Early diagnosis of invasive pulmonary aspergillosis in hematologic patients: an opportunity to improve the outcome

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Invasive pulmonary aspergillosis (IPA) is the leading invasive fungal disease in high-risk hematologic patients, including those with acute myeloid leukemia (AML) receiving chemotherapy for induction of remission, and allogeneic hematopoietic cell transplant (HCT) recipients.[1,2] Studies conducted in the 1990s and early 2000s reported crude mortality rates of 60-80%.[3] However, recent data suggest that the mortality rate of IPA has decreased.[4,5] Although the exact reasons for the improvement in the outcome are not clear, it is possible that this is partly due to the fact that IPA has been diagnosed at an earlier disease stage, after the introduction of routine chest computed tomography (CT) scans, and the use of serum biomarkers, such as galactomannan (GMI).

The concept of early diagnosis and improved outcomes is very familiar to hematologists since the outcome of hematologic malignancies is usually influenced by the tumor burden when treatment is started. For example, high white blood cell count at diagnosis of acute leukemia is associated with poor outcome,[6] disease stage strongly influences the outcome in Hodgkin's lymphoma,[7] and high tumor burden is associated with poor prognosis in multiple myeloma.[8] Therefore, it is tempting to speculate that fungal burden may influence the outcome of IPA, with poorer responses to treatment as long as the fungal burden increases.

The halo sign is the radiological representation of lung infarction that follows angioinvasion by hyphae. The nodule represents the coagulation necrosis, and the halo is the edema and hemorrhage that surrounds the zone of infarction. Although not specific, its presence in persistently febrile neutropenic patients must be interpreted as suggestive of an invasive mold disease. An important contribution to the management of IPA was made by studies showing the importance of the halo sign as the earliest detectable sign of disease. Caillot *et al*. analyzed the diagnosis of IPA in two periods. In the first, high-resolution chest computed tomography (CT) was obtained at the discretion of clinicians, based on clinical signs of infection, whereas in the second period CT scans were systematically performed, regardless of clinical signs. When CT scans were obtained upon clinical suspicion of IPA, the halo sign was detected in 13% of patients, compared with 92% when CT scans were systematically performed. This resulted in a reduction in the time to diagnosis, from seven to 1.9 days, and a marked improvement in the outcome.[9] More recently, the importance of the halo sign as an early sign of IPA was demonstrated in a study in which base-line chest CT scans of 235 patients who participated in a randomized clinical trial were reviewed. The halo sign was observed in 61% of cases, and patients with the halo sign had significantly better responses to treatment and greater survival than did patients with other images.[10] A similar finding was also observed in a smaller number of patients.[11] In these studies, the most reasonable explanation for the improved outcome was that the halo sign represented an earlier stage of IPA, allowing the initiation of antifungal therapy with a lower fungal burden.

Although the outcome of IPA has improved, the prognosis is still poor, particularly in some groups of patients, such as allogeneic HCT recipients. Therefore, attempts to further improve the outcome are needed. In order to do this, clinicians should be able to recognize IPA before the appearance of the halo sign. A detailed description of the events that occur early in the

