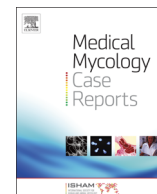




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## Why is biopsy of suspected fungal lung lesions necessary? <sup>☆</sup>

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### ARTICLE INFO

#### Article history:

Received 24 July 2013

Received in revised form

29 July 2013

Accepted 21 August 2013

#### Keywords:

Childhood

Invasive pulmonary infections

CT-guided biopsy

*Aspergillus flavus*

Amphotericin-B

### ABSTRACT

The recognition of antifungal resistance is necessary for the choice of the appropriate treatment in patients with invasive fungal disease. In this case report, the need for a computed tomography-guided percutaneous lung biopsy of a suspected fungal lesion in a patient treated for acute leukemia is demonstrated. Detection of Amphotericin-B resistant *Aspergillus flavus* infection has prompted the switch in antifungal therapy, followed by full resolution of symptoms, completion of chemotherapy and remission since then.

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## 1. Introduction

Several studies have been reported concerning the incidence of rare invasive fungal infections (IFIs) and the emergence of antifungal resistance in patients treated for cancer [1,2]. At the same time, numerous evidence-based recommendations have been proposed to simplify the diagnosis of these infections [3]. Unfortunately, there is little or no information on culture-based methods, as they remain largely underused and the impact of the tested antifungal agents on the outcome of the infections is still to be determined. In this way, a case report is presented, in which biopsy of a suspected fungal lesion has been performed and antifungal susceptibility testing has prompted the change in antifungal drugs.

## 2. Case

A 16-year old male adolescent was diagnosed with Philadelphia chromosome-positive acute lymphoblastic leukemia and subjected to treatment with the ESPHALL protocol for high-risk patients [4]. He achieved complete morphological remission in the peripheral blood on day 10 of treatment. On the day 27th of induction chemotherapy the patient developed his first febrile

episode. Physical examination was unremarkable. The laboratory tests showed leukopenia ( $0.5 \times 10^9/L$ ) with an absolute neutrophilic count of  $19/\mu L$ , but no elevation of inflammatory proteins (C-reactive protein  $< 0.06$  mg/dL) Fig. 1.

Invasive fungal infection was suspected by chest X-ray in a ALL patient on day 30 of induction chemotherapy and after 72 h of antibiotic resistant fever in neutropenia. CT scan of the chest on day 31 revealed a suspicious fungal mass associated with halo sign. Prophylactic treatment with Fluconazole was switched to empiric therapy with liposomal Amphotericin-B. 24 h later, CT-guided percutaneous lung biopsy was performed and direct examination confirmed the presence of *Aspergillus species*. Culture revealed growth of *Aspergillus flavus* highly resistant to Amphotericin-B. Treatment was immediately changed to Voriconazole.

Empirical antibiotic regimen was initiated with Meropenem (1 g three times daily) and Gentamycin (200 mg once daily). Because of continuous fever, Vancomycin (1 g twice daily) was added the day after. Both blood and urine cultures remained sterile. 72 h after developing fever, a chest X-ray was obtained and showed a distinctive mass in the middle of the right lung. Because the radiological image was ambiguous the diagnostics were extended by a chest computed tomography (CT) scan, which confirmed the large mass in the right upper lobe, surrounded by a wide zone of ground-glass attenuation, compatible with the halo sign.

Fluconazole (200 mg once daily), included in the treatment as a prophylactic measure at this time, was replaced by liposomal Amphotericin-B and administered at a dose of 3 mg/kg once daily. On the next day CT-guided biopsy was performed. No pneumothorax or hemorrhage was noted after the procedure. Immediate direct examination yielded dichotomously branching septated

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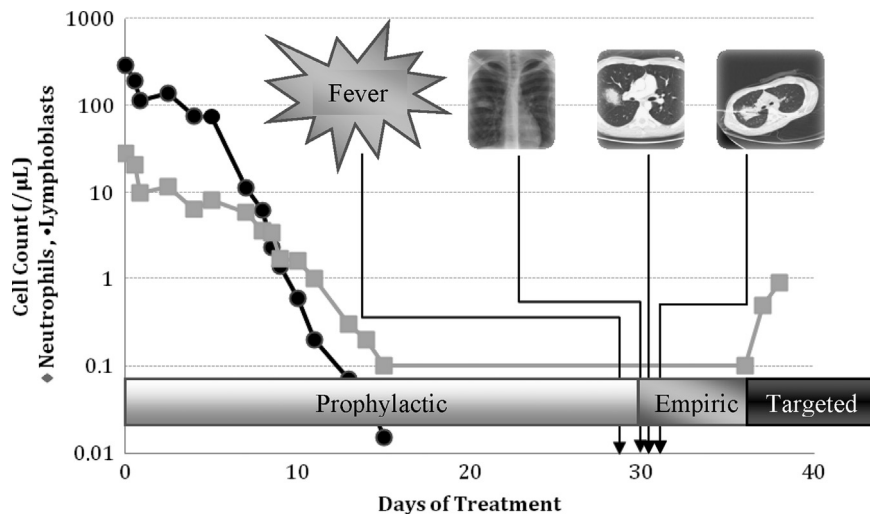


