Review

Invasive aspergillosis in hematology malignancies – a disease difficult to diagnose

Aspergiloza invazivă în bolile maligne hematologice – o boală greu de diagnosticat

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Abstract

Invasive fungal infections are a major threat to immunocompromised patients, particularly those with hematological malignancies and hematopoietic stem-cell transplant. Invasive aspergillosis (IA) represents the main cause of infections after hematopoietic stem cell transplant, with a high mortality rate among these patients. Clinical signs and symptoms of IA are non-specific. The traditional laboratory techniques have low sensitivity and late results, and thus a limited role in the diagnosis of IA. Therefore, improving the specific diagnosis of IA has become a common medical concern. Indirect mycological tests (detecting fungal antigens in serum) are useful instruments for early diagnosis of IA and they were widely studied. Galactomanan antigen (GM) Assay, which is the most standardized parameter, was implemented in Fundeni Clinical Institute starting Jan. 2011, as a routine test. First preliminary data suggest that, due to this test, an increased number of invasive aspergillosis was diagnosed in high risk patients.

Keywords: *invasive aspergillosis, hematological malignancies, allogenic stem cell transplant, galactomanan*

Rezumat

Infecțiile fungice invazive reprezintă o amenințare majoră pentru pacienții imunocompromişi, în special cei cu malignități hematologice şi transplant de celule stem hematopoietice. Aspergiloza invazivă (AI) reprezintă principala cauză de mortalitate infecțioasă post transplant de celule stem hematopoietice, având o rată mare de mortalitate la aceşti pacienți. Semnele şi simptomele clinice ale AI sunt nespecifice. Tehnicile tradiționale de laborator au o senzitivitate scăzută şi rezultate tardive, ceea ce limitează rolul lor în diagnosticul AI. Astfel, îmbunătățirea diagnosticului specific al AI a devenit o preocupare medicală comună. Testele micologice indirecte (detecția antigenelor fungice în ser) reprezintă instrumente foarte folositoare pentru diagnosticul precoce de AI şi sunt intens studiate. Testul de detecție al antigenului galactomanan, care reprezintă cel mai standardizat para-

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metru de diagnostic micologic indirect, a fost implementat în Institutul Clinic Fundeni începând cu Ianuarie 2011, ca un test de rutină. Analiza datelor preliminare sugerează că grație acestui test au fost diagnosticate un număr mai mare de cazuri de aspergiloză invazivă la pacienții cu risc crescut.

Cuvinte cheie: *aspergiloză invazivă, afecțiuni maligne hematologice, transplant alogenic de celule stem, galactomanan*

Introduction

The frequency of invasive fungal infections (IFI) has risen dramatically in recent years on account of mold infections, especially with *Aspergiluss spp.* (1-3), while the prevalence of all other IFI remained relatively constant (1). The majority of IFI occur in patients with hematological malignancies (HM), particularly in those with acute myeloid leukemia (AML) and those who have undergone allogeneic hematopoietic stem cell transplantation (allo-HSCT) (4-5). Protective isolation facilities, especially those that consist of high efficiency particulate air (HEPA) and laminar airflow (LAF) have been advocated for the prevention of IA in immunocompromised patients. Despite these facilities the overall incidence of *Aspergillus spp*. infections ranges from 0.3% to 20% depending on the underlying hematological condition (1-3).

Although many species of *Aspergillus* have been isolated in nature, *A fumigatus* is the most common cause of infection in humans, due to the ability of *A fumigatus* to grow at normal human body temperature. Human host defense against the inhaled spores begins with the mucous layer and the ciliary action in the respiratory tract. Macrophages and neutrophils encompass, engulf, and eradicate the fungus. However, many species of *Aspergillus* produce toxic metabolites that inhibit macrophage and neutrophil phagocytosis. Corticosteroids also impair macrophage and neutrophil function. Underlying immunosuppression also contributes directly to neutrophil dysfunction or decreased numbers of neutrophils. In individuals who are immunosuppressed, vascular invasion is much more common and may lead to infarction, hemorrhage, and lung tissue necrosis. The prognosis of invasive aspergillosis (IA) is grim, with a case mortality rate between 58- 98% (4). It is clear that successful management of IA is contingent on early detection, which, unfortunately, can be a difficult task in clinical practice.

