Aspergillus species are ubiquitous molds to which humans are commonly exposed. Of approximately 180 species, it is estimated that 34 are medically significant. Most persons who come in contact with the fungus remain asymptomatic.

DIAGNOSIS OF IA

IA is usually suspected when signs of infection refractory to broad-spectrum antibiotics develop in an immunocompromised patient. The gold standard for the diagnosis of IA is tissue biopsy demonstrating invasion on histopathological examination and identification of the organism in culture.

The finding of acute-angle branching, septated, nonpigmented hyphae on histopathological examination is not specific for IA because other molds, including *Fusarium*, *Paecilomyces*, and *Pseudallescheria boydii*, can have a similar appearance. For unclear reasons, growth of *Aspergillus* in culture occurs in only 30% to 50% of histopathologically suggestive cases. Obtaining tissue specimens for the diagnosis of IA is often difficult, because patients in whom IA is suspected often have medical conditions, such as thrombocytopenia, that preclude biopsy.

GALACTOMANNAN ANTIGEN TESTS

A recent diagnostic modality for IA is the galactomannan (GM) assay. GM is a cell wall component of many fungi, including *Aspergillus*, *Penicillium*, *Paecilomyces*, and *Geotrichum* species. An enzyme-linked immunosorbent assay-based kit is commercially available as the Platelia Aspergillus EIA (Bio-Rad Laboratories, Redmond, Wash) and was cleared by the FDA for diagnostic use in May 2003. GM antigen positivity is among the microbiological criteria proposed by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group for the diagnosis of IA.

The Platelia EIA is an immunoenzymatic sandwich microplate assay that uses monoclonal antibodies that bind to side-chain residues of the GM molecule. A sandwich enzyme immunoassay format is used for detection. The performance of the assay varies according to the threshold value used. When the Platelia EIA became available in Europe a decade ago, the manufacturer recommended an
optical density (OD) index (also called a GM index) of 1.5 as the cutoff between positive and negative results. Lowering the threshold to between 0.7 and 1.5 in an effort to improve sensitivity was validated, and an analysis of 986 serum samples determined that a cutoff of 0.5 increased sensitivity with minimal loss of specificity.\(^6,7\)

In the United States, the suggested OD index threshold is 0.5; in Europe, a cutoff of 0.7 is commonly used.\(^8\) The overall reported sensitivity of the Platelia EIA ranges from 30% to 90%, with a reported specificity of greater than 93%.\(^9,10\)

**Causes of false-positive GM assay results**
False-positive test results with the GM assay have been reported by a number of investigators (Table). Because *Penicillium* produces GM, it is not surprising that a number of -lactam antibiotics, including piperacillin/tazobactam, amoxicillin/clavulanate, ampicillin, and phenoxymethylpenicillin, have yielded positive Platelia EIA results.\(^11,12\)

Other reported causes of false-positive results include various foods, *Geotrichum capitatum* (a rare cause of invasive disease), *Bifidobacterium* species (frequent GI colonizers), and an electrolyte solution (containing sodium gluconate produced by *Aspergillus niger* fermentation) used for bronchoalveolar lavage (BAL).\(^13-16\)

**GM testing on nonserum specimens**
The GM assay has been validated only for serum samples. However, investigators have reported results of testing other body fluids, including BAL fluid, cerebrospinal fluid (CSF), and urine. Because 70% of cases of IA involve the lungs, testing of BAL fluid specimens for GM seems intuitive. Use of the Platelia EIA when testing BAL fluid in 49 cases of proven or probable IA and 47 control patients revealed a sensitivity of 76% (61% to 87%) and a specificity of 94% (84% to 99%).\(^17\) A similar study demonstrated a sensitivity of 100% when testing BAL fluid samples from 20 patients with proven or probable pulmonary IA.\(^18\)

Data on the use of the GM assay for testing of CSF are sparse. One study reported that CSF GM values in 5 patients with probable CNS IA were significantly higher than GM values in 16 control patients.\(^19\)

As with CSF sampling, a paucity of reports are available on the use of the GM assay for testing urine. In one study that used the Platelia EIA, only 2 of 6 patients with confirmed IA tested positive for GM in their urine.\(^20\)

**Use of serial GM testing**
The use of sequential serum GM assays as a strategy to improve sensitivity and specificity has been proposed. This approach was evaluated in a prospective study involving 88 neutropenic patients who had received chemotherapy for leukemia or myelodysplastic syndrome or myeloablative allogeneic hematopoietic stem cell transplant.\(^21\) Surveillance tests using Platelia EIA were performed 3 times a week, and treatment with amphotericin B was initiated only if 2 or more GM assays had positive results or chest CT findings suggested invasive fungal infection (supported by a culture or microscopic examination finding of molds). Application of these guidelines led to a 78% reduction in use of antifungals compared with use of antifungals for neutropenic fever when treatment decisions were based on standard guidelines.
Another study evaluated serial GM testing in 74 allogeneic stem cell transplant recipients who had serum GM assays performed twice weekly and who underwent chest CT if a fever of unknown focus persisted for more than 72 hours. 22 Nine patients had proven or possible IA. The GM test demonstrated a sensitivity of 100% and specificity of 93%, and the sensitivity of chest CT was comparable (100% had abnormal findings). Only 2 of the 9 patients died as a result of IA, leading to the conclusion that Aspergillus GM surveillance and early chest CT should be considered to detect IA in its initial stages.

CONCLUSION
The timely diagnosis of IA is problematic, and its delayed diagnosis is associated with high mortality rates. The GM assay offers the potential of securing a diagnosis through relatively noninvasive means. Clinicians should consider using this test when they suspect IA in immunocompromised patients. The test may prove especially helpful in patients in whom invasive tissue biopsy is contraindicated. There are published shortcomings of the GM test, and questions remain about GM detection in tissue and fluid other than serum and whether surveillance sampling in at-risk populations can both decrease use of empiric antifungal therapy and improve survival.

References:


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