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Invasive aspergillosis in neutropenic patients: rapid neutrophil recovery is a risk factor for severe pulmonary complications

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Abstract

Background In invasive aspergillosis, the duration of neutropenia is an accepted risk factor, and recovery from neutropenia is generally associated with a favourable outcome. However, the rapidity of granulocyte recovery may rarely be associated with adverse sequelae. The purpose of this study was to define the relationship between neutrophil (polymorphonuclear, PMN) recovery after chemotherapy-induced bone marrow aplasia and the occurrence of severe pulmonary complications (haemoptysis, pneumothorax and death) in patients with haematological malignancies who developed invasive fungal pneumonias.

Methods Twenty consecutive patients were retrospectively studied; eight of them had developed pulmonary events between 5 and 11 days after neutrophil recovery that followed deep neutropenia (PMN $< 100\,\mu L^{-1}$).

Results Five patients had haemoptysis (one of these also had pneumothorax) and three had pneumothorax. According to the multiplicative logistic model, the odds of occurrence of a pulmonary event increased significantly with increasing PMN count on the fifth day ($P < 0{\cdot}001$). Five of the eight patients who had pulmonary complications died. Also, the risk of death was larger in the presence of rapid neutrophil recovery, although the difference was not statistically significant ($P = 0{\cdot}111$). Analysis of clinical and laboratory data showed that the risk of pulmonary complications significantly increased when the neutrophil concentration was $> 4500\,\mu L^{-1}$ on day 5 after deep granulocyte neutropenia (PMN $< 100\,\mu L^{-1}$). There was no correlation between pulmonary complications, dosage of amphotericin B and deaths.

Conclusion The occurrence of life-threatening complications in patients with invasive fungal pneumonia is closely related to rapid PMN recovery.

Keywords Haemoptysis, invasive aspergillosis, neutropenia, neutrophil recovery, pneumothorax.
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Introduction

Invasive pulmonary mycoses, especially with *Aspergillus* spp., are a major cause of morbidity and mortality in immunocompromised hosts with protracted neutropenia [1–7], in patients with defects of phagocyte function [8] and in patients treated with high dosages of steroids [9,10], because steroids inhibit the bactericidal and fungicidal activities of phagocytes [11,12]. In patients with leukaemia, the prognosis of pulmonary aspergillosis is frequently related to the status of the underlying disease (i.e. whether the patient is in remission or relapse), and whether polymorphonuclear neutrophil (PMN) recovery occurs or is imminent. Indeed, it is frequently assumed that improvement in invasive pulmonary mycosis will occur if the patient achieves remission and/or haematological recovery. However, the massive leucocytosis that follows the period of profound neutropenia could paradoxically represent a hazard for some leukaemia patients with invasive fungal pneumonia. For example, three groups [13–15] observed the appearance of cavitary lesions and massive haemoptysis

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concomitant with bone marrow recovery. The aim of this study was to define whether a significant relationship between neutrophil recovery and the evolution of pulmonary infiltrates (i.e. cavitation and subsequent events such as life-threatening haemorrhage) exists and to determine the possible predictive factors for the outcome, in particular the rate of PMN recovery, expressed as PMN μL^{-1} on the fifth day after profound neutropenia (PMN $< 100\,\mu L^{-1}$) and antifungal therapy.

