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Invasive aspergillosis in acute leukemias: old and new risk factors and epidemiological trends

MORENA CAIRA, MARIO MANCINELLI, GIUSEPPE LEONE & LIVIO PAGANO
Department of Hematology, Università Cattolica del Sacro Cuore, Rome, Italy

In recent years, there has been increased interest in the development of prophylactic and diagnostic tools for patients at high risk for invasive aspergillosis (IA), resulting in a significant investment of human, technical, and economic resources. There are several classic risk factors for the development of IA, including neutropenia, graft-versus-host disease, and corticosteroid use. However, despite having similar risk profiles, only a subset of at-risk individuals will develop this fungal complication. At present, there is a significant expansion of the classically defined 'high-risk' group due to the ageing of the general population, the intensification of treatment strategies, and the introduction of new drugs into clinical practice (e.g., monoclonal antibodies, TNF inhibitors). Therefore, an improved categorization of patients would be useful in order to better target available resources and avoid the risk of potential overtreatment and toxicities.

Keywords aspergillosis, acute myeloid leukemia, epidemiology, risk factors

Introduction

Recent advances in medicine have resulted in an expansion of the population of immunocompromised patients and, consequently, the number of patients who develop invasive aspergillosis (IA) has dramatically increased worldwide. Reported incidence rates of IA are extremely variable in different categories of patients, affecting 24–40% of those suffering from chronic granulomatosis disease and acute leukemia, as well as heart and lung transplant recipients [1,2]. An assessment of the absolute number of cases of IA to prevent, diagnose and treat confirms that the impact of IA on the general population is extremely variable, but the majority of our resources are used in hematological departments. This allocation has been confirmed by the I3-*Aspergillus* Study Group: among 395 cases of IA, more than 60% occurred in hematological patients [3].

In two different multicenter surveys conducted in Italy from 1988–1997 and 1999–2003, IA was found to be the most frequent fungal complication in patients treated with conventional chemotherapies, particularly in those suffering from acute myeloid leukemia (AML) [4,5]. A further comparison between AML patients and allogenic hematopoietic stem cell transplant (allo-HSCT) recipients revealed significant differences: proven/probable mold infections were documented in 174 AML patients (incidence 10.9%) and 43 allo-HSCT recipients (6.3%, p-value < 0.001) [6]. Based on these results, AML patients should be considered a particularly high-risk population for IA.

In general, the major factors that have been recognized as influencing the likelihood of invasive fungal infection are a patient's immune status, the degree of any organ damage (e.g., mucositis), and overall microbial exposure (i.e., colonization, environment, and prior infection). Since the 1990s, different risk-stratification strategies have been evaluated in order to identify those patients who may benefit from intensive prophylactic and diagnostic measures [7,8]. However, despite having similar risk profiles, only a subset of at-risk individuals will develop IA.

One of the most exciting recent advances in the understanding of the epidemiology of aspergillosis is the recognition of the complexity of the host and the identification of new host-related risk factors.

Genetic risk factors

It has recently been hypothesized that genetic variation within key innate or adaptive immune response genes could influence the susceptibility to or the outcome of IA

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Correspondence: Morena Caira, Istituto di Ematologia, Università Cattolica del Sacro Cuore, Largo Francesco Vito, 1, I-00168 Roma, Italy. Tel: +39 (0)630154180; fax: +39 (0)63051343; E-mail: morenacaira@yahoo.it

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infection because most of the identified risk factors for this invasive fungal infection affect the immune system of the host.

Toll-like receptors are transmembrane proteins on the surface of immune cells that interact with several adapter proteins to activate transcription factors, resulting in the production of inflammatory cytokines and the activation of the adaptive immunity. In a recent study, single-nucleotide polymorphisms (SNPs) in TLR-4 gene haplotype S4 were found to be associated with an increased susceptibility to IA in recipients of unrelated allografts [9]. The S4 haplotype is characterized by four SNPs within or near the TLR-4 gene, two of which change the aminoacid sequence of TLR-4 to a lipopolysaccharide-hyporesponsive form. The association of the S4 haplotype with IA was confirmed in a validation study that compared 103 case patients with 263 matched controls.

Comparable results have been reported for polymorphisms in genes encoding TLR-1, TLR-6, IL-10, and the TNF-alpha receptor [10–12]. IL-10 acts as a regulator of inflammation by controlling the balance between inflammatory and humoral responses [11]. TNF-alpha is a pro-inflammatory cytokine with a central role in controlling invasive fungal infection. Differences in the TNF-alpha receptor type 2 promoter have also been shown to influence a patient's susceptibility to IA [12]. Similarly, Zaas and colleagues showed that SNPs in the gene encoding murine plasminogen were associated with an increased susceptibility to IA in mice, implicating the fibrinolytic system in the pathogenesis of this disease [13].

Exposure

Healthcare-associated outbreaks of IA have provided critical insight into its modes of exposure. In a review of more than 60 outbreaks of aspergillosis in healthcare facilities reported in the literature from the 1970s to the present, the most frequently identified outbreak sources were construction sites and air supply systems [14]. In recent years, however, a 'hospital independent' mode of exposure has been also described as a possible new risk factor for IA. In a recent survey, over 200 patients from various hospital departments were asked to complete a questionnaire about possible pre-hospital exposures to fungal sources; the requested information included details on working and living sites, personal habits, and exposure to different indoor and outdoor sources (e.g., pets, bio-waste containers). Specific subgroup analysis of AML patients revealed that within this group those who were smokers, lived in the countryside, or had two or more exposures to sources of fungus were at highest risk [15]. The impact of exposure on susceptibility could also explain the common observation that AML patients typically experience IA after the first cycle of chemotherapy, which is likely the first time colonized patients are severely immunocompromised [4,16].

