Le Infezioni in Medicina, Suppl. 2, 43-47, 2012

Successful management of pulmonary mucormycosis with liposomal amphotericin B and surgery treatment: a case report

Mucormicosi polmonare trattata con successo combinando Amfotericina B liposomiale e "curettage" chirurgico

Bianca Serio[1], Rosa Rosamilio[1], Valentina Giudice[1], Pio Zeppa[2], Silvano Esposito[3], Raffaele Fontana[1], Silvana Annunziata[1], Ida Lucia Ferrara[1], Carmine Selleri[1]

[1]Hematology;
[2]Pathology;
[3]Infectious Disease Branch, Department of Medicine, University of Salerno, Italy

INTRODUCTION

Invasive fungal infections (IFI) are frequent life-threatening complications in patients with hematologic malignancies [1-6]. The incidence of IFI has increased in recent years as a result of severe and long-lasting immunosuppression and neutropenia which such patients undergo, due to the use of high-dose chemotherapy, allogeneic (allo) and autologous hematopoietic stem cell transplantation (HSCT), ablative radiation therapy as well as the use of new and more potent immunosuppressant drugs [8-14].

Candida and *Aspergillus spp*. are the most common agents responsible for IFIs among patients with acute myeloid and lymphoblastic leukemia (AML, ALL) as well as in HSCT recipients [1-8, 14].

Other opportunistic fungal pathogens, such as *Fusarium spp.* and *Zygomycetes*, have also recently emerged, whereas the other fungal infections remain still rare [1-6, 7, 15].

Mucormycosis is a rare but still fatal IFI caused by various members of the filamentous fungi class *Zygomycetes*, especially *Mucoraceae*. The most commonly seen genera in mucormycosis cases are *Absidia*, *Rhizopus* and *Mucor* [16-18]. The presence of neutropenia, with an absolute neutrophil count of less than 1000/µl for 1 week, has been reported as the major risk for pulmonary mucormycosis which represents, together with rhinocerebral and sinus maxillofacial location, the most frequent clinical form observed in AML and HSCT recipients [1-6, 16-19]. In the pulmonary or sinus form, exposure occurs by inhaling aerosolized mucor spores from the environment.

Gastrointestinal mucormycosis, resulting from ingestion of spore-contaminated food, and cutaneous mucormycosis, due to direct skin implantation of fungal spores, only occasionally have been reported in acute leukemia and transplanted patients [16-19]. Mucor hyphae have a characteristic tendency to invade blood vessels resulting in vascular thrombosis of small to medium arteries, intraparenchymal bleeding and tissue necrosis. Generally, pulmonary mucormycosis is rapidly progressive, usually spreading to contiguous structures and occasionally complicated by haematogenous dissemination leading to multiorgan involvement with a devastating clinical course [14-19].

Here, we report a case of 32-year-old AML patient who developed, early after the onset of neutropenia in the first induction phase of chemotherapy, a rapidly progressive pulmonary mucormycosis, successfully treated with liposomal Amphotericin-B (LAmB) combined with surgical intervention, before undergoing consolidation therapy and allo HSCT.