Methods

Patients

Twenty consecutive neutropenic patients (10 women, 10 men, mean age 42 years, range 16–69 years) with acute leukaemia or high-grade lymphoma (acute myelogenous leukaemia 13, acute lymphoblastic leukaemia 4, blastic transformation of chronic myelogenous leukaemia 1, non-Hodgkin lymphoma 2) who had developed pulmonary infiltrates that were proven or likely to be due to fungal infection [14] were studied. *Aspergillus* pneumonia was defined as proven if histologically documented either *in vivo* or at autopsy, or as probable if diagnosed according to the criteria of Robertson & Larson [16]: clinical evidence and radiological evidence, i.e. presence of cavitation with 'fungus ball' on chest radiograph and/or findings on computerized tomography (CT) scan compatible with aspergillar lesions (long lesions with 'halo sign' and/or 'air crescent sign'), and/or microbiological evidence (sputum cultures and bronchoalveolar larage) but not histological evidence. Possible *Aspergillus* pneumonia was not considered. The patients were admitted to single-bed or double-bed rooms with reverse isolation precautions. The patients had no evidence of bacterial, viral or protozoal infections. All patients had normal platelet counts and normal coagulation tests. The first 17 episodes of pulmonary fungal infection occurred in patients who had not received antifungal prophylaxis, and the last three episodes occurred in patients who had received prophylactic oral itraconazole (200 mg daily) and nasal amphotericin B as reported elsewhere [17]. When patients became febrile and neutropenic they were evaluated and started on broad-spectrum parenteral antibiotic therapy. Patients with persistent fever (i.e. after a week of antibiotics) or who developed pulmonary infiltrate(s) were given i.v. amphotericin B (1 mg kg^{-1} per day). Patients did not receive granulocyte (G) or granulocyte–macrophage (GM) colony-stimulating factor (CSF).

Laboratory and clinical studies

Leucocyte, granulocyte and platelets counts were monitored daily. All patients had PMN counts less than $100\,\mu L^{-1}$. Dosages of i.v. amphotericin B on days 5 and 8 after neutrophil recovery were recorded. Chest radiography was carried out at least twice a week. The time of appearance of cavitation and the time of occurrence of pulmonary 'events' (either the appearance of severe haemoptysis and/or pneumothorax) in relation to PMN recovery was examined. In cases of fatal outcome, autopsy was performed.

Statistical analysis

Statistical analysis was performed by a multiplicative logistic model [18]: PMN count and amphotericin B dosage at 8 days were considered as independent variables, whereas the dependent variable was either the occurrence of a pulmonary event or death. The results of the analysis were synthesized using odds ratios, which were computed on the basis of an increase in the value of 1 SD. As the PMN count usually follows a Poisson distribution, a square-root transformation was applied to this variable before subsequent analysis [19]. The PMN count used as the cut-off to distinguish between low risk and high risk of pulmonary events was assessed by analysing clinical and laboratory data and using a decision level plot [20].

Results

Table 1 reports the main clinical data of the 20 patients studied. Before the appearance of fungal pneumonia, all 20 patients were profoundly neutropenic (PMN $< 100\,\mu L^{-1}$) as a consequence of previous chemotherapy. The granulocyte recovery always followed a prolonged neutropenia period. The median duration of neutropenia was 21 days (range 16–42 days). In 90% (18/20) of cases cavitation occurred in association with PMN recovery.

As shown in Fig. 1, the rapidity of PMN recovery was critical for adverse pulmonary events (haemoptysis, pneumothorax). In fact six out of eight patients who had rapid PMN recovery (from $< 100\,\mu L^{-1}$ to $4500\,\mu L^{-1}$ in 5 days) developed an event. In contrast, of 12 patients without PMN recovery or with slow PMN recovery, 2 out of 12 (17%) had an event ($P < 0{\cdot}001$).

The optimal cut-off point to distinguish between patients at low risk of a pulmonary event and patients at high risk was assessed using a decision level plot and was found to be 4500 PMN μL^{-1}.

Five of the eight patients (62·5%) who experienced a pulmonary event died because of haemoptysis (4) or haemoptysis plus pneumothorax (1), whereas none of the 12 patients who did not develop a pulmonary complication died. Table 2 shows that the risk of death was larger in the presence of rapid neutrophil recovery, although the difference was not statistically significant, probably because of the small number of cases ($P = 0{\cdot}111$).

The total dosages of amphotericin B received on day 8 are reported in Table 1, together with the occurrence of events and deaths.