Age and comorbidities

Age is a key risk factor for IA. The difference between juvenile and adult patients is well established and is likely due to both the juvenile patients' increased tolerance for intensive chemotherapy and their lower levels of exposure to sources of fungus. As a consequence, incidence rates in pediatric reports are lower than those in adult populations, even in cases of identical hematological malignancies [17]. Age continues to be a key risk factor through adulthood, with older adults at higher risk compared to their younger counterparts. The role of age as a risk factor is especially important when one considers that more than half of new AML diagnoses are made in patients older than 60 years and that the proportion of patients over 65 years old is expected to increase to 20% in 2030 due to the progressive aging of the general population [18,19].

These epidemiological data, as well as improvements in supportive care, have translated to a more frequent inclusion of elderly AML patients into intensive antineoplastic treatment programs and stem cell transplant regimens. To date, no standardized criteria for this selection process have been established. However many changes have been made since the 1980s, when advanced age was considered enough to exclude a patient from curative treatments. Several more complex scoring systems have been proposed in order to better characterize older AML patients and, in particular, to distinguish 'frail' from 'fit' patients. The hematopoietic cell transplantation comorbidity index (HCTCI) was developed in a large cohort of patients with hematopoietic malignancies and was found to predict non-relapse mortality and overall survival after stem cell transplantation [20]. The use of the HCTCI was then proposed for predicting the early death and survival of AML patients over 60 years of age [21]. By assigning weights to some scored conditions and adding a number of comorbidities of specific relevance to patients with AML (including infection, bleeding, and obesity), it was found that elderly patients can be better stratified and potentially considered fit for intensive chemotherapies despite their age.

Since the 1960s, much progress has been made in the treatment of AML, and we have moved towards a progressive intensification of our strategies, even in older patients. This is largely the result of improvements in both supportive care and our ability to control side effects. A recent study by the Swedish Adult Acute Leukemia Registry reported that, among 2,767 patients with acute leukemia diagnosed between 1997 and 2005, the proportion of patients over 55 years old considered fit for intensive

chemotherapy was 60% [22]. This finding is particularly interesting when compared to previous investigations; for example, one study conducted by Tailor *et al.* between 1988 and 1991 reported that only 42% of evaluated patients over 55 years old were considered fit for intensive chemotherapy [23].

The existence of comorbidities is specifically related to the aging of the general population and to the increasing number of older adults newly diagnosed with AML. The more significant comorbidities in elderly patients are expected to be associated with a higher risk of IA. For example, Weiser and colleagues demonstrated that in 274 patients with acute lymphocytic leukemia, hyperglycemia may be an independent predictor of treatment-related infectious complications in hospitalized patients without diabetes [24]. It had already been demonstrated that a threshold glucose concentration of 250 mg/dl results in impaired phagocytic function and bactericidal activity of polymorphonuclear leukocytes from non-diabetic patients [25].

Iron overload

Patients with acute leukemia or myelodisplastic syndrome and those undergoing allogenic HSCT receive frequent blood transfusions that can cause iron overload. This complication may increase the risk of infection – especially given the compromised state of these patients' defensive networks. Laboratory studies have demonstrated that iron is essential for *Aspergillus* growth and virulence. High levels of free iron may also worsen mucosal damage and impair cellular antimicrobial systems [26].

Kontoyiannis and colleagues compared 33 cases of IA in high-risk patients (acute leukemia and HSCT) with controls; bone marrow iron stores (BMIS) were used to assess potential iron overload. Uni- and multi-variate analyses revealed a significantly higher proportion of patients with high BMIS in those experiencing IA [27]. Earlier studies found similar results [28,29].

Neutrophil impairment

Neutropenia is usually held responsible for an increase in susceptibility to IA. Nearly all AML patients who receive intensive treatment experience a deep and long-lasting neutropenia. The prognostic role of the degree and duration of neutropenia has been recognized since the 1960s [30]. In a recent Italian study focusing on 140 patients with AML, a severe neutropenia at the onset of IA was reported in almost all cases (123/140, 95%). Furthermore, it lasted longer than 10 days in 69% of patients [16]. The scenario in AML patients is more complex, however, given that these patients exhibit not only a reduction in neutrophil count (due to both chemotherapy and bone marrow infiltration), but also reduced neutrophil activity. This reduced activity is potentially the result of coexisting myelodisplasia. Recent studies have demonstrated that dysplastic neutrophils exhibit a lower fungicidal activity against yeasts in comparison to normal control neutrophils. Similar studies with molds are ongoing [31].

Conclusions

Efforts to optimize the outcome of patients at high-risk for IA have included anti-mold prophylaxis and intensive diagnostic work-up, as well as empiric and pre-emptive treatment strategies [32]. Because the number of high-risk patients is constantly expanding, the last few years have seen a steadily increasing investment of human and economical resources into improving treatments. The use of a more complex risk stratification-grouping system may lead to a more targeted diagnostic and therapeutic strategy, avoiding the risk of potential overtreatment and toxicities as well as the incurrence of unnecessary expenses.

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