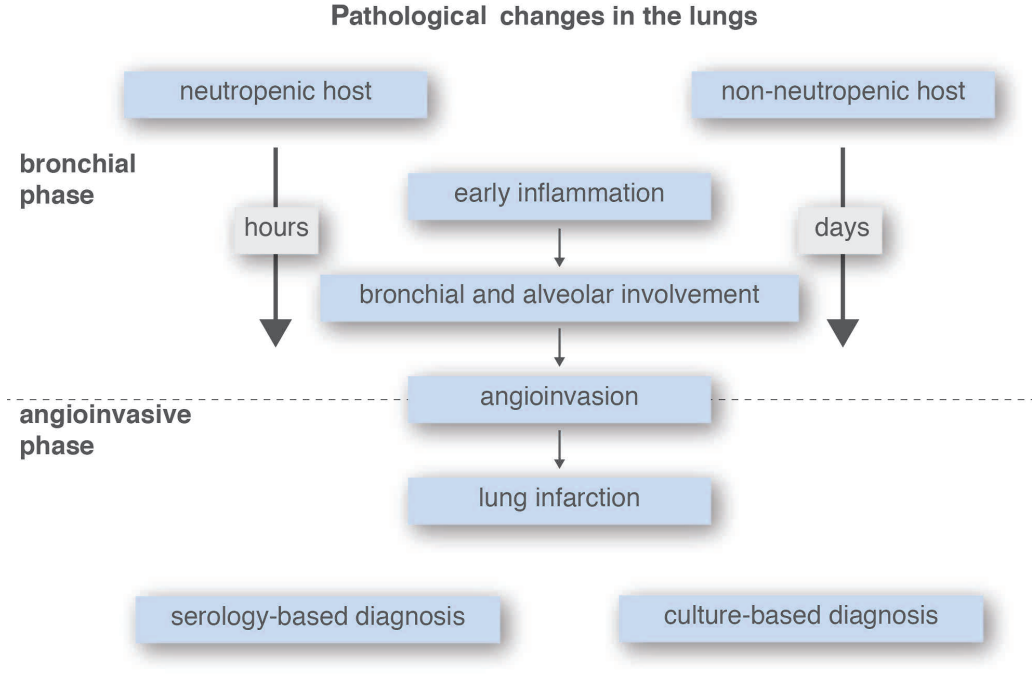

Figure 1. Evolution of invasive pulmonary aspergillosis in the bronchoalveolar and the angioinvasive phases in neutropenic and non-neutropenic patients.

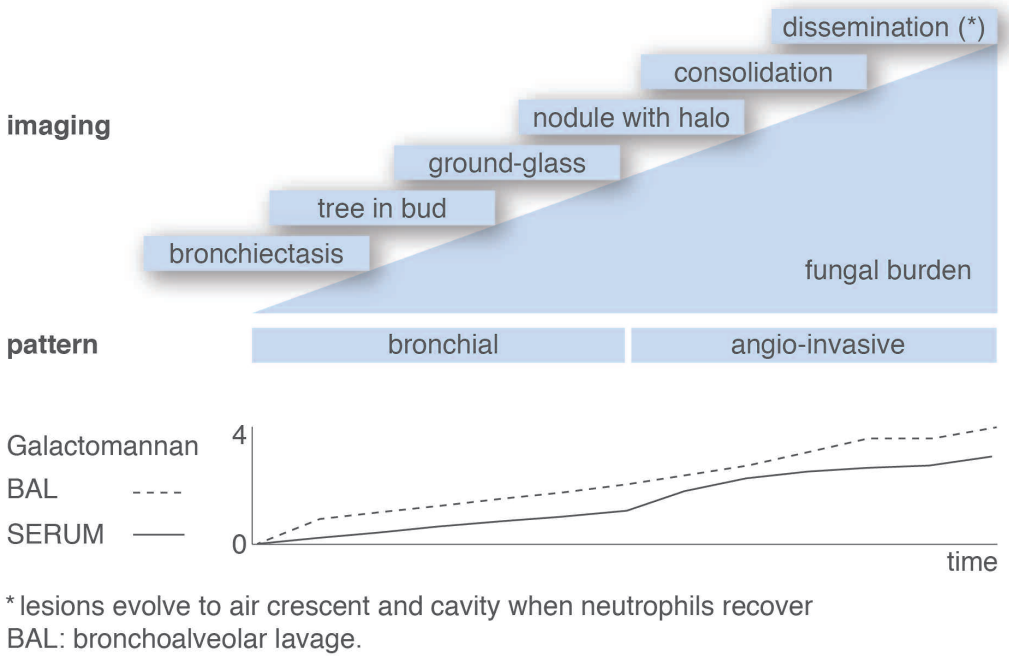

Figure 2. The clinical evolution of invasive pulmonary aspergillosis. *Lesions evolve to air crescent and cavity when neutrophils recover. BAL: bronchoalveolar lavage.

course of IPA was recently provided by an animal model of IPA in persistently neutropenic rabbits, which focused on the first 96 h of IPA.[12] In that study, rabbits were immunosuppressed with cytarabine and methylprednisolone, and challenged with $3x10^8$ conidia of *Aspergillus fumigatus* via endobronchial instillation. The histopathology of the lung was examined at different time points after inoculation of conidia, and showed various alterations in the lung parenchyma before the occurrence of angioinvasion and lung infarction. These changes included the phagocytosis of conidia by alveolar macrophages as early as 4 h after inoculation, and inflammatory infiltration, conidia germination and hyphae formation after 8 h. Lung infarction occurred after 24 h, with the peak infarct score occurring by 30 h after inoculation. Lung infarction was preceded by the detection of galactomannan in the bronchoalveolar lavage, which appeared as early as 8 h after inoculation, and in the serum, which became positive about 12 h after inoculation.