Autopsy –proven IFI

Many authors tried to establish the correct incidence of IFI in different categories of patients. The most reliable data come from autopsy studies, which reveal the fact that IFI is under diagnosed during the patients' life. Groll et al (6) analyzed data from 8124 autopsies performed between 1978 and 1992 on patients deceased at the University Hospital of Frankfurt/ Main and found that a total of 278 IFI were diagnosed: the highest rates occurred for aplastic syndromes (68%), followed by AML (25%). For the majority of cases (76%), IFI was reported as the immediate cause of death. A similar study, performed by Chamilos et al (7) evaluated the autopsy-proven invasive fungal infections (IFI) in patients with hematological malignancies over a 15-year period (1989-2003) and identified IFI in 314 (31%) of 1017 autopsies. A very important conclusion of this study was that most IFI (75%) escaped from being diagnosed antemortem.

Diagnosis of IA

The complex and evolving epidemiology of IFI in severely immunocompromised patients is not well captured by current diagnostic methods. In 2002, a consensus group of the European Organization for Research and Treatment of Cancer/ Invasive Fungal Infections Cooperative Group (EORTC) and the National Institute of Allergy and Infectious Diseases My-

coses Study Group (MSG) published the IFI standard definitions for clinical and epidemiological research (8). The definitions assigned 3 levels of probability to the diagnosis of IFI developed in immuno-compromised patients with cancer and in hematopoietic stem cell transplant recipients – namely "proven", "probable" and "possible" IFI. The definitions were revised in 2008 in order to reflect more accurately the disease process caused by fungal infection and the term of invasive fungal disease (IFD) was adopted instead of IFI (9). The category of possible IFD was revised to include only those cases that are highly likely to be caused by a fungal etiology, although mycological evidence is lacking. The criteria for proven and probable IFD, based on the same 3 elements as in the original definitions (host factors, clinical manifestations and mycological evidence) were modified to reflect the advances in indirect mycological tests.

Host factors

IA is usually suspected when signs of infection refractory to broad-spectrum antibiotics develop in an immunocompromised patient. Host factors are different from the risk factors; they are applicable to patients with malignant diseases, with hematopoietic stem cell transplants, solid organ transplant or patients who receive immunosuppressant therapy. The criteria recognized as host factors for IFD are: (1) deep neutropenia (<500 neutrophils/mm3 for >10 days); (2) receipt of an allogeneic stem cell transplant; (3) prolonged use of corticosteroids; (4) treatment with other recognized T cell immunosuppressants (cyclosporine, TNF-a blockers, specific monoclonal antibodies or nucleoside analogues) during the past 90 days; (5) inherited severe immunodeficiency (9-11).

Radiological exams

For pulmonary-localized mold infections, two specific radiological patterns are cited as being important diagnostic tools: the "halo sign", a typical precocious lesion, and the "air crescent sign", more prevalent later in the course of the disease.

The "halo sign", a macronodule (>1 cm in diameter) surrounded by a perimeter of ground-glass opacity, is regarded to be an early indicator of invasive pulmonary aspergillosis. The "air crescent sign" was defined as a crescentic pocket of gas occupying a separation interface between a lung sequestrum attributable to necrosis and a rim of viable lung. Both lesions have been incorporated into the EORTC criteria for IFD. The patients with severe T cell immunodeficiency, such as solid organ or allogeneic hematopoietic stem cell transplant recipients and patients with chronic hematologic malignancies may present with more inconspicuous images, including ill-defined consolidations, ground-glass attenuation, small nodules (< 1 cm), and other nonspecific lesions. High resolution computer tomography (CT) scan is informative for the type, number and localization of lesions, as well as their morphologic characteristics (12). CT scanning represents the elective method to investigate the lungs for an IA diagnosis. Prospective studies emphasized that serial chest CT-scans depict quite precisely the evolution of lesion volumes caused by pulmonary aspergillosis, being an useful tools both for the IA diagnosis and its outcome evaluation (13,14). Despite their importance, these characteristic radiological patterns do not allow the diagnosis for a specific aspergillosis because of their similarity to other angioinvasive fungi such as *Zygomycetes, Fusarium spp* or *Scedosporium spp.*

Mycological tests

The gold standard for the IA diagnosis remains the tissue biopsy demonstrating the fungus. Standard approaches to the laboratory diagnosis of invasive fungal infections include (i) direct microscopic visualization for the presence of organisms in freshly obtained body flu-

ids, (ii) histopathologic demonstration of fungi within tissue sections, and (iii) causative fungus cultivation and its subsequent identification. However, these approaches are often not sufficiently sensitive and/or specific to diagnose invasive fungal infections. The finding of angle branching, non-pigmented hyphae on histopathological examination is not specific for IA because other moulds, including *Fusarium* can have similar appearance (15, 16). Tarrand et al report that growth of *Aspergillus* in culture occurs in only 30% to 50% of histopathologically suggestive cases (17). The main problem for obtaining tissue specimens for the diagnosis of IA is thrombocytopenia, which often accompanies high risk patients, and precludes biopsy.

