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Original article

Evolution of procalcitonin, C-reactive protein and fibrinogen levels in neutropenic leukaemia patients with invasive pulmonary aspergillosis or mucormycosis

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Summary

Unlike bacterial infections, the value of procalcitonin (PCT) in detecting fungal infections in leukaemia patients is not clear. To determine whether the monitoring of PCT coupled with C-reactive protein (CRP) and fibrinogen (Fib) could be helpful in the management of pulmonary aspergillosis (IPA) or mucormycosis (PM), we retrospectively analysed the evolution of PCT, CRP and Fib levels in 94 leukaemia patients with proven/probable IPA ($n = 77$) or PM ($n = 17$) from D−12 to D12 relative to IFI onset defined as D0. Overall, 2140 assays were performed. From D−12 to D0, 12%, 5% and 1.4% of patients had PCT >0.5, 1 and 1.5 μg l^{-1}, respectively, while CRP was >50, 75 and 100 mg l^{-1} in 84%, 70% and 57% and Fib was >4, 5 and 6 g l^{-1} in 96%, 80% and 61% of cases respectively ($P < 10^{-7}$). The same trends were observed from D1 to D12. Overall, between D−12 and D12, only 6.4% of patients had PCT >1.5 μg l^{-1}, while CRP >100 mg l^{-1} and Fib >6 g l^{-1} were observed in 80% and 75% of cases respectively ($P < 10^{-7}$). In leukaemia patients, IPA or PM was accompanied by a significant increase in CRP and Fib while PCT remained low.

Key words: Procalcitonin, aspergillosis, mucormycosis, leukaemia, neutropenia.

Introduction

Invasive fungal infections (IFI), especially invasive pulmonary aspergillosis (IPA) and mucormycosis (PM), are a major cause of morbidity and mortality in haematological malignancies.[1] The prevalence ranges from 2% to 40%, depending on the disease and treatment administered.[2] Mortality rates can reach 40% to 60%.[3] The incidence of IPA or PM has increased during the last two decades especially following prolonged neutropenia induced by intensive cytotoxic chemotherapies.[4]

Early recognition and effective antifungal treatment are necessary to improve the prognosis.[5,6] However, the diagnosis of IPA or PM is often delayed or difficult to establish with certainty because there is no reliable method of diagnosis. Clinical symptoms are variable, non-specific and often become manifest only once the infection is well established. The gold standard is based on tissue biopsies, but these are not always feasible. More recently, a diagnostic approach using high-resolution computed tomography and the measurement of serum markers such as galactomannan, β 1-3 glucan, and even polymerase chain

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reaction have allowed earlier diagnosis but these methods present flaws.[5,7–11]

Some studies have reported the usefulness of serological markers such as C-reactive protein (CRP) and Fibrinogen (Fib) in identifying patients at risk of IFI.[5] Because of its excellent sensitivity and specificity, procalcitonin (PCT), a 116-amino-acid precursor of calcitonin, marked a major turning point in the diagnosis of infections, in particular, to distinguish between early systemic bacterial infection and non-infectious inflammatory conditions.[12,13] It is also useful to guide decisions to initiate antibiotherapy and establish its duration. However, its value in the diagnosis of IFI, especially during neutropenia, remains limited because of the small number of studies and their controversial findings.[14]

The aim of this study was to know if PCT coupled with other markers of inflammation (CRP and Fib) could be an aid in the diagnosis of IPA or PM in high-risk leukaemia patients.

Patients and methods

Between January 2009 and May 2015, we retrospectively studied patients who had received intensive chemotherapies (inducing prolonged and deep neutropenia defined as a white blood cells (WBC) count below 500 mm^{-3}) with curative intent for acute myeloblastic (AML) or lymphoblastic leukaemia (ALL) at the Department of Clinical Haematology, University Hospital of Dijon, France. We included leukaemia patients who had contracted proven or probable IPA or PM defined by the revised European Organization for Research and Treatment of Cancer-Mycosis study group (EORTC-MSG) criteria.[15] Only patients who benefited from PCT assays were analysed. The first day of IFI was defined as day 0 (D0). D0 was the day with first CT scan evidence of IFI after the onset of clinical symptoms (e.g. CT halo sign in aspergillosis or reversed halo sign in mucormycosis).[8,16] The local institutional review board approved the study.

