immunosorbent assay (ELISA) was developed for detecting galactomannan antigenemia in the serum. This assay is an important advancement in the nonculture diagnosis of invasive aspergillosis. The level of galactomannan in the serum may be an important indication of therapeutic response. In this review, the role of galactomannan in the diagnosis of IA and its significance has been highlighted.

Keywords: Galactomannan, aspergillosis, interpretation, ELISA, diagnosis

Introduction

One of the most important problems in medical mycology is the inability to consistently make a convincing and early diagnosis of invasive fungal infection (Marr *et al*., 2002; Rex, 2006). Conventional diagnosis methods such as culture-based approaches are limited by the insufficient accessibility sensitivity and non-culture-based approaches based on the detection of specific fungal antigens, fungal metabolites, and fungal DNA are limited by the slow speed (Rex, 2006).

Invasive aspergillosis (IA) is an important cause of mortality in immunocompromised patients and hematopoietic cell transplant

recipients (Fisher *et al*., 2013). The high mortality rates can be attributed partially to the delay in establishing a reliable diagnosis, especially in patients receiving cytoreductive or marrow-ablative therapy (Maertens *et al*., 2001). The available diagnostic standards usually require invasive procedures to obtain specimens for histological examination and culturing (Walsh *et al*., 1994).

Galactomannan (GM) is a carbohydrate that is released in the serum by *Aspergillus* during its growth and invasion in the host. This antigen serves as a biomarker of *Aspergillus* disease (Fisher *et al*., 2013). GM detection in the blood circulation and in tissues has emerged as a valuable, non-invasive method for culturing and histopathology-based methods in the diagnosis of invasive aspergillosis. Some recent studies have demonstrated that a decrease in the serum GM indices (GMI) during the early therapy can be correlated with successful clinical outcomes (Boutboul *et al*., 2002; Chai *et al*., 2012; Hadrich *et al*., 2012). However, it remains unclear whether the site of detection (serum vs. airway) or the magnitude of the GMI at the time of diagnosis has prognostic significance (Maertens *et al*., 2001). Recent developments in the detection of GM antigenemia by double-sandwich enzyme-linked immunosorbent assay (ELISA) are extremely vital in nonculture diagnosis of IA (Verweij *et al*., 1997; Verweij *et al*., 1998).

In the current review, the role of GM detection in the interpretation of positive IA has been discussed.

Invasive aspergillosis

The incidence of IA has increased among immunocompromised patients, particularly among hematopoietic stem cell and solid-transplant recipients in the recent years (Marr *et al*., 2002). IA has been reported to be the most important cause of death in allogeneic bone marrow transplant patients (Marr *et al*., 2002; Wald *et al*., 1997). Neutropenia, long-term antibiotic therapy, and corticosteroids are the main risk factors of IA (Marr *et al*., 2002). IA is significantly associated with morbidity and mortality, as approximately 30–70% of IA patients from among transplant recipients die despite the advances in therapeutic methods (Fukuda *et al*., 2003).

Diagnostic Methods of IA

Diagnosis of IA is truly challenging, as clinical and radiological signs of this disease are extremely insensitive and/or nonspecific. Tissue biopsy is not always possible, especially in individuals with thrombocytopenia (Aisner *et al*., 1977). Since early diagnosis leading to prompt decision-making regarding an appropriate therapy may result in improved patient outcomes, much attention is being focused on developing a non-invasive test for the diagnosis of IA (Pfeiffer *et al*., 2006).

Insisting on irrefutable evidences of systemic tissue infection before undergoing any therapeutic procedures exposes patients to invasive, potentially life-threatening tests, which results in an unacceptable delay of potentially life-saving therapy. On the other hand, a certain substantial diagnostic is important to avoid over-treatment or wrong treatment (Maertens *et al*., 2001).

GM Test and Possible Pitfalls

GM is a component of *Aspergillus spp.* cell wall. This polysaccharide is released into the surrounding environment during the growth or tissue invasion of the fungus. GM is composed of a mannan matrix with galactose side groups (Bart-Delabesse *et al*., 2005).

GM s is usually added to food products as a stabilizer and to increase the viscosity of water phase. Several food items like pasta and rice contain GM (Matsudomi *et al*., 2002).

Until date, monitoring of *Aspergillus* GM has been found to be promising only in non-invasive test for diagnosing IA (Bart-Delabesse *et al*., 2005). It has been documented that serum GM can be detected at approximately 7–14 days before other diagnostic clues become apparent, and, therefore, monitoring of GM may allow initiation of pre-emptive antifungal therapy before establishment of the corresponding life-threatening infection (Cummings *et al*., 2007).

Antigen detection is an important approach in this respect, it should be avoided during the investigation of urine specimens with unabsorbed sera obtained from conventionally raised rabbits (Dupont *et al*., 1987). Sometime, *Aspergillus* serum specimens for the detection of antibodies against it may be absorbed with *Escherichia coli*, *Staphylococcus saprophyticus*, and *Klebsiella pneumonia*. Therefore, *Aspergillus* detector sera should be examined before using it in immunoblot tests for detecting the presence of common antibodies organisms causing urinary tract infections antibodies (Kawamura *et al*., 1999; Yamakami *et al*., 1998).