All these inflammatory changes that occur before hyphae invade the blood vessels and cause lung infarction correspond to the "bronchoalveolar" phase of IPA. This phase is not recognized by clinicians because IPA is only diagnosed when the halo sign appears. The bronchoalveolar phase of IPA has been described in non-neutropenic patients, and presents different and usually 'non-specific' radiological findings, including areas of peribronchial consolidations, micro (<1 cm) centrilobular nodular opacities, ground-glass infiltrates, branching linear or nodular opacities with a 'tree-in-bud' appearance, and focal areas of bronchiectasis.[5,13]

The impact of neutrophil counts in determining the predominant form of IPA was described in a recently published paper by Bergeron *et al.* that analyzed the radiological picture of 55 patients with proven or probable IPA.[14] Patients were divided into three groups: allogeneic HCT recipients (23 patients), acute leukemia (22 patients) and others (10 patients, all with other hematologic diseases). An angioinvasive pattern was present in 45% of acute leukemia patients and in 13% of HCT recipients. By contrast, an 'airway-invasive' (or bronchoalveolar) pattern (with centrilobular micronodules and tree-in-bud infiltrates without large nodules with the halo sign) was present in 44% of allogeneic HCT recipients and in 14% of patients with acute leukemia. Significant differences between these two groups were a higher proportion of prior corticosteroid exposure in HCT recipients, and of neutropenia in acute leukemia patients.

The neutrophil count seems to drive the predominant pattern of IPA. The neutrophils are key elements for containing the invasion of conidia into the bloodstream.[15] Therefore, the more severely neutropenic the host is, the faster and more likely is the angioinvasion, making the early bronchoalveolar phase very short. In the persistently neutropenic rabbit animal model,[12] the bronchoalveolar phase lasted approximately 24 h. In patients with various degrees of neutropenia and T-cell mediated immunodeficiency, the bronchoalveolar phase may be of a longer duration, opening a window of opportunity for early diagnosis of IPA.

The speed with which the angioinvasive phase evolves also explains the performance of diagnostic tests in neutropenic and non-neutropenic patients (Figure 1). With regards to this, the performance of the tests may be seen as a function of the duration of the bronchoalveolar phase, with a higher proportion of patients having positive cultures of respiratory secretions in the non-neutropenic setting (because the bronchoalveolar phase is long enough to spill out fungal elements) and a higher proportion of neutropenic patients having positive serum galactomannan as the main mycological criterion for the diagnosis. This was shown in the paper by Bergeron *et al.*[14] who reported that positive cultures from respiratory secretions occurred in 83% of patients with radiological signs of bronchoalveolar IPA and in 17% of patients with the angioinvasive form of IPA. On the other hand, since the angioinvasive phase occurs earlier the more severely neutropenic the patient is, serum galactomannan is more likely to be positive. By contrast, in patients with higher neutrophil counts the angioin-

vasive phase is delayed (or even does not occur at all) and serum galactomannan is less likely to be positive.[16]

We have recently proposed the inclusion of a new category of invasive aspergillosis which we called probable invasive aspergillosis without pre-specified radiological findings.[5] We analyzed 125 episodes of invasive aspergillosis in patients with hematologic malignancies (mostly multiple myeloma). Patients were monitored after the start of antineoplastic therapy with serial (typically 3 times/week) serum galactomannan. Patients in the category of probable aspergillosis without pre-specified radiological findings were similar to those with probable invasive aspergillosis, with the same host, mycological and clinical features. The only exception was in the radiological appearance: none of them presented with the images that qualify for the EORTC/MSG diagnostic criteria of probable aspergillosis (i.e. well circumscribed lesions with or without the halo sign, air crescent or cavity).[17] Instead, radiological findings consisted mostly of ill-defined consolidations and ground-glass infiltrates. Interestingly, 26 of 53 cases originally classified as probable aspergillosis without pre-specified radiological findings had repeated imaging about two weeks after the first CT examination. Eleven of these 26 patients (42%) were reclassified as probable aspergillosis by EORTC/MSG definitions because repeated images showed well-circumscribed consolidations and / or marconodules, suggesting that this category may represent an earlier stage of aspergillosis.

In another paper, Girmenia *et al.* analyzed 109 episodes of IPA (56 with AML, 31 with lymphoproliferative diseases, and 22 allogeneic HCT recipients).[18] Seventy-six episodes were classified as proven or probable IPA, and 33 were probable aspergillosis without pre-specified radiological findings. All clinical and mycological criteria were similar between the two groups, with the exception of the radiological findings. Marconodules with halo sign were more likely to be present in neutropenic than in non-neutropenic patients (32.9% *vs.* 5.4%). Repeat imaging showed an evolution from the early images to the EORTC/MSG-defined images in 42.9% of AML patients, 50% of patients with lymphoproliferative diseases, and in 63.6% of allogeneic HCT recipients, confirming that the non-specific images represent an earlier stage of IPA.