Mortality was lower for patients who received a higher (> 500 mg) total dose of i.v. amphotericin B at PMN recovery than those who received lower dosages of amphotericin B (14% vs. 50%, respectively), although the difference did not reach conventional significance.

Table 1 Major clinical data of the patients of this study.

Patient	Cavitation	Event	Death	Phagocyte count (μL) (PMN + monocytes) Day 5	Phagocyte count (μL) (PMN + monocytes) Day 8	Amphotericin B (mg) Day 5	Amphotericin B (mg) Day 8	Neutropenia total duration (day < 500)	Time of phagocyte recovery (days) From 500 μL^{-1} to 4500 μL^{-1}	Time of phagocyte recovery (days) From 500 μL^{-1} to max
1 LS	Yes	Yes	No	10000	18000	850	1000	17	3	11
2 GA	Yes	Yes	Yes	5000	12050	280	400	17	5	9
3 GC	Yes	No	No	1500	2000	900	1000	38	–	10
4 FR	Yes	No	No	1200	1800	500	650	24	19	20
5 MR	Yes	Yes	No	12000	20000	280	600	17	3	9
6 BG	Yes	No	No	700	900	600	750	21	–	6
7 FR	Yes	No	No	5500	7000	350	560	16	6	7
8 CM	Yes	Yes	Yes	5000	NE	500	NE	16	5	5
9 GC	Yes	Yes	Yes	9300	12000	30	130	16	3	7
10 BC	Yes	No	No	800	1200	600	700	40	–	14
11 CZ	Yes	No	No	1000	1500	1055	1200	17	–	7
12 CC	Yes	Yes	Yes	7200	10000	280	400	18	5	9
13 IM	Yes	No	No	2500	4500	240	390	18	10	19
14 CG	Yes	No	No	1700	2700	900	1110	16	–	8
15 CP	Yes	Yes	No	1800	2300	600	750	17	8	8
16 PD	No	No	No	8000	9500	150	360	20	3	12
17 PS	Yes	No	No	2000	3000	50	230	19	15	17
18 DF	No	Yes	Yes	2320	NE	1450	NE	25	–	5
19 LL	Yes	No	No	3500	4500	600	800	16	–	6
20 LL	Yes	No	No	70	90	830	950	42	–	–

From 5 to 8 days after phagocyte nadir.
NE, not evaluable (dead); PMN, polymorphonuclear.

Discussion

A recent review of the literature [15] showed that massive haemoptysis is an undervalued cause of death in patients with acute leukaemia in remission and pulmonary filamentous mycosis. Our results are in agreement with this, and the data presented in this paper allow us to address the question of the possible mechanism(s) of severe pulmonary complications in these patients. Just as the onset and duration of neutropenia are widely accepted risk factors for infections, including invasive mycoses, recovery from neutropenia is also generally associated with an improved outcome. Although these two tenets are true for the vast majority of patients, there may be times when granulocyte recovery may actually be associated with adverse sequelae [13,14]. The pulmonary complications we described suggest that this may be true for patients with invasive mycosis, and this may have particular relevance for occasions when rapid granulocyte recovery occurs.

The pathogenesis of pulmonary cavitation is probably related to both the tissue damage produced by the organism and to the host reaction. As already pointed out by Panos *et al*. [21], a fatal haemoptysis in patients with fungal pneumonia may occur by two different mechanisms. During profound neutropenia, the pulmonary lesions may be due to direct vascular damage by the fungus, possibly through the release of proteolytic enzymes by *Aspergillus* itself. A second mechanism is the disruption of vascular structures by necrotizing acute inflammation. We suggest that the second mechanism is prevalent in patients who are affected by pulmonary complications