CASE REPORT

A 32-year-old man with intermediate risk AML (M4), developed non productive cough, fever (>39°C) and chest pain during neutropenia (neutrophils <500/ml for 10 days), 19 days after the initiation of first remission induction chemotherapy with cytarabine, etoposide and high-dose cytarabine, according to the treatment trial of EORTC/GIMEMA AML12.
At this time, the patient was receiving standard antibacterial and antifungal prophylaxis with fluoroquinolone and itraconazole and routine laboratory investigations showed an elevated C-reactive protein (CRP), while the chest radiograph documented a slight right sided pleural effusion.
Five days later, since the fever was not responding to five days broad spectrum antibacterial therapy, the patient performed high resolution computerized tomography (HRCT) revealing in the lower lobe of the right lung a 3.5 cm peripheral pleural-based nodule with surrounding ground-glass halo associated with a slight pleural effusion, suggesting invasive mold infection.
We did not detected neither an HRCT finding highly suggestive for mucormycosis, such as the reversed halo (circular focus of ground-glass density within a ring of dense consolidation) or the air crescent sign, more common in invasive aspergillosis (IA).
During febrile neutropenia, Aspergillus galactomannan (GM) antigen, tested in serum specimens twice a week (Platelia®, Aspergillus, Bio-Rad), gave negative results [20, 21]. Empirical treatment with liposomal amphotericin B at dose of 3 mg/kg/day for 21 days and granulocyte-colony stimulating factor (G-CSF) was started; three days later, fever disappeared concomitantly with the complete hematological recovery, further confirmed by marrow examination showing complete disease remission (CR).
Pulmonary HCRT scans sequentially performed 30, 37, and 45 days after starting induction chemotherapy showed at beginning an increase of nodular lesion (4,5 cm) and then a progressive infiltrate regression with CRP values normalization.
At this time, a CT-guided fine needle biopsy of the pulmonary lesion and bronchoalveolar lavage (BAL) specimens, tested for Aspergillus GM antigen, failed to document any fungal infection [22-25]. As HRCT scan 45 days after 1st induction did not shown fully regression of lung nodule (2 cm), 7 days later, the patient underwent a limited toracothomy, leading to a complete surgical removal of the lung infiltrate.
Fungal culture of lung specimens remained negative, but histological examination revealed non-septate fungal hyphae with branches occurring at right angles suggestive for mucormycosis.
Three weeks after surgery, being pulmonary HRCT scans completely negative for fungal infection, the patient performed consolidation chemotherapy with daunorubicin and standard-dose cytarabine.
Ninety-eight days after 1st induction therapy, in CR for AML disease, the patient received a myeloablative (BUCY2 regimen) allo HSCT from a sibling HLA-matched related donor, using graft-versus-host disease (GVHD) prophylaxis with cyclosporine A and short course methotrexate.
During both consolidation chemotherapy and allo HSCT, secondary prophylaxis with LAmB was applied for additional 21 days, until hemopoietic recovery.
The patient well tolerated the long course of LAmB. During the antifungal therapy the creatinine values remained within the normal range with a transient increase of bilirubin and alkaline phosphatase (two times over the upper normal limit), and their normalization when LAmB treatment was stopped. Although the patient experienced, after HSCT, a grade II acute GVHD, successfully treated with immunosuppressive therapy, any IFI recurrence was not seen.

DISCUSSION

Patients with hematologic malignancies are at high risk of IFI, more often caused by molds than by yeasts, and the incidence of IFI is highest among patients with AML and after allo HSCT, due to deep and prolonged neutropenia and to the breakdown of immune defenses which they undergo during high-dose chemotherapy and immunosuppressive treatment [1-14].
Clinical, radiological and microbiological criteria for an IFI diagnosis have been categorized by IFIs Cooperative Group of European Clinical, Organization for Research and Treatment of Cancer (EORTC) and the Mycoses Study Group (MSG) of the National Institute of Allergy and Infectious Diseases (NIAD) [26, 27]. Ac-

cording to the consensus criteria proposed by the EORTC/MSD, IFI is defined as "possible" in the presence of clinical criteria for an IFI, "probable" when radiological findings suggestive for an IFI are also associated, and "proven" with the addition of positive microbiological sample obtained from the site of disease, plus positive histopathologic examination of infected tissues [1-6, 22].

Here we report a case of 32-year-old AML who developed ten days after the onset of neutropenia during 1st induction therapy, clinical and radiological findings of a possible IFI, such as a non productive cough, broad spectrum antibiotic resistant fever, prolonged neutropenia associated to a rapidly progressive peripheral pleural-based nodule with surrounding ground-glass halo and a slight pleural effusion. Approximately half of nodular infiltrates in AML therapy and HSCT recipients are the result of pulmonary invasive aspergillosis (IA), with the halo signs most likely detected early in infection and air-crescent signs much later, typically found at time of neutrophil recovery. In our AML patient, the biweekly serum GM monitoring together with BAL GM testing, which have been reported to be highly sensitive and specific for IA, gave negative results as well as fungal blood cultures.

Although IA is the most likely cause of halo lesions in AML and HSCT recipients, it is important to note that the halo sign is not be specific for IA: other pathogens, such as *Pseudomonas aeruginosa* and other less common molds, can also give rise to halo infiltrates such as the agents of mucormycosis [1-6, 14-19, 22, 26]. We did not detected neither an HRCT finding highly suggestive for mucormycosis, such as the reversed halo (circular focus of ground-glass density within a ring of dense consolidation) or the air crescent sign, more common in IA.