During hospitalisation, Fib testing and Aspergillus antigenemia (Platelia Aspergillus®, Bio-Rad, Marnes la Coquette, France) was routinely performed three times a week. In addition, for all patients, after informed consent had been obtained, serum samples were taken and frozen at −20 °C on entry to the Department and then five times a week until hospital discharge (for possible retrospective assays).

Since 2011, when IFI was suspected, CRP and PCT were assayed either sequentially over the days after IFI diagnosis or retrospectively (for the days preceding IFI) on frozen blood samples. For the remaining patients (seen before 2011), CRP and PCT were assayed retrospectively on frozen blood samples when available.

Procalcitonin was measured by a sensitive immunofluorescence method on fresh or frozen serum, with the Brahms Kryptor compact analyzer® (BRAHMS GmbH, Hennigsdorf, Germany). The maximum determined threshold was 0.5 μg l^{-1}, (as defined by manufacturer). CRP was measured by nephelometry. The normal threshold of 3.2 mg l^{-1} was replaced in this study by 50 mg l^{-1}, which is the clinically significant value in sepsis.[17] The maximum threshold for Fib was 4 g l^{-1}. The comparisons between the different levels of the three biomarkers were done with cut-offs of 0.5, 1 and 1.5 μg l^{-1} for PCT; 50, 75 and 100 mg l^{-1} for CRP and 4, 5 and 6 g l^{-1} for Fib.

Antifungal prophylaxis policy varied over time. Before 2011, prophylaxis was uncommon and since 2011 prophylaxis mainly relied on Posaconazole or Itraconazole in AML patients and Fluconazole in ALL patients.

SAS version 9.2 was used for the statistical analysis. The *Chi-square test* was applied as indicated. Non-parametric tests were applied due to the non-Gaussian distribution of the quantitative data of the study population. No log-transformation of the variables was attempted before moving to the non-parametric analysis. As the measured parameters were not normally distributed, all the values described in the text are expressed as medians with quartiles values. The comparison over time used mixed models on repeated measures (ANOVA test). Survival was measured from January 1st, 2009 and estimated according to Kaplan–Meier estimates: the log-rank test was for comparisons between groups.

Results

Characteristics of patients and diagnosis of IFI are summarised in Table 1 and Fig. 1. During the studied period, 563 patients with either AML ($n = 440$) or lymphoblastic ($n = 123$) leukaemia received 1068 courses of intensive chemotherapy, inducing prolonged neutropenia. Among these patients, 90 cases of IPA and 20 cases of PM (proven or probable) were identified. The frequencies of IPA and PM were 8% and 2% of the neutropenia episodes respectively. Sixteen patients were excluded from the study as they did not have a PCT assay and no frozen serum samples were available. Finally, 94 patients with PCT assays [60 men and 34 women, age range 23 to

Table 1 Characteristics of 94 leukaemia patients with invasive pulmonary aspergillosis (IPA) or mucormycosis (PM).