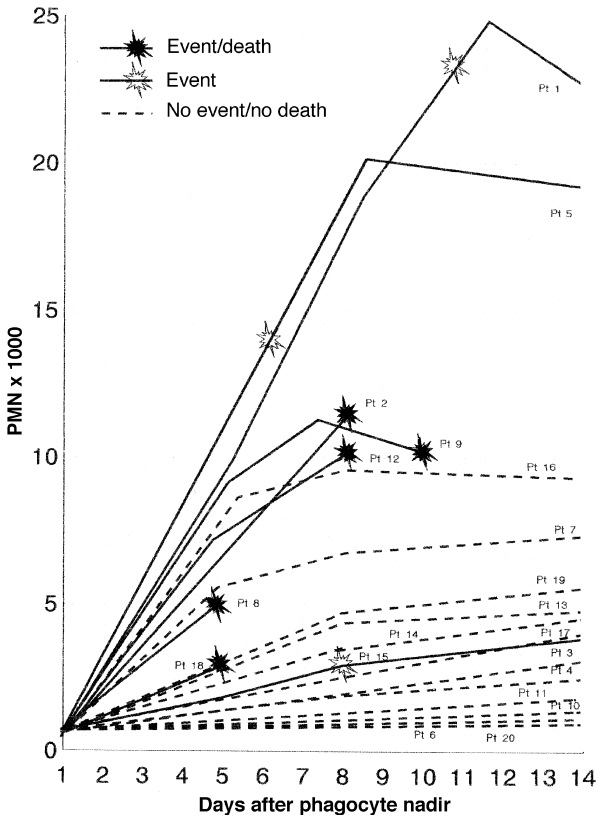

Figure 1 Kinetics of blood PMN counts and major pulmonary complications in the patients of this study.

Table 2 Correlation between PMN* at fifth and at eighth day after nadir, dosage of amphotericin B† at fifth day after nadir and outcome.

	Odds ratio (95% CI)		
	Not adjusted	Adjusted for the other variable	*P*
Day 5			
Outcome = event			
Amphotericin B	0·91 (0·36–2·31)	3·54 (0·56–22·41)	0·102
PMN	5·11 (1·30–20·1)	13·78 (1·35–140·8)	<0·001
Outcome = death			
Amphotericin B	0·84 (0·29–2·46)	1·34 (0·39–19·02)	0·642
PMN	2·32 (0·76–7·08)	2·68 (0·73–9·81)	0·111
Day 8			
Outcome = event			
Amphotericin B	0·99 (0·99–1·00)	1·00 (0·99–1·01)	0·862
PMN	1·07 (1·01–1·13)	1·07(1·00–1·15)	0·002
Outcome = death			
Amphotericin B	0·714(0·044–2·245)		0·563
PMN	1·04 (0·99–1·08)	1·21(0·85–1·73)	0·039

*PMNs more than 4500 μL vs. less than 4500 μL^{-1}.
†Amphotericin B more than 500 mg vs. less than 500 mg.

during or just after bone marrow recovery. A massive influx of PMNs into the site of fungal lesions, attracted and primed by chemotactic factors released by *Aspergillus* and by humoral inflammatory mediators, may be detrimental for the residual healthy pulmonary tissue [22,23]. We have previously demonstrated that mature neutrophils that regenerate after marrow aplasia are functionally active and produce oxygen radicals when suitably activated [24]. It is highly conceivable that the interaction between functional PMN and a large amount of infectious agent and cell debris induces the stimulation of cell metabolism with release of reactive oxygen intermediates and proteolytic enzymes, including collagenase and elastase. In addition, stimulated neutrophils may inactivate protease inhibitors [25] and plasminogen activator inhibitor [26]. As a consequence, an outburst of the inflammatory process develops in a few hours or a few days, which can eventually propagate to the neighbouring structures that have been invaded by *Aspergillus*, and causes massive haemorrhage and/or pleural rupture.

Our experience demonstrates that the degree of lung damage and the occurrence of haemorrhage and pneumothorax are strongly correlated with the blood PMN count and the rapidity of their recovery. The risk of death was not significantly affected by the extent of PMN recovery, probably because of the low number of subjects: 20 patients and 5 casualties. Larger samples are needed to clarify this point.