In hematological malignancies, the most frequently involved site in mucormycosis is the lung. Neutropenic patients are at a high risk of developing a disseminated mucormycosis. Since rapidly progressive dissemination occurs in up to 40% of mucormycosis in patients with hematologic malignancies, and the mortality exceeds 50%, early diagnosis and initiation of effective antifungal therapy is mandatory for a successful outcome [16, 19, 26].

Many new antifungal drugs such as voriconazole and caspofungin, which are useful in treatment of IA, have no role in treatment of mucormycosis [1-6]. Clinical experience with posaconazole, documented effective in *in vitro* and *in vivo* studies against mucor, is limited in pulmonary mucormycosis; it is still used as a second line therapy [28].

The only effective antifungal agent for treatment of mucormycosis is amphotericin B [3-6, 16-18, 29].

In our patient, LamB therapy was applied at standard dose of 3 mg/kg/day for 21 days during 1st induction therapy.

As another important factor for successful treatment of mucormycosis is the resolution of predisposing neutropenia, LamB was combined with G-CSF, resulting in rapid hematologic recovery and progressive regression of lung infiltrate at HRCT scans.

Since it was still needed to establish species definition of patient's IFI and full regression of pulmonary infiltrate was not achieved after LAmB, the patient underwent limited open toracothomy biopsy, completely removing the lung nodule.

LamB was then successfully used as a secondary prophylaxis treatment for IFI, during both consolidation chemotherapy and myeloablative allo HSCT, for additional 21 days [30-31]. Although the patient experienced, after HSCT, a grade II acute GVHD and a limited chronic GVHD, successfully treated with immunosuppressive therapy [32], any IFI recurrence was not seen.

Our case report further provide evidence that the combined surgical and antimicrobial therapy with liposomal amphotericin B is an effective and safe choice in haematological immunocompromised patients both for clearly establishing IFI diagnosis and for the management of a potentially letal mould infection such as pulmonary mucormycosis.

Keywords: Mucormycosis, allogeneic stem cell transplant, fungal prophylaxis.

Conflict of interest disclosure
The authors declare that the article has not been sponsored, that no financial support has been given and finally that there is no conflict of interest.

SUMMARY

Mucormycosis is an increasingly recognized invasive fungal infection (IFI) in patients with acute myeloid leukemia (AML) and after allogeneic (allo) stem cell transplantation (HSCT); it is mainly due to the severe and prolonged neutropenia related to high-dose chemotherapy. In such patients, the lung is the most frequently involved site in mucormycosis.
Since rapidly progressive dissemination may occur after pulmonary mucormycosis in hematologic malignancies, early diagnosis and prompt initiation of an effective antifungal therapy is mandatory for a successful outcome.
We report the case of a young AML patient who developed, early after the onset of neutropenia in the first induction phase of chemotherapy, a rapidly progressive pulmonary IFI, successfully treated with liposomal Amphotericin-B (LAmB) and then with a limited open toracothomy biopsy, clearly establishing diagnosis of mucormycosis and removing lung infiltrate. Secondary prophylaxis with LamB, applied during both consolidation therapy and myeloablative sibling allogeneic HSCT, was effective to prevent IFI recurrence despite the development of grade I acute graft-versus-host disease (GVHD) and limited chronic GVHD requiring immunosuppressive treatment.
Our case report further provide evidence that the combined surgical and LAmB therapy is an effective and safe choice for the management of pulmonary mucormycosis in hematological immunocompromised patients.

SOMMARIO

La mucormicosi è una emergente infezione fungina invasiva (IFI) nei pazienti con Leucemia Mieloide Acuta (LMA) e dopo trapianto di cellule staminali emopoietiche (TCSE). La severa e prolungata neutropenia secondaria a chemioterapia ad alte dosi è il più importante fattore di rischio di una IFI.
In tali pazienti, la più frequente sede coinvolta è quella polmonare. Noi riportiamo il caso di un giovane di 32 anni con una LMA che sviluppò, precocemente dopo l'inizio di una neutropenia durante il primo ciclo di induzione, una mucormicosi polmonare, trattata con successo con Amfotericina B liposomiale (LAmB) e poi con una toracotomia limitata in grado di rimuovere completamente l'infiltrato polmonare.
La profilassi secondaria con LAmB, instaurata sia nel corso del primo ciclo di consolidamento che durante il TCSE allogenico, è stata efficace nel prevenire qualsiasi altra IFI, nonostante lo sviluppo di malattia da trapianto (GVHD) contro l'ospite acuta di grado II e una limitata GVHD cronica, responsiva al trattamento immunosoppressivo. Il nostro caso documenta che la terapia chirurgica combinata alla terapia con LAmB è una sicura e efficace scelta terapeutica nella mucormicosi polmonare.