Indirect mycological tests

Another approach is to diagnosis IFI in host body fluids using immunologic tests able to detect and quantify fungal antigens or fungal cell-wall constituents. The ideal antigenic IFI markers should have many characteristics: non transient, not associated with colonization, no cross-reaction with other human and microbial antigens, conserved within the fungal species of interest and present quite early for the starting of antifungal therapy. Moreover, the technique to detect these antigens should be easy to perform, as a routine test and not be subject to significant variation between laboratories (18, 19). At present, two indirect mycological tests are recognized by the EORTC criteria: the Galactomanan (GM) Antigen Assay for Aspergillosis – detect the Galactomannan antigen in plasma, serum, bronchoalveolar lavage fluid, or CSF and B-D-glucan (BG) Assay for Invasive fungal disease other than cryptococcosis and zygomycoses - detect the B-D-glucan Antigen in serum. In practice, routine follow up of high risk patients should include determination of GM and BG twice weekly as a screening. A positive result should trigger further evaluation for disease (imaging tests) while a negative result should lead to the aggressive search for other etiologies. In addition, the GM testing represents a very useful marker in evaluation of the treatment response.

The Galactomanan (GM) Antigen Assay

GM is part of the outer layer of the *aspergillus* cell wall, and it is released during the fungus growth at the tips of hyphae (20, 21). GM is also a cell wall component of many other fungi, including *Aspergillus, Penicillium* and *Geotrichum spp*. The GM antigen assay can provide valuable information if the specimens are tested upon collection. A positive GM test represents also an early diagnosis tool taking in account that the fungal antigen can be detected in its very early stages: 5-8 days (average) before any clinical signs develop (in 65.2% of patients), chest X-ray patterns become visible (in 71.5% of patients) and culture results become positive (in 100% of patients) (22). A negative GM test may provide support for more aggressive traditional diagnostic protocols, especially in patients with syndromes compatible with aspergillosis.

The GM antigen can be detected using a commercially available sandwich ELISA (Platelia Aspergillus, BioRad, France) (PA-ELISA), which employs a monoclonal antibody (EB-A2) that binds galactofuran epitope of the GM antigen (24). The assay has been extensively studied and is now commonly used to monitor patients at high risk of invasive aspergillosis (23,25). When the Platelia EIA became available in Europe, the manufacturer recommended an optical density (OD) index (also called a GM index) of 1, 5 as the cut-off between positive and negative results. It was demonstrated that lowering the threshold to a cut-off of 0.5 the sensitivity will increase with minimal loss of specificity (26, 27) and that is why the suggested OD index threshold is 0.5 both in United States and Europe. The performance of the GM ELISA test was reported to range from 50% to 100% in sensitivity and from 92% to 100% in specificity (28-31). In these reports,

sensitivity varied considerably, while specificity remained more consistent and was usually greater than 85%. When the test is applied to clinical diagnosis, double-checking of positive samples might be necessary because of the possible lack of reproductibility (30). IA should be considered when 2 consecutive positive samples from a patient have been obtained. In patients with only single positive samples, the sensitivity is low, perhaps around 40% or less (15). It was described many instances for false negative results but the main reasons are exposure to antifungal agents and high cut-off values. The use of antifungal agents may lower antigen levels by decreasing the fungal load, making the test less useful in patients receiving antifungal prophylaxis. The sensitivity of the galactomannan testing is lower in patients with non- or minimally invasive manifestation of aspergillosis. Galactomannan detection is not useful in patients suffering from cavitary pulmonary aspergillosis or allergic bronchopulmonary aspergillosis. False positive results have been observed in all studies, but the prevalence has varied considerably. The use of antibiotics was the most studied. GM was detected in several drugs that originated from fungal organism, including piperacillin, piperacillin/tazobactam and uricase (30). False positive results may occur more frequently in children. Some suggest that GM present in milk, rice, or protein-rich nutrients is the cause of false-positive results in children, a conclusion that cannot explain the high rate of false-positivity in premature infants who do not receive cereal. The false-positive rate was reported to be high in up to 83% of newborn babies (15). Besides GM of food origin, lipoteichoic acid of *Bifidobacterium spp.*, which heavily colonizes the neonatal gut, might cause ELISA reactivity in infants after translocation through immature intestinal mucosa (32). False-positive results have also been reported in neutropenic patients with bacteremia caused by *staphylococci, enterococci, Corynebacterium jeikeium, Pseudomonas, Escherichia coli* and fungemia with *Candida albicans*.