	IPA *n* = 77	PM *n* = 17	All patients *n* = 94
Clinical data			
Median age (years) (range)	64 (23–83)	59 (33–75)	64 (23–83)
Sex ratio (male/female)	1.85	1.43	1.76
Patients with AML and ALL	62 and 15	13 and 4	75 and 19
Patients with progressive leukaemia (failure or relapse)	32 (42%)	5 (29%)	37 (39%)
Median duration (days) of hospital stay before IFI (range)	18 (0–47)	16 (0–37)	18 (0–47)
Median duration (days) of hospital stay after IFI (range)	12 (4–42)	15 (2–34)	12 (2–42)
Median duration (days) of neutropenia (range)	23 (7–130)	16 (7–106)	23 (7–130)
Before IFI diagnosis	14 (0–80)	11 (0–96)	14 (0–96)
After IFI diagnosis	8 (0–59)	7 (2–65)	8 (0–65)
Patients with fever >39° before IFI	35 (48%)	13 (76%)	48 (51%)
Patients receiving azole prophylaxis (>7 day) before IFI	34 (44%)	8 (47%)	42 (45%)
Radiological and mycological data associated with IPA			
Patients with unilateral pulmonary involvement on CT	38 (49%)	–	–
Initial CT scan with halo sign	66 (86%)	–	–
Patients with positive antigenemia (≥0.5)[1]	45 (58%)	–	–
Positive culture of BAL for *Aspergillus* strains	15/70 (21%)	–	–
Positive *Aspergillus* antigen (≥0.5) on BAL[1]	62/70 (89%)	–	–
Positive PCR for *Aspergillus sp* on BAL	35/49 (71%)	–	–
Positive tissue biopsy (pathological exam and/or culture and/or PCR)	10 (13%)	–	–
Radiological and mycological data associated with PM			
Patients with unilateral pulmonary involvement on CT	–	16 (94%)	–
Initial CT scan with reversed halo sign	–	16 (94%)	–
Positive culture of BAL for *Mucorales* strains	–	3/13 (23%)	–
Positive PCR for *Mucorales sp* on BAL	–	1/2 (50%)	–
Positive detection of circulating *Mucorales* DNA by qPCR on serum	–	15/16 (94%)	–
Positive tissue biopsy (pathological exam and/or culture and/or PCR)	–	12/13 (92%)	–
Level of confidence of IPA or PM diagnosis[2]			
Patients with definite diagnosis of IFI	10 (13%)	12 (71%)	22 (23%)
Patients with probable diagnosis of IFI	67 (87%)	5 (29%)	72 (77%)
Antifungal treatment of IFI			
Voriconazole alone or combined with other antifungal agent	66 (86%)	–	–
Other antifungal agent alone or in combination	11 (14%)	–	–
Ambisom® alone or combined with Posaconazole	–	17 (100%)	–
Haematological response and outcome			
Haematological CR after IFI	53 (68%)	13 (76%)	66 (70%)
Survival at 3 months after IFI	64 (83%)	14 (82%)	78 (83%)
Survival at 1 year after IFI	38/73 (52%)	9/15 (60%)	47/88 (53%)

AML, acute myeloblastic leukaemia; ALL, acute lymphoblastic leukaemia; IFI, invasive fungal infection; CR, complete response.

[1]Platelia *Aspergillus*® test.

[2]Revised EORTC-MSG criteria.

83 years (median, 64 years)] were included in the study. All of these 94 patients had only one episode of IFI. Seventy-five were treated for AML and 19 for ALL. Thirty-seven patients (39%) had progressive haematological disease while the remaining patients were on first line of haematological therapy. All the patients had severe neutropenia [polymorphonuclear (PMN) < 0.5 G l^{-1}] during the hospitalisation and at D0, 88/94 (94%) of patients had been neutropenic for a median of 14 days (0–96) before the IFI. IPA or MP was accompanied by a febrile episode in only 51% of patients. At time of IFI diagnosis, 42 patients (45%) received antifungal azole prophylaxis (as primary the prophylaxis in 40 cases) lasting more than 1 week (Fluconazole, Itraconazole, Posaconazole and Voriconazole in 22, 11, eight and one cases, respectively). In addition, in the week preceding IFI diagnosis, four patients had concomitant bacterial septicaemia [due to *Enterococcus* ($n = 2$) and gram negative bacillus ($n = 2$)]. Overall, clinical and mycological characteristics of the 94 studied patients and the 16 excluded patients were similar.