The increased frequency of pulmonary events when neutrophil recovery is higher than 4500 PMN μL^{-1} in 5 days may have particular relevance for the management of these high-risk patients. Three major points can be discussed in this respect. First, the awareness of the high probability of the occurrence of pulmonary events can lead the clinician to make ready all the emergency procedures, including surgical intervention, for the treatment of this kind of life-threatening event. Second, although we could not demonstrate significant differences in outcome based on the dosage of amphotericin B, probably because of the limited number of cases, data from the literature stress the importance of an early and intensive amphotericin B use in the presence of fever not responsive to broad spectrum antibiotics, mainly if associated with pulmonary infiltrates of suspected fungal origin [27,28].

Finally, the clear correlation between PMN recovery and pulmonary complications connected with aspergilloma raises the question about the use of haemopoietic growth factors. Our data, together with those of Groll *et al*. [29], suggest that in some patients the use of these agents, which are known to prime the neutrophil respiratory burst [30], may be detrimental. Accordingly, the beneficial effects generally associated with phagocyte recovery may need to be modulated in patients with invasive pulmonary aspergillosis.

In conclusion, the present work supports the view that abrupt neutrophilia, after a period of neutropenia and associated fungal infections, can cause more lung damage than when the return of neutrophils is relatively slower, and indicates that the rate of PMN recovery is a clinically significant variable in the follow-up of patients recovering from bone marrow ablative chemotherapy.

References

1 Young RC, Bennet JE, Vogel CL, Carbone PP, De Vita VT. Aspergillosis: the spectrum of the disease in 98 patients. *Medicine*, 1970; **49:** 143–7.
2 Arnow PM, Andersen RL, Mainous PD, Smith EJ. Pulmonary

