

CASE REPORT

Organising pneumonia mimicking invasive fungal disease in patients with leukaemia

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Abstract

Clinical charts from 63 consecutive highly immunocompromised haematologic patients presenting with pulmonary nodular lesions on CT scan, classified as either probable or possible invasive fungal disease (IFD) according to the revised EORTC/MSG classification, were retrospectively studied. Histopathological analysis of lung tissues, available for 23 patients, demonstrated proven IFD in 17 cases (14 invasive aspergillosis and 3 invasive zygomycosis), diffuse alveolar damage in one and organising pneumonia (OP) in five cases. In the OP cases, three of which have been defined as probable IFD according to EORTC/MSG classification, extensive immunohistochemical, molecular and immunological analyses for fungi were negative. Our case descriptions extend the notion that OP may be encountered as a distinct histopathological entity in pulmonary nodular lesions in patients with leukaemia with probable/possible IFD.

Key words invasive fungal disease; leukaemia; organising pneumonia; lung histopathology

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Accepted for publication 4 February 2010

doi:10.1111/j.1600-0609.2010.01427.x

Introduction

Organising pneumonia (OP), a non-specific inflammatory pulmonary process, histopathologically characterised by granulation tissue plugging into the lumens of small airways, extending, in a continuous fashion, into alveolar ducts and alveoli, occurs rarely in patients with haematologic malignancies (1–3). While the most common radiological pattern of OP on chest computerised tomography (CT) scan is of multiple, patchy alveolar opacities, with a peripheral and bilateral distribution, and a density varying from ground-glass to consolidation, in 10 to 15% of cases OP presents as nodular lesions, occasionally characterised either by cavitation or by halo sign (1, 4). When these latter radiological findings are disclosed on chest CT scan, in high-risk immunocompromised

patients, an invasive filamentous fungal disease (IFD), especially invasive aspergillosis (IA), is usually suspected (5, 6). We have retrospectively analysed 63 highly immunocompromised patients affected with haematologic malignancies, consecutively observed at our Institution between January 2007 and October 2009, because of either probable or possible pulmonary IFD according to the revised EORTC/MSG classification (5) (Fig. 1). The histopathological analysis of a lung tissue specimen was available for 23 of 63 patients, demonstrating proven IFD in 17 cases, while in five patients with acute myeloid leukaemia (AML) histological features consistent with OP, in the absence of fungi, were observed. Issues of diagnosis and clinical management of this uncommon and possibly under-recognised histopathological entity in patients with leukaemia are discussed.

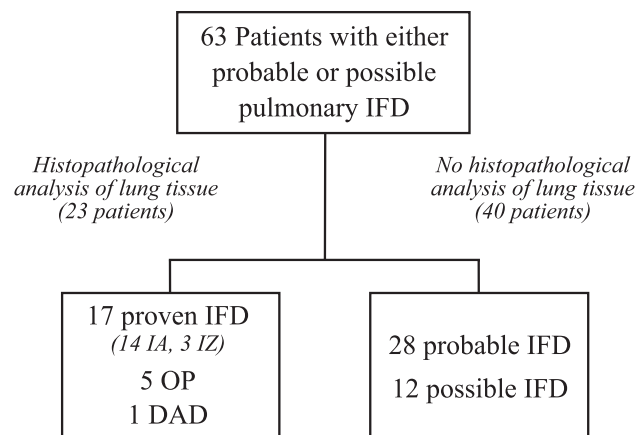


Figure 1 Lung tissue samples from 23 of 63 immunocompromised patients with haematologic malignancies, classified as either probable or possible IFD, were collected for histopathological examination by either open lung biopsy (video-assisted thoracoscopic surgery or thoracotomy), or transbronchial or CT-guided percutaneous lung biopsy, or autopsy. IFD, invasive fungal disease; IA, invasive aspergillosis; IZ, invasive zygomycosis; OP, organising pneumonia; DAD, diffuse alveolar damage.