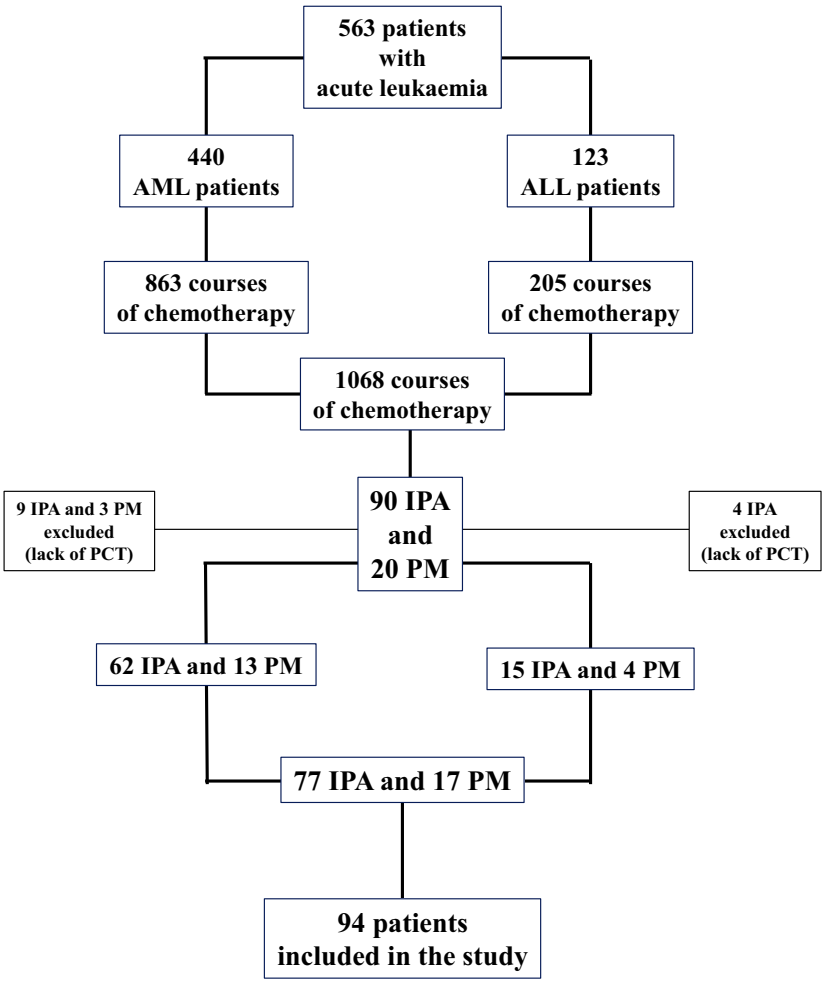


Figure 1 Flow diagram of the study. AML, acute myeloblastic leukaemia; ALL, acute lymphoblastic leukaemia; IPA, invasive pulmonary aspergillosis; PM, pulmonary mucormycosis.

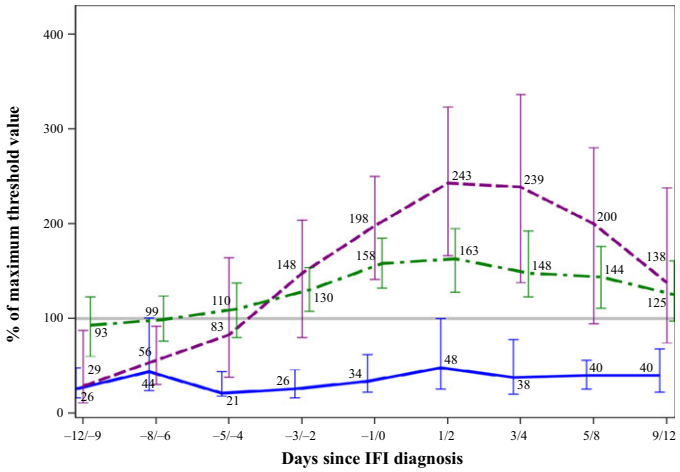
Seventy-seven patients had IPA, including 10 proven (13%) and 67 probable (87%). All patients had at least one thoracic CT scan and a CT halo sign was recorded in 86% of cases on the first CT. The 11 remaining patients had less specific CT images such as wedge-shaped infiltrates and segmental or lobar consolidation. *Aspergillus* antigenemia was positive in 45 of the 77 patients (58%). A bronchoalveolar lavage (BAL) was performed in 70 patients (91%). BAL culture was positive for *Aspergillus* in 15 cases (21%) while *Aspergillus* antigen was positive (≥0.5) in BAL in 62/70 cases (89%).