GM and Diagnosis of IA

Primary diagnostic assays of IA including latex agglutination had little or poor sensitivity (Verdaguer *et al*., 2007). The Aspergillus GM enzyme-linked immunosorbant assay (EIA) has been demonstrated to facilitate rapid and sensitive detection of IA (Cummings *et al*., 2007). Recently, the use of a double-sandwich ELISA incorporating β1→5 galactofuranose –specific EBA2 monoclonal antibody (as both detector and acceptor for GM) showed a high sensitivity with a threshold of 0.5 ng/ml; this test was 15–30-times more sensitive than the previous latex agglutination assay (Mennink-Kersten *et al*., 2004; Rovira *et al*., 2004; Stynen *et al*., 1995). This method was confirmed by the US Food and Drug Administration (FDA) for use with serum samples. However, the sensitivity and specificity of this enzyme immunoassay (EIA) varies with the type of transplant recipient (Singh *et al*., 2005). Application of GM EIA for the early diagnosis of IA in neutropenic patients has been demonstrated across several studies (Kawazu *et al*., 2004; Machetti *et al*., 1998; Maertens *et al*., 1999., Maertens *et al*., 2001; Maertens *et al*., 2002; Rovira *et al*., 2004; Sulahian *et al*., 2001). However, it remains unclear whether detection of GM is useful in the lung and liver transplant recipients (Singh *et al*., 2005). Moreover, it has been reported that monitoring of antigenemia is important for predicting the therapeutic outcomes in IA patients (Boutboul *et al*., 2002; Bretagne *et al*., 1997).

Positive results support diagnosis of IA, although those should be remarked in conjunction with other diagnostic procedures, including microbiologic culturing, histopathological examination, and radiographical testing (Verdaguer *et al*., 2007). Meanwhile, negative results did not rule out diagnosis of IA. In case of negative results, the examination should be repeated (Pinel *et al*., 2003). Patients at a high risk of IA should be subjected to a baseline serum test and should be monitored twice a week for increasing GM antigen levels.

Specificity of GM detection in the serum has not been fully evaluated in fungal species

except *Aspergillus*. However, cross-reaction has been noted with five fungal species, including *Blastomyces dermatitidis*, *Nigrospora oryzae*, *Paecilomyces lilacinus*, *Penicillium chrysogenum*, and *Trichothecium roseum* (Cummings *et al*., 2007).

It is well-known that damage to the gastrointestinal tract wall by cytotoxic therapy, irradiation, or graft-versus-host disease enables translocation of the GM from the GI lumen into the blood, which may be partially responsible for the high false-positive rate of this assay (Matsudomi *et al*., 2002).

Some genera of fungi such as *Penicillium spp.* and *Paecilomyces spp.* have shown reactivity with rat EBA-2 monoclonal antibody used in the assay (Wallis *et al*., 2001). Nevertheless these species are rarely involved in invasive fungal diseases. Cross-reactivity with *Alternaria spp.* has also been reported. Semisynthetic antibiotics such as piperacillin, amoxicillin, and augmentin, which are based on natural compounds derived from the genus *Penicillium*, have been observed to cross react with the rat EBA-2 Mab applied in this test. The specificity of GM detection in sera for *Aspergillus* spp. does not exclude the involvement of other fungal pathogens with similar clinical presentations, such as *Fusarium spp.*, *Alternaria spp.*, and *Mucorales* (Adam *et al*., 2004). The performance of the GM measurement by ELISA has not been evaluated with infant serum specimens or in combination with plasma or other specimen types such as urine and cerebrospinal fluid (Maertens *et al*., 2001). Moreover, ELISA may exhibit reduced detection of GM in patients with chronic granulomatous disease and Job's syndrome (Rovira *et al*., 2004; Singh *et al*., 2005).

The concomitant use of antifungal therapy in some patients with IA may result in reduced sensitivity of the ELISA test. False-positive GM results are possible in patients receiving plasma-lyte for intravenous hydration or for Broncho alveolar lavage. Specimens containing *Histoplasma capsulatum* antigen may cross-react in the *Aspergillus* GM assay (Adam *et al*., 2004).

The data on the use of serum GM antigen test for serological diagnosis of chronic pulmonary aspergillosis (CPA) are limited. In a recent study, the serum GM antigen test was evaluated in patients with CPA. Positive results were observed among 23% of CPA patients and in 15% patients without CPA. In this study, the sensitivity of the serum GM antigen test was 23% (95% confidence interval [CI], 17e30%), with specificity of 85% (95% CI, 79e90%), and positive and negative predictive values of 60% (95% CI, 47e72%) and 52% (95% CI, 46e58%), respectively. The accuracy of this test was 54%. The authors therefore concluded that serum GM antigen is not reliable for the serological diagnosis of CPA (Shin *et al*., 2014).

Conclusion and Perspectives

Detection of GM in patient serum by sandwich ELISA is the most reliable and non-invasive approach for the detection of IA in intensive care units. However, false-positive results have been reported at the rate of 8–14% for this assay. We therefore conclude that, although GM detection in the serum is a useful method for early diagnosis of IA, the GM levels should be interpreted with results from other examinations such as radiology. More studies are necessary to introduce a novel kit with higher sensitivity and minimal false-positive results for precise diagnosis of IA.

Conflicts of Interest

The authors declare no conflicts of interest.

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