Fig. 1. Diagnosis and treatment of *Aspergillus flavus* infection in a patient treated for leukemia.

hyphae consistent with *Aspergillus species*. Culture was obtained 7 days after biopsy and demonstrated the growth of *Aspergillus flavus* by repetitive-sequence-based polymerase chain reaction (PCR). According to high minimum inhibitory concentrations for Amphotericin-B, antifungal therapy was switched to Voriconazole (6 mg/kg twice a day) for 8 weeks intravenously and then orally to the same dose until the 12th week. CT imaging studies confirmed a gradual recession of the lesion. Two months later, the patient underwent right-sided thoracotomy with wedge resection of the fungal mass. Post-operative course was uneventful and no recurrence of fungal infection was noted. He underwent allogeneic hematopoietic stem cell transplantation under prophylactic antifungal treatment with Voriconazole (6 mg/kg twice a day) and has been in complete molecular remission since then.

### 3. Discussion

The widespread use of aggressive chemotherapies, including myeloablation for hematopoietic stem cell transplantation together with the use of targeted compounds, causes prolonged immunosuppression and puts the patients at higher risk for developing invasive mycoses [5]. The morbidity of these infections is high and the prognosis is poor [6], unless they are diagnosed early and treated promptly. Strategies to optimize management of fungal infections include prophylactic therapy and early empiric treatment of antibiotic resistant fever during periods of prolonged neutropenia [3,7]. Alternatively, antifungal drugs can be initiated preemptively, based upon the results of serial screening for galactomannan and/or fungal deoxyribonucleic acid in human serum [3,7]. However this approach might be limited by low sensitivity and can be restricted by reduced specificity. Theories to explain false-positive results include cross-reactivity with transfused blood products [8], milk-based formula [9], *Penicillium species* [10], recent use of chemotherapy [11] and the concurrent use of Piperacillin/Tazobactam [12]. Randomized trials are currently being performed, but so far, no overall clinical or survival benefit to a preemptive therapy, that involved serial PCR [13] or the serum galactomannan assay in combination with other clinical indicators [14] in hematologic patients, has been demonstrated. Thus, safety and efficacy of preemptive strategies remain elusive, and this approach has to be further elaborated.

Although non-culture methods for the diagnosis of IFIs have largely been promoted and there is no clear winner among the different methods, we suggest that biopsy of suspected fungal

lesion should be stressed as the gold standard for the accurate diagnosis of IFIs. This is particularly true for the fact, that rare and atypical fungal pathogens, such as *Mucorales*, *Aspergillus terreus* and *Fusarium spp.* have continuously been noted in immunocompromised patients [15]. Failure to respond to empiric or preemptive therapy may suggest the presence of unusual fungal pathogens for which a definitive diagnosis should be pursued aggressively, including a CT-guided biopsy, if available. Depending on antifungal susceptibility testing, the antimycotic regimen can then be readjusted. This is critical for optimal response to therapy, e.g. for patients with large necrotic lesions, in which surgical debridement, such as thoracotomy followed by resection of the fungal mass, has to be performed. For patients who are effectively been treated for fungal infection, they still might be at risk for relapse during periods of increased immunosuppression. This is critical for patients who undergo intensified chemotherapy and allogeneic stem cell transplantation. In these cases secondary prophylaxis is recommended and might be adjusted depending on the susceptibility testing.

In summary, we demonstrate a case, in which biopsy of a large fungal lesion is required for accurate diagnosis, safety of this procedure is guaranteed and culture of Amphotericin-B resistant *Aspergillus flavus* infection has been noted. Antifungal therapy has been adjusted to treatment with Voriconazole and infection has completely resolved. Chemotherapy was continued timely, including myeloablation followed by allogeneic stem cell transplant. The patient is alive, doing well without any sequelae. In conclusion, our experience should encourage clinicians to obtain *in vitro* antifungal testing, which is critical for the choice of appropriate therapy in patients with suspected fungal infections.

### Conflict of interest statement

There are none.

### Acknowledgments

This work was supported by the Children's Aid Society Tirol and Vorarlberg.

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