Seventeen patients had PM, including 12 proven (71%) and five probable (29%). All patients had a thoracic CT scan and a reversed halo sign (RHS) was recorded in 16 patients (94%). Culture of BAL was positive for *Mucorales* strains in three cases. The search for circulating *Mucorales* DNA by qPCR on at least two sera was found to be positive in 15 of 16 tested patients.[18] In addition, a total of 12 tissues biopsies (eight from surgical pulmonary resection and four from thoracic CT guided biopsy) were positive by pathological exam, culture or PCR.

From D0, all of the patients received antifungal treatment. All of the patients with PM received Ambisom® and patients with IPA were mainly treated with Voriconazole. A haematological complete response (CR) was achieved in 70% of patients. Survival at 3 months (83%) and overall survival (OS; median = 13 months) were only influenced by the achievement of a haematological CR.

Evolution of inflammatory markers from D−12 to D12

Biomarkers were studied from D−12 to D12 and included 2140 assays of three biological markers (PCT = 459, CRP = 607 and Fib = 1074) (Fig. 2 and Table 2). PCT and Fib assays were performed in all 94 patients with a median of four PCT assays (1–17) and 12 Fib assays (5–20) per patient respectively. CRP



Days	–12 to –9	–8 to –6	–5 to –4	–3 to –2	–1 to 0	1 to 2	3 to 4	5 to 8	9 to 12
No of PCT assays	36	22	17	20	70	115	77	76	26
No of CRP assays	45	48	32	39	82	115	85	107	54
No of Fib assays	162	174	87	87	100	102	98	164	100

Figure 2 Evolution of median PCT, CRP and Fib per period for all pulmonary IFI (n = 94). Results are given in % compared with normal maximum threshold for PCT and Fib and relevant threshold for CRP (see *Methods*). Blue curve represents the evolution of median PCT; the green curve shows the evolution of median Fib and the purple curve, the evolution of median CRP. For each period, vertical lines define the quartiles.

assay was done in 82 patients [median = 6 (0–18) assays per patient].

For all 94 IFI (Fig. 2), the evolutions of median PCT, CRP and Fib values when compared with the maximum normal threshold (0.5 μg l^{-1}, 50 mg l^{-1} and 4 g l^{-1} for PCT, CRP and Fib respectively) showed:

- From D−12 to D0, a marked increase in CRP and Fib levels, reaching a median value at D0 of 108 mg l^{-1} (5–326) and 6.4 g l^{-1} (2–10.5), respectively, while median PCT levels remained well below the cut-off of 0.5 μg l^{-1} [with median value at D0 of 0.18 μg l^{-1} (0.05–3)]. In the same period, only 12%, 5% and 1.4% of tested patients had PCT above 0.5, 1 and 1.5 μg l^{-1} respectively. Conversely, CRP above 50, 75 and 100 mg l^{-1} was observed in 84%, 70% and 57% of cases and Fib above 4, 5 and 6 g l^{-1} was seen in 96%, 80% and 61% of cases respectively ($P < 10^{-7}$ for each comparison).
- From D1 to D12, median levels of CRP and Fib remained high (over 150% of normal maximum value) while median PCT was still below the normal value (Fig. 2). In this period, 30%, 13% and 6% of tested patients had PCT above 0.5, 1 and 1.5 μg l^{-1}, respectively. On the other hand, CRP above 50, 75 and 100 mg l^{-1} was observed in 90%, 83% and 72% of cases and Fib above 4, 5 and 6 g l^{-1} was seen in 97%, 83% and 66% of cases respectively ($P < 10^{-7}$ for each comparison).
- Overall, from D−12 to D12, only 6/94 patients (6.4%) had PCT values >1.5 μg l^{-1}, while CRP >100 mg l^{-1} and Fib >6 g l^{-1} were observed in 66/82 (80%) and 70/94 (75%) of patients respectively ($P < 10^{-7}$ for each comparison).