Case reports

Clinical information on the five patients with leukaemia with OP observed in our series is reported in detail in Table 1. All but patient 4 underwent aggressive chemotherapeutic regimens. The five patients were all in neutropenic phase, either because of chemotherapy administration or because of leukaemia at diagnosis, and febrile, except for patient 3. Before the disclosure of pulmonary lesions, all the patients were receiving antimould active prophylaxis with itraconazole oral suspension, except for patient 4, in whom the radiological findings were disclosed at AML diagnosis, presenting with pancytopenia (WBC count $1.8 \times 10^9/L$ with a neutrophil count of $0.3 \times 10^9/L$). The patients were diagnosed as having pulmonary lesions, being either focal, single (Fig. 2a,c) or multiple, bilateral (Fig. 2b,d,e) dense well-circumscribed nodules, with or without halo sign, radiologically suggestive for IFD. While three subjects (patients 1, 2, 5) showed increase in the size of lung nodular lesions on subsequent chest CT scan evaluation, neither cavitation nor air-crescent sign was observed in our series. All, except for patient 3, underwent bronchoscopy with bronchoalveolar lavage (BAL), whose cultural examinations did not disclose any bacterial, viral or fungal agent, while Galactomannan antigenemia (GM) on BAL samples resulted positive in two cases (patients 1, 2). On the contrary, the GM on serum samples (cut-off ≥ 0.5), performed twice a week, was repeatedly negative, in all patients, except for patient 2, whose serum GM resulted positive in 6 of 16 samples, immediately before undergo-

ing lung tissue biopsy. Of interest, in this latter patient and in patient 3, GM tested on lung tissue (7, 8) resulted positive (6.17 and 1.26, respectively). Only in patients 1 and 4, the cytological examination of BAL fluid detected septate, acutely branching, hyphae, which, because of negative culture results, were presumed to belong to an *Aspergillus* species. Based upon the host factors, namely neutropenia (< 0.5 neutrophils $\times 10^9/L$ for > 10 days), the radiological pulmonary findings and the mycological criteria, our patients were classified as having either probable (patients 1, 2, 4) or possible (patients 3, 5) IFD, before histopathological examination of lung tissue. Of note, patient 5 was classified as possible IFD, because *Aspergillus niger* and *Acinetobacter baumannii* were recovered by sputum culture, 10 days after the performance of lung biopsy. All the patients received broad spectrum antibiotic and antifungal treatment, for a median duration of 25 days (range 5–48) before the collection of lung tissue samples by video-assisted thoracoscopic surgery (VATS), transbronchial or CT-guided percutaneous lung biopsy. In patients 1 and 3, complete surgical resection of the single nodular lung lesion was obtained. None of the patients showed any other extrapulmonary site of infection. Cultural examinations on lung tissue samples were available for patients 1, 2, 3 and resulted unrevealing, for either bacterial or fungal species. Histological examinations on haematoxylin-eosin staining revealed for all the patients a reactive inflammatory picture with buds of granulation tissue in the distal air-spaces, consistent with OP, while neither Periodic acid-Schiff nor Grocott's methenamine silver staining detected fungal hyphae in the lung tissue (Fig. 2f). Moreover, neither *Aspergillus spp* RT-PCR, performed with the use of a commercially available diagnostic kit (*Aspergillus spp*. Q-PCR Alert Kit Nanogen, Turin, Italy), nor a nested pan-fungal PCR (9, 10), performed on formalin-fixed, paraffin-embedded lung tissue biopsies, detected fungal DNA. Furthermore, we have explored the presence of either *Aspergillus*-specific T cells, producing permissive interleukin-10 [TH2(IL-10)] or *Aspergillus*-specific T cells, producing protective interferon-gamma [TH1(IFN- γ)] (11), through an enzyme-linked immunospot (ELISPOT) assay previously described (12), on serial peripheral blood samples of the five patients. *Aspergillus*-specific ELISPOT assays resulted repeatedly negative in patients 2 and 3, while patient 1 showed one single sample positive for protective [TH1(IFN- γ)]. Unfortunately, for patient 4, both samples were not evaluable for [TH2(IL-10)], because of insufficient T-cell responses in wells with phytohemagglutinin (PHA), while patient 5 showed one positive and three negative results, respectively, in the four samples evaluable for [TH2(IL-10)], with repeatedly negative results for [TH1(IFN- γ)]. Patients 1 and 3 completed chemothera-

Table 1 Characteristics of five patients with leukaemia with histopathological diagnosis of organising pneumonia on lung biopsy

