Aspergillus antigen detection in invasive aspergillosis

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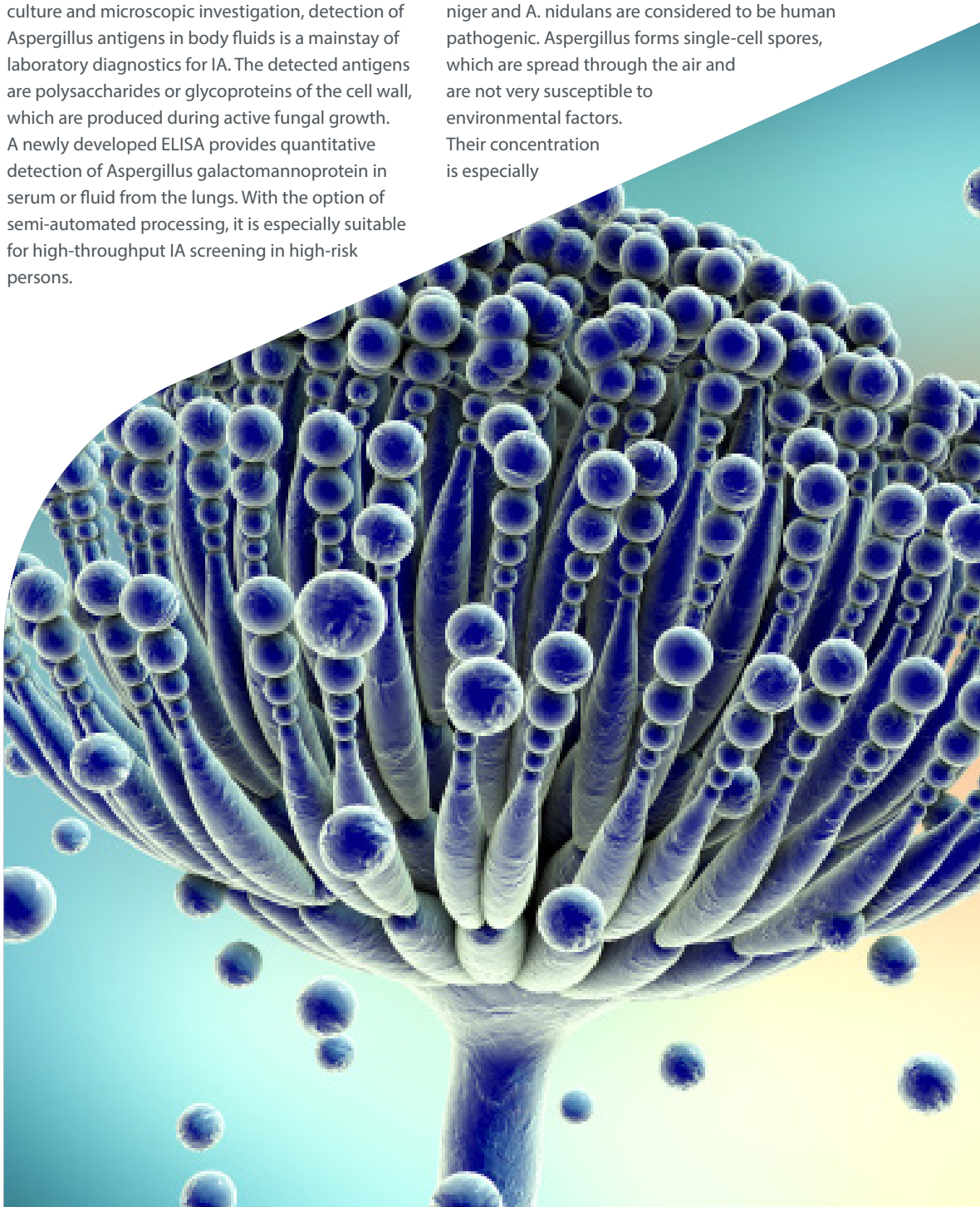
Invasive aspergillosis (IA) is a severe and often life-threatening infection in immunocompromised individuals. Early diagnosis is critical to enable prompt initiation of treatment and prevention of fatalities. Alongside culture and microscopic investigation, detection of Aspergillus antigens in body fluids is a mainstay of laboratory diagnostics for IA. The detected antigens are polysaccharides or glycoproteins of the cell wall, which are produced during active fungal growth. A newly developed ELISA provides quantitative detection of Aspergillus galactomannoprotein in serum or fluid from the lungs. With the option of semi-automated processing, it is especially suitable for high-throughput IA screening in high-risk persons.

Aspergillosis

Aspergillus spp. are ubiquitous sac fungi which are found, for example, in soil, compost or on damp walls. Almost 200 species have been described, of which A. fumigatus, A. terreus, A. flavus, A. niger and A. nidulans are considered to be human pathogenic. Aspergillus forms single-cell spores, which are spread through the air and are not very susceptible to environmental factors. Their concentration is especially



An estimated 200,000 to 300,000 cases of life-threatening IA occur each year worldwide.