Table 2 Evolution of inflammatory parameters from day −12 to day 12 since IFI diagnosis for all patients.

	D−12 to D0 n = 93	D1 to D12 n = 94
PCT level (normal value <0.5 μg l^{-1})		
No of patients with PCT > 0.5 μg l^{-1}	9/73 (12%)	26/88 (30%)
No of patients with PCT >1 μg l^{-1}	4/73 (5%)	11/88 (13%)
No of patients with PCT >1.5 μg l^{-1}	1/73 (1.4%)	5/88 (6%)
CRP level (significant threshold <50 mg l^{-1})		
No of patients with CRP >50 mg l^{-1}	56/67 (84%)	73/81 (90%)
No of patients with CRP >75 mg l^{-1}	47/67 (70%)	67/81 (83%)
No of patients with CRP >100 mg l^{-1}	38/67 (57%)	58/81 (72%)
Fib level (normal value <4 g l^{-1})		
No of patients with Fib >4 g l^{-1}	89/93 (96%)	91/94 (97%)
No of patients with Fib >5 g l^{-1}	74/93 (80%)	78/94 (83%)
No of patients with Fib >6 g l^{-1}	57/93 (61%)	62/94 (66%)

IFI, invasive fungal infection.

The same trends were observed in both IPA and PM patients. In addition, evolution of the three biomarkers over time was not different in patients with or without antifungal prophylaxis.

Evolution of inflammatory markers from D−2 to D2

From D−2 to D2, 702 assays of the three biomarkers were performed (PCT = 200, CRP = 216 and Fib = 286) (Table 3). During this 5-day period, 24%, 11% and 3.4% of patients had PCT >0.5, 1 and 1.5 μg l^{-1} respectively. At the same time, 92%, 85% and 74% of patients had CRP >50, 75 and 100 mg l^{-1} and 97%, 85% and 65% of patients had Fib >4, 5 and 6 g l^{-1} ($P < 10^{-7}$ for each comparison).

In the same period, 20%, 6% and 1.5% of PCT assays were above 0.5, 1 and 1.5 μg l^{-1}, respectively, while CRP assays were above 50, 75 and 100 mg l^{-1} in 88%, 75% and 60%, respectively, and Fib assays were above 4, 5 and 6 g l^{-1} in 88%, 74% and 51%, respectively ($P < 10^{-7}$ for each comparison).

Between D−2 and D+2, in an ANOVA model on repeated measures, PCT values did not increase significantly ($P = 0.13$) whereas CRP and Fib values increased significantly ($P < 0.001$ in each case).

Discussion

Patients with acute leukaemia have long phases of chemotherapy-induced neutropenia (>10 days), which puts them at a higher risk of IFI.[19] These infections in patients with neutropenia are life-threatening.[1] Over the last 30 years, improvements in haematological treatments have been accompanied by an increase in the frequency of IFI in these patients especially invasive pulmonary fungal infection. Early detection and prompt initiation of antifungal therapy are critical factors that could decrease mortality.

Currently, the use of CT scan, the detection of mycological biomarkers such as *Aspergillus* galactomannan or β 1-3 glucan or molecular biology techniques can be helpful for IFI diagnosis. However, diagnosis is often delayed by a lack of efficiency and false positives and negatives.[20] Moreover, some methods are expensive and not available in all centres. Pathological evaluation and culture remain the cornerstones for the definite diagnosis, but the methods are time-consuming and cannot always be performed in cases of IFI, as patients may be unstable, hypoxic or with coagulopathy. In addition, clinical signs of IPA or PM are not very specific and are not always associated with fever. This was the case in our study, in which only half of the patients experienced fever.