REFERENCES

[1] Martino R., Subirà M. Invasive fungal infections in hematology: new trends. *Ann. Hematol*., 81, 233-243, 2002.
[2] Parody R., Martino R., Sánchez F., Subirá M., Hidalgo A., Sierra J. Predicting survival in adults with invasive aspergillosis during therapy for hematological malignancies or after hematopoietic stem cell transplantation: single-center analysis and validation of the Seattle, French, and Strasbourg prognostic indexes. *Am. J. Hematol.* 84, 571-578, 2009.
[3] Pagano L., Caira M., Candoni A., et al. The epidemiology of fungal infections in patients with hematologic malignancies: the SEIFEM-2004 study. *Haematologica* 91, 1068-1075, 2006.
[4] Caira M., Mancinelli M., Leone G., Pagano L. Invasive aspergillosis in acute leukemias: old and new risk factors and epidemiologicl trends. *Med. Mycol.* 49, Suppl.1, 13-16, 2011.
[5] Caira M., Girmenia C., Fadda R.M., et al. Invasive fungal infections in patients with acute myeloid leukemia and in those submitted to allogeneic hemopoietic stem cell transplant: who is at highest risk? *Eur. J. Haematol.* 81, 242-243, 2008.
[6] Maertens J., Marchetti O., Herbrecht R., et al. European guidelines for antifungal management in leukemia and hematopoietic stem cell transplant recipients: summary of the ECIL 3-2009 update. *Bone Marrow Transplant*. 46, 709-718, 2011.
[6] Avvisati G., Petti M.C., Lo-Coco F., et al. Induction therapy with idarubicin alone significantly influences event free survival duration in patients with newly diagnosed hypergranular acute promyelocytic leukemia: final result of the GIMEMA randomized study LAP 0389 with 7 years of minimal follow-up. *Blood* 100, 3141-3146, 2002.
[7] Bassetti M., Taramasso L., Nicco E., et al. Epidemiology, species distribution, antifungal susceptibility and outcome of nosocomial candidemia in a tertiary care hospital in Italy. *PLoS One.* 6, e24198, 2011.