Table 1. Comparable performance of Aspergillus Antigen ELISAs*

	EUROIMMUN Aspergillus Antigen ELISA (cut-off: ratio 0.4**)	Bio-Rad Platelia Aspergillus Ag ELISA (cut-off: ratio 0.5)
Sensitivity	56%	47%
Specificity	97%	99%

*Dichtl et al., J. Clin Microbiol, Apr 2019

**Varies from the cut-off recommended by the manufacturer

elevated in summer.

In individuals with an intact immune system the inhalation of spores does not generally cause any health problems, although a permanent load can lead to sensitivity or allergic reactions. Immunocompromised individuals, however, cannot mount an adequate immune response, resulting in severe or even life-threatening infections. The spores are deposited in the lung tissue, germinate and grow hyphae. These penetrate the tissue and spread to other parts of the body via the blood. This disseminated form, known as IA, represents the most severe clinical manifestation of Aspergillus infection. It most often affects the nervous system, eyes, heart, kidneys and skin. The symptoms, which are often unspecific at onset, encompass cough, breathing difficulties, dyspnoea with hypoxia, sustained fever of longer than 72 hours, lung infiltration under antibiotic treatment, pain in the chest and haemoptysis. An estimated 200,000 to 300,000 cases of life-threatening IA occur each year worldwide. Mortality is high, ranging from 40 to 95 per cent depending on the immune status of the patient, distribution of the infection and drug treatment. IA with involvement of the central nervous system is nearly always fatal.

High-risk patients

Persons with neutropaenia, leukaemia, or chronic granulomatous disease (CGD) are considered at high risk of IA. It is also a frequent life-threatening complication in advanced stages of AIDS, during chemotherapy or following bone marrow or organ transplants.

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The incidence of IA amounts to 40 per cent in CGD patients and 12 per cent in AIDS patients. Seven to 13 per cent of allogenic bone marrow transplant recipients develop IA, either after two weeks of aplasia or around three months later during immunosuppressive therapy. IA also affects 0.5 to 8 per cent of autologous transplantation patients during neutropaenia, as well as 2 to 3 per cent of organ recipients, with lung transplant patients at highest risk. Further risk factors for IA are chronic lung diseases, CMV infection, tumours and autoimmune diseases. In recent years an increased number of nosocomial infections in patients in intensive care has been observed.

IA diagnostics

IA can be difficult to diagnose due to the unspecific initial symptoms such as fever or inflammatory reactions. Diagnosis is based on a combination of clinical manifestation, radiology, culture, microscopy and serology. Since clinical and radiological signs of IA are often non-specific, laboratory tests are nearly always required to substantiate diagnosis. Culture of Aspergillus spp. in combination with histopathologic evidence of tissue invasion by hyphae provides a definitive evidence of IA. However, culture is time-consuming and has a success rate of only around 50 per cent, and biopsy is not always feasible due to the risk of complications. In vitro detection of antigen biomarkers provides a first-line, non-invasive method to screen patients for IA.

Antigen detection yields results much faster than culture and is thus particularly helpful for early diagnosis. For this reason it has been incorporated into guidelines of the European Organization for Research and Treatment of Cancer (EORTC) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) as a criterion for probable IA. In high-risk patients regular analysis of blood for Aspergillus antigen is recommended. Polymerase chain reaction (PCR) is not recommended for routine IA diagnostics as there are few standardised test systems and PCR does not allow differentiation between non-invasive and invasive infections.

Aspergillus Antigen ELISA

The new CE-marked Aspergillus Antigen ELISA from EUROIMMUN provides detection of extracellular galactomannoprotein of different growing Aspergillus spp. The

analysis is performed on patient serum or bronchoalveolar lavage (BAL) fluid samples. Results can be evaluated either quantitatively in pg/ml using a 6-point calibration curve or semiquantitatively by means of a cut-off ratio. The test can be semi-automated, for example, on the EUROIMMUN Analyzer I, increasing the efficiency of screening.

Sensitivity and specificity

In the most comprehensive clinical study to date based on 120 sera from 45 patients with proven IA as well as control sera, the EUROIMMUN Aspergillus Antigen ELISA yielded comparable sensitivity and specificity to another commercially available Aspergillus antigen test (Platelia Aspergillus Ag Assay, Bio-Rad). The EUROIMMUN assay identified 56 per cent of the cases, while the Bio-Rad test detected 47 per cent (Table 1). The specificity amounted to 97 per cent for the EUROIMMUN assay and 99 per cent for the Bio-Rad test. To overcome the relatively low sensitivity of Aspergillus antigen detection, the author recommends serial testing of patients at risk. Overall, the positive predictive value of the EUROIMMUN Aspergillus Antigen ELISA was 77.8 per cent for serum and 86.2 per cent for BAL, while the negative predictive value was 92.9 per cent for serum and 95.2 per cent for BAL.

Perspectives

In recent decades the incidence of IA has surged due to the rising number of patients undergoing organ and stem cell transplantation or aggressive cancer chemotherapy regimes. Given the growing population of chronically ill and elderly individuals, the burden of IA will likely increase further in the future, including in critical care settings. Deciding on the appropriate antifungal medication, dose and duration relies on fast and reliable diagnosis. Aspergillus antigen detection has been integrated into diagnostic algorithms and is now employed in most medical centres in Europe for routine diagnostics and surveillance of high-risk patients. The antigen test can also play a role in trials of antifungal drug efficacy, strategy trials and epidemiological studies.

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