Indeed, the use of biomarkers such as PCT, CRP and fib could lead to a suspicion of IPA or PM in high-risk haematology patients and in particular, in patients with prolonged neutropenia.

In this study, in accordance with the literature, the incidence of IPA or PM was approximately 10%.[1] We

Table 3 Evolution of inflammatory parameters from day −2 to day +2 since IFI diagnosis.

	IPA *n* = 77	PM *n* = 17	All patients *n* = 94
PCT level (normal value <0.5 μg l^{-1})			
No of patients with PCT >0.5 μg l^{-1}	16/72 (22%)	5/15 (33%)	21/87 (24%)
No of patients with PCT >1 μg l^{-1}	6/72 (8%)	4/15 (27%)	10/87 (11%)
No of patients with PCT >1.5 μg l^{-1}	1/72 (1.4%)	2/15 (13%)	3/87 (3.4%)
No of assays with PCT >0.5 μg l^{-1}	33/166 (20%)	6/34 (18%)	39/200 (20%)
No of assays with PCT >1 μg l^{-1}	8/166 (5%)	4/34 12%)	12/200 (6%)
No of assays with PCT >1.5 μg l^{-1}	1/166 (0.6%)	2/34 (6%)	3/200 (1.5%)
CRP level (significant threshold <50 mg l^{-1})			
No of patients with CRP >50 mg l^{-1}	55/61 (90%)	13/13 (100%)	68/74 (92%)
No of patients with CRP >75 mg l^{-1}	51/61 (84%)	12/13 (92%)	63/74 (85%)
No of patients with CRP >100 mg l^{-1}	44/61 (72%)	11/13 (85%)	55/74 (74%)
No of assays with CRP >50 mg l^{-1}	158/178 (89%)	32/38 (84%)	190/216 (88%)
No of assays with CRP >75 mg l^{-1}	136/178 (76%)	27/38 (71%)	163/216 (75%)
No of assays with CRP >100 mg l^{-1}	109/178 (61%)	21/38 (55%)	130/216 (60%)
Fib level (normal value <4 g l^{-1})			
No of patients with Fib >4 g l^{-1}	75/77 (97%)	16/17 (94%)	91/94 (97%)
No of patients with Fib >5 g l^{-1}	65/77 (84%)	15/17 (88%)	80/94 (85%)
No of patients with Fib >6 g l^{-1}	48/77 (62%)	13/17 (76%)	61/94 (65%)
No of assays with Fib >4 g l^{-1}	207/237 (87%)	46/49 (94%)	253/286 (88%)
No of assays with Fib >5 g l^{-1}	170/237 (72%)	42/49 (86%)	212/286 (74%)
No of assays with Fib >6 g l^{-1}	117/237 (49%)	28/49 (57%)	145/286 (51%)

IFI, invasive fungal infection; IPA, invasive pulmonary aspergillosis; PM, pulmonary mucormycosis; PCT, procalcitonin assay; CRP, C-reactive protein assay; Fib, fibrinogen assay.

observed that IPA and PM were accompanied by no increase or only a slight increase in PCT, which remained well below the threshold of 0.5 μg l^{-1}, while CRP and Fib rose significantly during the 8 days before the infection. When we focused on D−2 to D2, we found that only 24%, 11% and 3.4% of patients had a PCT >0.5, 1 and 1.5 μg l^{-1}, respectively, whereas CRP and Fib were most often above the maximum threshold. PCT production is stimulated by pro-inflammatory cytokines and is inhibited by gamma-interferon, which is one of the main factors that control IFIs and may decrease levels of PCT in IPAs.[21]

To date, few studies have investigated the interest of PCT in the diagnosis of IFIs,[13,14] and these studies generated controversial findings and lacked power because of the small sample size.