[8] Esposito S., Esposito I., Leone S. Considerations of antibiotic therapy duration in community- and hospital-acquired bacterial infections. *J. Antimicrob. Chemother*. Jul 24, 2012 [Epub ahead of print].
[9] Crocchiolo R., Zino E., Vago L., et al. Nonpermissive HLA-DPB1 disparity is a significant independent risk factor for mortality after unrelated hematopoietic stem cell transplantation. *Blood* 114, 1437-1444, 2009.
[10] Rigacci L., Puccini B., Dodero A., et al. Allogeneic hematopoietic stem cell transplantation in patients with diffuse large B cell lymphoma relapsed after autologous stem cell transplantation: a GITMO study. *Ann. Hematol.* 91, 931-939, 2012.
[11] Patriarca F., Bacigalupo A., Sperotto A., et al. Allogeneic hematopoietic stem cell transplantation in myelofibrosis: the 20-year experience of the Gruppo Italiano Trapianto di Midollo Osseo (GITMO). *Haematologica* 93, 1514-1522, 2008.
[12] Specchia G., Lo-Coco F., Vignetti M., et al. Extramedullary involvement at relapse in acute promyelocytic leukemia patients treated or not with all-trans retinoic acid: a report by the Gruppo Italiano Malattie Ematologiche dell'Adulto. *J. Clin. Oncol.*19, 4023-4028, 2001.
[13] Selleri C., Serio B., Risitano A.M. Novel immunosuppressive strategies for bone marrow failure syndromes: a focus on alemtuzumab. *Mini Rev. Med. Chem.* 11, 536-543, 2011.
[12] Risitano A.M., Selleri C., Serio B., et al. Alemtuzumab is safe and effective as immunosuppressive treatment for aplastic anaemia and single-lineage marrow failure: a pilot study and a survey from the EBMT WPSAA. *Br. J. Haematol*. 148, 791-796, 2010.
[13] Risitano A.M., Serio B., Selleri C., Rotoli B. Valganciclovir as CMV reactivation prophylaxis in patients receiving alemtuzumab for marrow failure syndromes. *Ann. Hematol.* 88, 1261-1262, 2009.
[14] Pagano L., Caira M., Nosari A., et al. Fungal infections in recipients of hematopoietic stem cell transplants: results of the SEIFEM B-2004 study - Sorveglianza Epidemiologica Infezioni Fungine Nelle Emopatie Maligne. *Clin. Infect. Dis.* 45, 1161-1170, 2007.
[15] Girmenia C, Pagano L, Martino B, et al. Invasive infections caused by Trichosporon species and Geotrichum capitatum in patients with hematological malignancies: a retrospective multicenter study from Italy and review of the literature. GIMEMA Infection Program. *J. Clin. Microbiol*. 43: 1818-1828, 2005.
[16] Skiada A., Pagano L., Groll A., et al. Zygomycosis in Europe: analysis of 230 cases accrued by the registry of the European Confederation of Medical Mycology (ECMM) Working Group on Zygomycosis between 2005 and 2007. *Clin. Microbiol. Infect*. 17, 1859-1967, 2011.
[17] Pagano L., Valentini C.G., Posteraro B., et al. Zygomycosis in Italy: a survey of FIMUA-ECMM (Federazione Italiana di Micopatologia Umana ed Animale and European Confederation of Medical Mycology). *J. Chemother*. 21, 322-329, 2009.
[18] Pagano L, Valentini CG, Caira M, Fianchi L. Zygomycosys: current approaches to management of patients with haematological malignancies. *Br. J. Haematol.* 146, 597-606, 2009.
[19] Ribes J.A., Vanover-Sams C.L., Baker D.J. Zygomycetes in human disease. *Clin. Microbiol. Rev* 13, 236-301, 2000.
[20] Ji Y., Xu L.P., Liu D.H., et al. Positive results of serum galactomannan assays and pulmonary computed tomography predict the higher response rate of empirical antifungal therapy in patients undergoing allogeneic hematopoietic stem cell transplantation. *Biol. Blood Marrow Transplant*. 17, 759-764, 2011.
[21] Zou M., Tang L., Zhao S., et al. Systematic review and meta-analysis of detecting galactomannan in bronchoalveolar lavage fluid for diagnosing invasive aspergillosis. *PLoS One*, 7, e43347, 2012.
[22] Zeppa P. Haematocytopathology: why? *Cytopathology*. 23, 73-75, 2012.
[24] Schmitt F., Vielh P., Zeppa P. Cytology for pathologists: two sides of the same coin or different views of the same side? *Cytopathology*. 23, 345-346, 2012.
[25] Vigliar E., Cozzolino I., Fernandez L.V., et al. Fine-needle cytology and flow cytometry assessment of reactive and lymphoproliferative processes of the breast. *Acta Cytol.* 56, 130-138, 2012.
[26] Ascioglu S, Rex J.H., de Pauw B., et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin. Infect. Dis*. 34, 7-14, 2002.
[27] Wingard JR, Hiemenz JW, Jantz MA. How I manage pulmonary nodular lesions and nodular infiltrates in patients with hematologic malignancies or undergoing hematopoietic cell transplantation. *Blood* 120, 1791-1800, 2012.
[28] Vehreschild J.J., Birtel A., Vehreschild M.J., et al. Mucormycosis treated with posaconazole: review of 96 case reports. *Crit. Rev. Microbiol.* Aug 24., 2012. [Epub ahead of print]
[29] Skiada A., Lanternier F., Groll A.H., et al. Diagnosis and treatment of mucormycosis in patients with haematological malignancies: guidelines from the 3rd European Conference on Infections in Leukemia (ECIL 3). *Haematologica* Sep 14, 2012. [Epub ahead of print]
[30] Messina G., Giaccone L., Festuccia M., et al. Multicenter experience using total lymphoid irradiation and antithymocyte globulin as conditioning for allografting in hematological malignancies. *Biol. Blood Marrow Transplant*. 18, 1600-1607, 2012.
[31] Majolino I., Davoli M., Carnevalli E., et al. Reduced intensity conditioning with thiotepa, fludarabine, and melphalan is effective in advanced multiple myeloma. *Leuk. Lymphoma* 48, 759-766, 2007.
[32] van Lint M.T., Milone G., Leotta S., et al. Treatment of acute graft-versus-host disease with prednisolone: significant survival advantage for day +5 responders and no advantage for nonresponders receiving anti-thymocyte globulin. *Blood* 107, 4177-4181, 2006.