More recently, Kyo *et al.* analyzed 30 cases of probable IPA in patients with acute leukemia.[19] All patients were neutropenic, and had repeated CT scans performed every three days if fever persisted after treatment initiation, or if galactomannan serum was more than 0.4 while on treatment. Each patient had a median of eight CT scans. All patients exhibited a bronchoalveolar pattern of IPA preceding the angioinvasive phase. All patients survived. The authors concluded that the bronchoalveolar pattern seems to be the first CT abnormality of IPA, and that prevention of the angioinvasive phase may have contributed to the recent decrease in mortality of IPA.

How can we make a diagnosis of early IPA? The first step is to apply serial serum serology monitoring with galactomannan (3 times/week), and establish aggressive criteria for ordering a chest CT scan. The second step is to recognize the images that precede the halo sign and to interpret them as early IPA in the context of positive serum galactomannan (Figure 2). In non-neutropenic patients, since serum galactomannan may be negative, a bronchoalveolar lavage should be obtained and the diagnosis of IPA established on the basis of direct exam, culture and / or positive galactomannan.

As stated above, diagnosing IPA at an earlier stage will result in the initiation of appropriate antifungal therapy when the fungal burden is still low. This may potentially further improve the outcome. In addition, by starting treatment earlier and preventing the occurrence of the angioinvasive phase of IPA it is possible that a shorter period of treatment will be needed. For example, in a cohort of 115 hematologic patients with invasive aspergillosis (32% with probable aspergillosis without pre-specified radiological findings), 37% received treatment for 14 days or under, with a 69% 6-week probability of survival in the entire cohort.[20]

In summary, the application of serial serum galactomannan and an aggressive strategy of early chest CT scan in neutropenic patients may allow clinicians to diagnose IPA with a low fungal burden, before the appearance of the halo sign, and thus prevent the occurrence of lung infarction. The potential benefits and costs of such an approach should be evaluated by prospective studies.

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Financial and other disclosures provided by the author using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are available with the full text of this paper at www.haematologica.org.

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A niche for every cell, for every function

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Acute or chronic blood loss reduces the numbers of red blood cells (RBCs) in the circulation, reducing oxygen delivery and producing tissue hypoxia. One of the first cellular responses to hypoxia is increased production of reactive oxygen species (ROS) by mitochondria. With evolution, organisms have developed an efficient and rapid way to respond to erythroid challenges by using ROS as the 'ignition key' that activates the engine, defined stress erythropoiesis, which induces hematopoietic/stem progenitor cells to produce RBCs to compensate losses.[1,2]

Much has been discovered in recent years on the mechanism(s) that regulate stress erythropoiesis. ROS stimulates kidney cells to produce greater levels of erythropoietin (Epo), the hormone that specifically stimulates RBC production.[2] In addition, ROS stimulates hematopoietic niches in the marrow to produce factors that induce hematopoietic stem cells to generate 'stress-specific erythroid progenitors' with enhanced ability to produce RBCs.[3] In mice, 'stress-specific erythroid progenitors' egress from the marrow into the blood to home in 'stress-specific' niches in the spleen where they continue their maturation.[4,5] The development of mechanisms that use 'ROS' as the sensor to increase RBC production in response to erythroid challenges is an evolutionary winner that has an important drawback. As described by Ulyanova *et al.* in this issue,[6] maturation of erythroid cells is uniquely associated with accumulation of hemoglobin, the oxygen carrier molecule with the intrinsic property of increasing ROS production. Since above a 'critical' threshold, ROS activates the p53-dependent pathway of cell death, during their maturation, erythroid cells must maintain ROS constantly low by increasing production of enzymes, such as catalase, that are able to degrade ROS. The expression of these enzymes is controlled by the transcription factor forkhead-box protein O3 (FoxO3) activated by AKT[7] (Figure 1). Therefore, in contrast to stem/progenitor cells, hypoxia-induced increases of ROS are deleterious to erythroblasts. Hence the need for these cells to have a safety circuitry up-regulating expression of enzymes that catabolize ROS under conditions of hypoxia. Without this circuitry, stress-specific erythroblasts would die in great numbers, eliminating the expected increases in RBC production mediated by ROS-induced proliferation of hematopoietic stem/progenitor cells.

Studies have been performed to clarify how increases in ROS production as a result of hypoxia induce hematopoietic stem cells to generate stress-specific progenitor cells.[1-3] ROS induce the formation of 'stress-specific' niches that through specific adhesion receptors attract hematopoietic stem cells exposing them to growth factors that, in synergy with Epo, induce them to generate stress-specific progenitor cells. In contrast, little is known about how the control of ROS production becomes more stringent in erythroblasts maturing under conditions of hypoxia.

Integrins are heterodimers composed by an alpha(α) and a beta(β) subunit that interact with counter receptors present on niche cells and/or with fibronectin, a component of