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Age (ys)/Sex	53/M	49/M	59/M	73/F	74/M
Haematologic diagnosis	AML	AML	AML	AML	AML
Comorbidities	Arterial hypertension Diabetes Latent tubercular infection	No	Latent tubercular infection	Arterial hypertension COPD Depression	Prostatic carcinoma Arterial hypertension Latent tubercular infection
Antileukaemic treatment	Induction chemotherapy regimen	Induction chemotherapy regimen	Consolidation chemotherapy regimen	Supportive care	Induction chemotherapy regimen
Radiological features of lung nodule(s) on chest CT scan at onset/Time of onset (days from start of chemotherapy)	Dense nodule with halo sign at right lower lobe/day +34	Multiple, bilateral, dense nodular lesions without halo sign/Before starting chemotherapy	Dense nodule without halo sign at left upper lobe/day +39	Two dense nodules plus one dense nodule (with halo sign) on left upper and lower lobes, and right upper lobe, respectively/At AML diagnosis	Multiple, bilateral, dense nodular lesions with halo sign/Before starting chemotherapy
Radiological evolution of nodule(s) at subsequent chest CT scans, performed before histological examination	Increase in size after 11 and 21 days	Increase in size of some lesions after 4 days. Reduction in size of some lesions after 19 days. Appearance of new lesions after 37 days.	NA	Slight reduction in size of left lung lesions, unchanged the right nodule, after 17 days.	Slight increase in size of some lesions, unchanged other lesions, after 15 days.
GM antigenemia on serum/BAL/lung tissue	Neg./6.02/NA	Pos. in 6 of 16 serum samples/3.68/6.17	Neg./NA/1.26	Neg./0.17/NA	Neg./0.14/NA
Detection of fungal hyphae on BAL cytological examination	Yes	No	NA	Yes	No
Aspergillus spp RT-PCR and pan-fungal PCR on lung tissue biopsies ¹	Neg.	Neg.	Neg.	Neg.	Neg.
Cultural examination (sample/tissue)	Neg. on BAL and lung biopsy	Neg. on BAL and lung biopsy	Neg. on lung biopsy	Neg. on BAL	Neg. on BAL/Positive for Aspergillus niger on sputum (detected after lung biopsy)
IFD classification according to EORTC/MSG, before histological examination	Probable	Probable	Possible	Probable	Possible
Antifungal therapy and duration of treatment (days) before histological examination	Yes (caspofungin, voriconazole)/48	Yes (liposomal amphotericin-B, voriconazole, posaconazole)/39	Yes (voriconazole)/14	Yes (caspofungin)/20	Yes (voriconazole)/5
Lung tissue samples collection	Complete surgical resection on VATS	Lung biopsy on VATS	Complete surgical resection on VATS	Transbronchial biopsy	CT-guided percutaneous lung biopsy

Table 1 (Continued)

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Aspergillus-specific ELISPOT assay	TH2(IL-10): neg. (3); NI (2) TH1(IFN- γ): neg. (4); 80 SFC/10 ⁶ PBMCs (1)	TH2(IL-10): neg (5); NI (4) TH1(IFN- γ): neg. (9)	TH2(IL-10): neg. (3) TH1(IFN- γ):neg. (3)	TH2(IL-10): NI (2) TH1(IFN- γ):neg. (2)	TH2(IL-10): neg. (3); 250 SFC/10 ⁶ PBMCs (1); NI (1) TH1 (IFN- γ):neg. (5)

AML, Acute myeloid leukaemia; COPD, Chronic obstructive pulmonary disease; CT, Computerised tomography; GM, Galactomannan (cut-off ≥ 0.5); BAL, Bronchoalveolar lavage; Neg, negative; Pos, Positive; NA, Not available; NI, Not informative; RT-PCR, Real-time Polymerase chain reaction; IFD, invasive fungal disease; EORTC/MSG, European Organization for Research and Treatment of Cancer/Mycoses Study Group; VATS, Video-assisted thoracoscopic surgery

¹Aspergillus spp RT-PCR and pan-fungal PCR were performed on formalin-fixed, paraffin-embedded lung tissue biopsy. Only for patient 3, the same molecular examinations were also performed on fresh lung tissue.

ELISPOT, enzyme-linked immunospot; [TH2(IL-10)], *Aspergillus*-specific T cells, producing interleukin-10; [TH1(IFN- γ)], *Aspergillus*-specific T cells, producing interferon-gamma; SFC, spot-forming cells; PBMCs, peripheral blood mononuclear cells. Numbers of tested samples in parenthesis.

peutic treatments under voriconazole, without experiencing further lung complications, while patient 2 was maintained on posaconazole, with progressive resolution of bilateral lung infiltrates, as documented on subsequent CT scans. On the other hand, patient 4 started to receive itraconazole instead of caspofungin, and died few weeks later because of leukaemia progression, while patient 5, treated with liposomal amphotericin-B, azithromycin and prednisone (40 mg/day) after lung biopsy, with initial clinical and radiological improvement, presented with febrile neutropenia twenty days later (on day +10 of chemotherapy induction regimen). Two dense nodular lesions with halo sign on left upper lobe and one area of patchy consolidation on right upper lobe were disclosed on chest CT scan, and the patient died of respiratory failure. Unfortunately, no invasive diagnostic approach was performed, and consent for necropsy was not obtained.

