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Researchers identify tests to diagnose invasive aspergillosis with 100 percent accuracy

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The fungal infection invasive aspergillosis (IA) can be life threatening, especially in patients whose immune systems are weakened by chemotherapy or immunosuppressive drugs. Despite the critical need for early detection, IA remains difficult to diagnose. A study in *The Journal of Molecular Diagnostics* compared three diagnostic tests and found that the combination of nucleic acid sequence-based amplification (NASBA) and real-time quantitative PCR (qPCR) detects aspergillosis with 100% accuracy.

IA is caused by the fungus *Aspergillus fumigatus*, which is considered by many pathologists to be the world's most harmful mold. "Traditional diagnostic methods, such as culture and histopathology of infected tissues, often fail to detect *Aspergillus*," comments lead investigator Yun Xia, PhD, of the First Affiliated Hospital of Chongqing Medical University, Chongqing, China.

In this retrospective study, scientists evaluated the diagnostic performance of two nucleic acid amplification assays (qPCR and NASBA) and one antigen detection method (galactomannan enzyme-linked immunosorbent assay [GM-ELISA]) using blood samples collected from 80 patients at high risk of IA. Of the 80 patients, 42.5% had proven or probable IA. The patients came from intensive care, hematology, neurology, nephrology, geriatrics, and other hospital departments.

The tests were evaluated singly and in combination. Individually, NASBA had the highest sensitivity (76.47%) whereas qPCR offered the highest specificity (89.13%). NASBA also was the test that best indicated that a patient did not have the infection (negative predictive value). NASBA and qPCR each had a high Youden index, a measure of the effectiveness of a diagnostic marker.

Combining the tests improved the outcomes. The combination of NASBA and qPCR led to 100% specificity and 100% positive predictive value (the probability that subjects truly have the infection).

"Because each test has advantages and disadvantages, a combination of different tests may be able to provide better diagnostic value than is provided by a single test," says Dr. Xia. The combination of NASBA and qPCR should be useful in excluding IA in suspect cases, thus reducing both suffering and expense for immunocompromised patients. On the other hand, the combination of NASBA and qPCR could be more suitable for screening patients suspected of infection, because this assay had the highest sensitivity."

The authors note that NASBA offers the advantages of rapid amplification (90 minutes) and simple operation with low instrument cost compared with qPCR and GM-ELISA. They caution that although GM-ELISA is widely and routinely used for aspergillosis diagnosis, this study indicates that it had low sensitivity (52.94%) with reasonable specificity (80.43%), making it "inferior to both NASBA and qPCR."

The A. fumigatus mold is ubiquitous in the environment and is found on decaying plant matter. For healthy individuals exposure to the fungus can be inconsequential, but it can cause significant morbidity and mortality for those with compromised immune systems, including patients who have undergone organ transplants or have advanced AIDS. Even patients with more modest immune impairments, such as diabetes,

poor nutrition, steroid use, or lung disease, can become severely infected. Symptoms may include fever, cough, difficulty breathing, chest pain, seizures, and focal neurological problems.

The criteria for high risk for IA included high (1,3)-β-D-glucan levels (>60 pg/mL), immunocompromised status, and one of six conditions (recipient of an allogeneic stem cell transplant, hematological disease, severe immunodeficiency, prolonged use of corticosteroids, fever or chest infiltrate unresponsive to routine antibiotics, or radiological indication of fungal disease). Patients did not receive any antifungal therapy until after blood samples were collected.

The first test was the measurement of GM, a polysaccharide component of the fungal cell wall, which can be released into serum and bronchoalveolar lavage fluid during infection. GM was measured using a commercially available ELISA kit (Platelia *Aspergillus*; Bio-Rad Laboratories, Hercules, CA). In this study, a GM index (GMI) of 0.5 was used.

The second test was an *Aspergillus* DNA extraction and real-time qPCR assay. The DNA was extracted from plasma using a QIAamp blood mini kit (Qiagen, Hilden, Germany). The purified DNA was then amplified by an *Aspergillus* genus-specific qPCR assay using SYBR Green chemistry and primers targeting the 28S rRNA gene. The lower limit of detection was empirically determined to be 10 colony-forming units of *Aspergillus* conidia per reaction.

The third test was an *Aspergillus* RNA extraction and NASBA assay. Total RNA was extracted from plasma using a blood/liquid sample total RNA rapid extraction kit (BioTeke, Beijing, China). A highly conserved 18S rRNA region specific for the *Aspergillus* genus was chosen as the detection target. It was then amplified using a pair of primers. Blank control, negative control (RNA extracted from patients without

Aspergillus infection) and positive control (RNA extracted from *Aspergillus* in pure culture) were included in each run.

More information: "Retrospective Comparison of NASBA, Real-time PCR, and Galactomannan Test for Diagnosis of Invasive Aspergillosis," by Lipeng Wang, Yunyan He, Yun Xia, Xiaoyan Su, Huijuan Wang, and Shumei Liang, DOI: dx.doi.org/10.1016/j.jmoldx.2014.05.001. Published online ahead of The *Journal of Molecular Diagnostics*, Volume 16, Issue 5 (September 2014).

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