aspergillosis during hospital renovation. *Am Rev Respir Dis* 1978; **118:** 49–53.
3 Fischer B, Armstrong D, Yu B, Gold JV. Invasive aspergillosis. Progress in early diagnosis and treatment. *Am J Med* 1981; **71:** 571–7.
4 Rinaldi MG. Invasive aspergillosis. *Rev Infect Dis* 1983; **5:** 1061–77.
5 Rhame FS, Streifel AJ, Kersey JH, McGlave PB. Extrinsic risk factors for pneumonia in the patient at high risk of infection. *Am J Med* 1984; **15:** 42–52.
6 Gerson SL, Talbot GH, Hurwitz S, Strom BL, Lusk EJ, Cassileth PM. Prolonged granulocytopenia: the major risk factor for invasive pulmonary aspergillosis in patients with acute leukemia. *Ann Intern Med* 1984; **100:** 345–51.
7 Denning WD, Stevens DA. Antifungal and surgical treatment of invasive aspergillosis: review of 2121 published cases. *Rev Infect Dis* 1991; **12:** 1147–201.
8 Cohen MS, Isturiz RE, Malech HL, *et al*. Fungal infection in chronic granulomatous disease. The importance of the phagocyte in defense against fungi. *Am J Med* 1981; **71:** 59–71.
9 Weiland D, Ferguson RM, Peterson PK, Snover DC, Simmons RL, Najarian JS. Aspergillosis in 25 renal transplant recipients: Correlations with corticosteroid therapy. *Ann Surg* 1983; **198:** 622–9.
10 Gustafson TL, Schaffner W, Lavely GB, Stratton CW, Johnson HK, Hutcheson RH. Invasive aspergillosis in renal transplant recipients: correlation with corticosteroid therapy. *J Infect Dis* 1983; **148:** 230–8.
11 Rinehart JJ, Sagone AL, Balcerzak SP, Ackerman GA, LoBuglio AF. Effects of corticosteroids therapy in human monocyte function. *N Engl J Med* 1975; **292:** 236–41.
12 Bowen DL, Fauci AS. Adrenal corticosteroids. In: Gallin JI, Goldstein, IM, Snyderman R, editors. *Inflammation: basic principles and clinical correlates*. New York: Raven Press; 1984. p. 877–95.
13 Albelda SM, Talbot GH, Gerson SL, Miller WT, Cassileth PA. Pulmonary cavitation and massive hemoptysis in invasive pulmonary aspergillosis. Influence of bone marrow recovery in patients with acute leukemia. *Am Rev Resp Dis* 1985; **131:** 115–20.
14 Martino P, Girmenia C, Venditti M, *et al*. Spontaneous pneumothorax complicating pulmonary mycetoma in patients with acute leukemia. *Rev Infect Dis* 1990; **12:** 4.
15 Pagano L, Ricci P, Nosari AM, *et al*. Fatal haemoptysis in pulmonary filamentous mycosis: an undervaluated cause of death in patients with acute leukemia in haematological complete remission. A retrospective study and review of the literature. *Br J Haematol* 1995; **89:** 500–5.
16 Robertson MJ, Larson RA. Recurrent fungal pneumonias in patients with acute non lymphocytic leukemia undergoing multiple courses of intensive chemotherapy. *Am J Med* 1988; **84:** 233–9.
17 Todeschini G, Murari C, Bonesi R, *et al*. Oral itraconazole plus nasal amphotericin B for prophylaxis of invasive aspergillosis in patients with haematological malignancies. *Eur J Clin Microbiol Infect Dis* 1993; **12:** 614–8.
18 Clayton D, Hills M. *Statistical models in epidemiology* . Oxford: Oxford Science Publications; 1993.
19 Fleiss JL, *The design and analysis of clinical experiments*. New York: J. Wiley & Sons; 1986.
20 Pellar TG, Leung FY, Henderson ARA. Computer program for rapid generation of receiver operating characteristic curves and likelihood ratios in the evaluation of diagnostic tests. *Ann Clin Biochem* 1988; **25:** 411–6.
21 Panos RJ, Barr LF, Walsh TJ, Silverman MD. Factors associated with fatal hemoptysis in cancer patients. *Chest* 1988; **94:** 1008–13.
22 Weiss SJ. Tissue destruction by neutrophils. *N Engl J Med* 1989; **320:** 365–76.
23 Smith JA. Neutrophils, host defense, and inflammation: a double-edged sword. *J Leukocyte Biol* 1994; **56:** 672–86.
24 Todeschini G, Zeni L, Bellavite P. Follow-up of superoxide production by phagocytes in whole blood of leukaemic patients during therapy. *Acta Haematol* 1988; **79:** 38–40.
25 Ossanna PJ, Test ST, Matheson NR, Reggiani S, Weiss SJ. Oxidative regulation of neutrophil elastase-alpha-1-proteinase inhibitor interactions. *J Clin Invest* 1986; **77:** 1939–51.
26 Lawrence DA, Loskutoff DJ. Inactivation of plasminogen activator inhibitor by oxidants. *Biochemistry* 1986; **25:** 6351–5.
27 Karp JE, Burch PA, Merz WG. An approach to intensive antileukemia therapy in patients with previous invasive aspergillosis. *Am J Med* 1988; **85:** 203–6.
28 Walsh TJ, Lee J, Lecciones J, *et al*. Empiric therapy with amphotericin B in febrile granulocytopenic patients. *Rev Infect Dis* 1991; **13:** 496–503.
29 Groll A, Renz S, Gerein V, *et al*. Fatal haemoptysis associated with invasive pulmonary aspergillosis treated with high-dose amphotericin B and granulocyte-macrophage colony-stimulating factor (GM-CSF). *Mycoses* 1992; **35:** 67–75.
30 Weisbart RH, Kwan L, Golde DW, Gasson JC, Human GM-CSF. Primes neutrophils for enhanced oxidative metabolism in response to the major physiological chemoattractants. *Blood* 1987; **69:** 18–21.