Discussion

OP may be either cryptogenetic or secondary to other causes, including lung infections, mainly bacterial. In this latter instance, OP may represent a sequela in non-resolving pneumonia, where, despite clearance of the infectious agents by antibiotics, the inflammatory process remains active, with further organisation of the intra-alveolar fibrinous exudates (1).

We have reported here on five patients with leukaemia with pulmonary either probable or possible IFD, under antibiotic and antifungal treatment (median duration 25 days, range 5–48), whose histopathological examination of lung tissue detected OP, in the absence of angioinvasion by fungal hyphae. Of note, neither cavitation nor air-crescent formation, hallmarks of angioinvasive aspergillosis, occurred on subsequent chest CT scan evaluations of the nodular lesions, despite antifungal therapy and neutrophil count recovery.

Kim *et al.* (13) previously described seven cases of OP over 31 patients with haematologic malignancies, suspected as having IA only based on clinical and radiologic grounds, but lacking any data about non-cultural-based diagnostic markers of IFD, who underwent open lung biopsy (OLB) on average 12 days (range 1–29) after the disclosure of the radiological findings. Rickerts *et al.* (14) reported on 56 immunocompromised patients, with suspected IFD, receiving antifungal treatment since 9.3 days (range 1–63) before undergoing lung biopsy. In 46% of cases, classified as possible or probable IFD, non-specific histological results were obtained. Among these latter cases, while some biopsies showed inflammatory changes consistent with fungal pneumonia, without detection of hyphae and with negative results of PCR assays, only

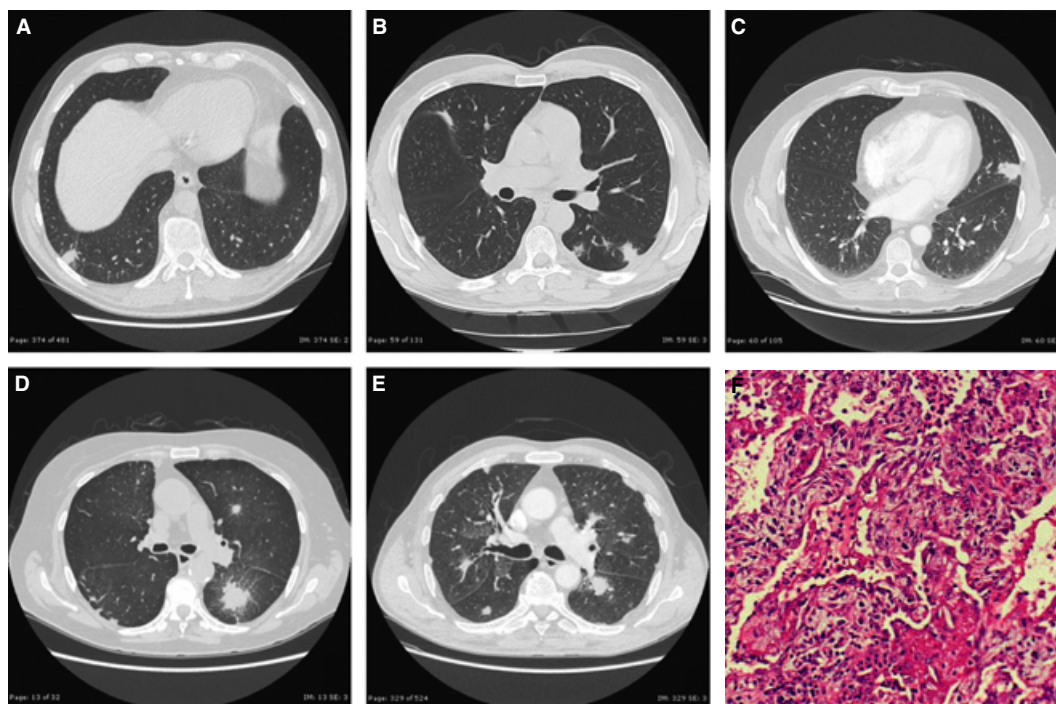


Figure 2 Radiological and histological findings of organising pneumonia, mimicking invasive fungal disease in leukaemic patients. Chest CT scans showed either focal, single (a, c) or multiple, bilateral (b, d, e) dense well-circumscribed nodules in patients 1, 3 and 2, 4, 5, respectively, with or without halo sign, radiologically suggestive for IFD. Histological examination (f) revealed for all the patients a reactive inflammatory picture with buds of granulation tissue plugging into the lumens of the distal airways, in the absence of fungal hyphae (haematoxylin-eosin staining, magnification $\times 100$).

one sterile sample revealed OP on histopathological analysis, concurrently with positivity for *Zygomycetes* PCR.