Most of these studies were conducted in non-neutropenic patients, many in ICUs and mostly focused on candidaemia. Most ICU-based studies concluded that PCT was lower during IFIs than in bacterial infections. Indeed in this setting, PCT levels in clinical sepsis are markedly higher in patients with bacteraemia than in those with candidaemia. A threshold value of procalcitonin at 5.5 μg l^{-1} was proposed to distinguish between fungal and bacterial infections. This cut-off had a negative predictive value of 100% and a positive predictive value of 65%.[22,23] Similar findings were observed in a recent study with a cut-off at 6.08 μg l^{-1}.[24]

Conversely, in a series of 34 patients, of whom 13 had IFI, it was shown that fungal infection could be suspected when PCT values <0.5 μg l^{-1} were combined with CRP <300 mg l^{-1}.[25]

In the setting of neutropenia, the review published by Sakr *et al.* [26] reported PCT cut-offs ranging from 0.5 to 1.3 μg l^{-1} to discriminate between bacterial and non-bacterial infection. However, some studies provided controversial results on the usefulness of PCT for the diagnosis of IFI in immunocompromised patients. In this setting, Petrikkos *et al.* [27] comparing 44 IFI with 47 bacterial infections, found that PCT <0.5 μg l^{-1} suggested IFI. In contrast, Ortega *et al.* [28] reported 77 allogeneic stem-cell transplant recipients with 14 bacterial infections, two candidaemia and five IPA, in which PCT exceeding 3 μg l^{-1} had 80% sensitivity and 100% specificity for IFI. At the same time, it was suggested that a delayed PCT peak higher than 5 μg l^{-1} observed beyond 3 days of persistent fever during neutropenia is helpful for the diagnosis of IFI.[29] The potential heterogeneity of the studied populations in these reports could partly explain these conflicting results.

Our study reports on homogenous and specific population of acute leukaemia patients with prolonged neutropenia. In these patients with IPA or PM, we found that PCT above 1.5 μg l^{-1} was observed in less than 5% of cases, either in the 12 days before IFI diagnosis or in the 2 days preceding or following the diagnosis while, in the same period, CRP and Fib were most often at a high level. It suggests that a PCT cut-off value of 1.5 μg l^{-1} could be helpful in the diagnosis of IPA or PM in the setting of neutropenic patients with other elevated markers of inflammation.

As in a previous report we found that Fib can reach very high values at the time of filamentous IFI.[5] Though Fib and CRP levels increase from 8 days before the IFI is suspected, their reliability as a marker of infection is hampered by very low specificity.[30] Curiously, we found that, the evolution of the three biomarkers before IFI diagnosis was not influenced by the antifungal prophylaxis. Nevertheless, among patients with prophylaxis, more than 50% of them received Fluconazole, which is not effective against IPA or PM.

In our study, the large cohort of neutropenic leukaemia patients is a major point. In this setting, our conclusions suggest that IPA or PM could be suspected when a low PCT is combined with increased CRP and Fib. Nevertheless, most of our patients had a probable diagnosis of IPA. Therefore, we cannot exclude the possibility that some of these patients had another type of IFI (e.g. invasive fusariosis with a false positive result for *Aspergillus* antigenemia).

However, the findings of our study are limited by the lack of a comparative cohort study. Currently, we are not able to determine the evolution of the three biomarkers in neutropenic leukaemia patients without IFI. In this setting, we can only suppose that CRP, Fib and PCT increase significantly in cases of bacterial infection. It would be interesting to conduct a prospective multicentre study in which all leukaemia patients receiving myeloablative chemotherapy had systematic PCT, CRP and Fib assays during neutropenia episodes. The comparison of PCT evolution between patients with IFI and those without IFI could be helpful to highlight the place of PCT in the diagnostic strategy. In this setting, a PCT cut-off value of 1.5 μg l^{-1} could be helpful to determine sample sizes.

Conclusion

In patients with acute leukaemia, the occurrence of IPA or PM was accompanied by a significant increase

in CRP and Fib while PCT remained low. These results support the use of combined PCT, Fib and CRP monitoring as an aid to such IFI diagnosis in high-risk haematological patients.

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Conflict of interest

All authors report no potential conflicts.

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