Beta glucan Assay (BG)

The beta-glucan test proved to be comparable with the GM assay in diagnosing IA both in animals models and in clinical trials, appearing earlier in the course of infections. The cut-off for a positive result is > 80 pg/ml. As with GM, variable results have been reported for BG assay, with a high sensitivity and specificity, range from 70% to 90% (33). In a recent study, based on autopsy studies the BG test evaluation proved to be effective in diagnosing IFI (31,34). False positive results appear more often than in the GM test, and are reported in bacteriemic patients, patients treated with fungal derived antibiotics, cirrhosis, abdominal surgery, mucositis, enterocolitis etc.

Molecular methods

A range of polymerase chain reaction (PCR) based methods have been developed with the goal of offering a highly specific, sensitive and rapid method of *Aspergillus* s*pp*. identification. Although PCR has been studied for years, the lack of standardization and clinical validation has led to its exclusion from consensus criteria for defining IFD. In a meta-analysis of *Aspergillus* PCR tests for IA diagnosis, Mengoli et al. (35) concluded that a single PCR negative test is sufficient to exclude IA, whereas two PCR positive tests are required to confirm disease. Real-time PCR is more frequently used than the classical PCR (36).

Invasive Aspergillosis in hematological patients in Romania

There are no published data related to epidemiology of IFD in hematology patients in Romania. A retrospective survey of IFD in patients with hematological malignancies admitted to the Fundeni Hematology Clinic could evaluate only proven IFD (histopathology/ culture positive). The incidence of proven IA

during 2006-2010, in patients with HM and allo-HSCT hospitalized in Fundeni Hematology Clinic was 3 / 68510 admissions, a result that indicates an extremely low incidence that does not correlate with the data reported for the same category of patients in other European and USA centers. We found several explanations for the situation: (1) the patients with clinical signs of IFD were often transferred in the Infectious Diseases Units for diagnosis and treatment; (2) most of the biopsies required for diagnosis were not performed due to the patients' medical conditions (mainly thrombocytopenia); (3) the autopsies required also for diagnosis purposes in this cathegory of patients were not performed for various reasons (mainly the refusal of the patients' family); (4) lack of the indirect mycological tests. Starting with 2011, when we introduced the indirect mycological tests (GM from January 2011 and BG from March, 2011), it has become possible to assess the probable IFD (host criteria + imaging criteria + indirect positive mycological tests). Thus, by using the indirect mycological tests, in just 6 months (January to June 2011), a total of 7 cases of IA were diagnosed and treated early. These preliminary data demonstrate the importance of introducing the indirect mycological tests for both diagnosis and treatment purposes.

Conclusions

Invasive aspergillosis represents a serious and often fatal infection for the hematological and stem cell transplant patients. Its diagnosis based on the clinical and radiographic findings is not specific enough and prone to lead to empiric or toxic therapies. That is why improved methods of diagnosis like indirect mycological tests, especially the Platelia *Aspergillus* galactomannan antigen assay, are desperately required, because they represent the main tool for IA early diagnosis. So far these tests do not replace the standard IFD diagnosis, but they have the ability to indicate a probable IFD and allow for the initiation of early antifungal therapy, a clue factor in reducing the IA mortality rates.

Taking in account that the life-threatening opportunistic mycosis represents an increasing pathology in Romania, mainly because of the growing number of allogenic transplants, we are entitled to believe that the indirect mycological tests represent an imperative need for our hematologists in order to ensure a good standard of care for their high risk recipients.

Abbreviations

IA = Invasive Aspergillosis
GM = Galactomanan antigen
IFI = Invasive Fungal Infections
HM= Hematological Malignancies
AML= Acute Myeloid Leukemia
allo-HSCT = Allogeneic Hematopoietic Stem Cell Transplantation

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