While OP has been described in association with fungal pathogens, namely *Cryptococcus neoformans*, *Pneumocystis jirovecii* and *Penicillium janthinellum*, angioinvasive fungal infections, caused by moulds, are not notoriously characterised by histopathological features consistent with OP (1, 15). Nevertheless, airway-invasive aspergillosis, characterised by invasion of *Aspergillus* hyphae to the basement membrane of the airways, with a neutrophil reaction, may present with OP surrounding the involved airway (16, 17). Our patients, with nodular lesions and positive mycological markers of infection, may potentially have presented with airway-invasive aspergillosis, but fungal hyphae were not detected in lung tissue, concurrently with negative PCR results. Nevertheless, it should be recognised that the detection of fungal DNA through PCR assays still has limitations, related either to difficulties in understanding the fungal DNA release and kinetics or to technical barriers (18, 19), so that this diagnostic approach has not so far been included in EORTC/MSG classification of IFD. Furthermore, ELISPOT assay repeatedly failed to detect *Aspergillus*-specific T cells, at least in the patients in which several samples, collected at different time points, resulted evaluable. Although the

diagnostic value of the ELISPOT assay has not been consistently validated in large series, the existence of adaptive immunity contributing to the host defence against *Aspergillus* species has been recently described in both mice and humans (11, 12, 20–23). Of note, the production of pro-inflammatory cytokines such as IFN- γ against *Aspergillus* species, which has also been reported in healthy subjects as indicative of a protective immunity (11, 12), has not been revealed by immunological analyses in the cases reported here. Similarly, the TH2 cell immunity, producing IL-10, which has been correlated with the progression of IA in immunocompromised and in non-neutropenic patients (11, 22, 24), has not been documented even by a sensitive ELISPOT assay in the same cases. Although not conclusive, the negative findings by both PCR and ELISPOT, at least argue against a fungal aetiology of the lung infiltrates in these patients.

Overall, even if a preceding IA, eventually resulting in OP after antifungal therapy, cannot be definitively excluded, the negativity of immunohistochemical, molecular and immunological analyses renders unlikely the diagnosis of IA in our patients. Of interest, even if combined antifungal and antibiotic therapies may have potentially eliminated or obscured pulmonary pathogens, the negative results disclosed in our five cases do not

seem to be simply related to the long-lasting antifungal therapies, because proven IFD was disclosed on histological and immunohistochemical examination of lung tissues in other 17 patients of our series (Fig. 1), who previously underwent antifungal treatments of similar duration (median 26 days, range 1–70 days).

On the other hand, at least for the two patients (4 and 5) who did not undergo a complete surgical resection of the nodular lesion, we cannot exclude that the infectious focus has been missed by biopsy procedure, with OP representing a peripheral reactive region, surrounding the necrotising pneumonia. Of note, OLB may show up to 40% of non-specific inflammatory processes, mainly when necrotic tissue has been obtained or biopsy has been taken very late after onset of infiltrates and after prolonged antibiotic/antifungal treatments (25).

The prosecution of antifungal treatment in this clinical setting is still a matter of debate. In a report by Nosari *et al.* (26), percutaneous lung biopsies provided non-specific results in 4 of 17 haematologic patients with suspected IFD, after a median of 15 days (range 0–90) of empirical antifungal therapy, which was continued in those cases, until disappearance of nodular lesions. We also decided to continue the antifungal therapy in patients with histological diagnosis of OP, undergoing intensive chemotherapeutic courses. Nevertheless, the withdrawal of antifungal treatments in immunocompromised patients, resulting as having OP or other reactive pictures on histopathological analysis of lung tissue, may also be attempted with a careful observation of clinical, radiological and immunological evolution.

In conclusion, our case descriptions, although anecdotal, extend the notion that OP may be encountered as a distinct histopathological entity in pulmonary nodular lesions detected on chest CT scan, also in patients with leukaemia classified as having either probable or possible IFD according to the EORTC/MSG classification before histopathological examination of lung tissue. Further clinical and pathological investigations into larger series are needed to exactly define the incidence of OP among immunocompromised subjects presenting with probable/possible pulmonary IFD.

Acknowledgements

This study was supported by grant supports from Associazione Italiana Lotta alle Leucemie, Linfomi e Mieloma (AIL)-Modena-ONLUS (FF and LP), and the Programma di Ricerca Regione Università (PRU) 2007–2009 (GT).

Disclosures of conflict of interest

Mario Luppi received grants for research laboratory activity from Merck Sharp & Dohme, Gilead and Scher-

ing Plough. The other authors indicated no potential conflicts of interest.

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