ORIGINAL ARTICLE MYCOLOGY

Breakthrough invasive fungal disease in patients receiving posaconazole primary prophylaxis: a 4-year study

N. Lerolle[1], E. Raffoux[2], G. Socie[3], S. Touratier[4], H. Sauvageon[4], R. Porcher[5], S. Bretagne[6], A. Bergeron[7], E. Azoulay[8], J.-M. Molina[9] and M. Lafaurie[1]

1) Infectious Diseases Intervention Unit, Hôpital Saint-Louis, APHP, University of Paris Diderot Paris 7, Paris, 2) Department of Haematology, Hôpital Saint-Louis, APHP, University of Paris Diderot Paris 7, Paris, 3) Haematology-Bone Marrow Transplantation Unit, Hôpital Saint-Louis, APHP, University of Paris Diderot Paris 7, Paris, 4) Pharmacy, Hôpital Saint-Louis, APHP, University of Paris Diderot Paris 7, Paris, 5) Centre d'Epidémiologie Clinique, Centre Cochrane Français, Hôpital de l'Hôtel-Dieu, INSERM U738, APHP, Paris, 6) Parasitology and Mycology Laboratory, Hôpital Saint-Louis, APHP, University of Paris Diderot Paris 7, Paris, 7) Department of Pneumology, Hôpital Saint-Louis, APHP, University of Paris Diderot Paris 7, Paris, 8) Department of Intensive Care Unit, Hôpital Saint-Louis, APHP, University of Paris Diderot Paris 7, Paris and 9) Department of Infectious Diseases, Hôpital Saint-Louis, APHP, University of Paris Diderot Paris 7, Paris, France

Abstract

Posaconazole (PSC) is currently recommended as primary prophylaxis in neutropenic patients with acute myeloid leukaemia (AML) and in allogenic haematopoietic stem cell transplantation (AHSCT) recipients with graft-versus-host disease (GVHD). Studies focusing on breakthrough invasive fungal disease (IFD) upon PSC prophylaxis show disparate results. In order to evaluate the incidence of IFD in patients on PSC prophylaxis and identify IFD risk factors, we carried out a retrospective study of all consecutive patients on PP from January 2007 to December 2010 in our hospital. Breakthrough IFDs were identified from the database of the central pharmacy and the French administrative database (PMSI), registering final medical diagnoses of hospitalized patients. Medical data were reviewed to study proven or probable IFD, according to EORTC/MSG definition. PSC plasma concentrations (PPC) were also retrieved. Poisson models were used for statistical analysis. Two hundred and seventy-nine patients received PSC prophylaxis for a median duration of 1.4 months (range 0.2–17.9). Proven (n = 6) or probable (n = 3) IFDs were diagnosed in nine cases (3.2%). IFD incidence rate per 100 person-month was 1.65 (95% CI, 0.79–2.97). IFDs were candidaemia (*Candida glabrata*, n = 2), pulmonary invasive aspergillosis (n = 3), disseminated fusariosis (n = 2) and pulmonary mucormycosis (n = 2). Seven deaths were reported, directly related to IFD in three patients (33.3%). First dosage of PPC under 0.3 mg/L was the single significant risk factor for IFD (RR, 7.77; 95% CI, 1.30–46.5; p 0.025). Breakthrough IFD in patients receiving PSC prophylaxis is rare but associated with a poor outcome. Low PSC plasma concentrations are associated with an increased risk of IFD.

Keywords: Antifungal prophylaxis, candidaemia, fusariosis, invasive aspergillosis, invasive fungal disease, mucormycosis, posaconazole, therapeutic drug monitoring
Original Submission: 21 February 2014; **Revised Submission:** 12 May 2014; **Accepted:** 17 May 2014
Editor: E. Roilides
Article published online: 26 May 2014
Clin Microbiol Infect 2014; **20**: O952–O959
10.1111/1469-0691.12688

Corresponding author: N. Lerolle, Infectious Diseases Intervention Unit, Hôpital Saint-Louis, APHP, University of Paris Diderot Paris 7, 75010 Paris, France
E-mail: nathalie.lerolle@gmail.com

Introduction

Despite improvement in the management of acute leukaemia (AL) and allogenic haematopoietic stem cell transplantation (AHSCT), invasive fungal infection (IFD) remains an important cause of morbidity and mortality in these settings [1,2]. Identified risk factors for IFD, such as prolonged and profound neutropenia, corticosteroids and immunosuppressive therapies, recent surgery, exposure to invasive devices, broad-spectrum antibiotic therapy, older age, diabetes mellitus, renal failure, viral infections and fungal colonization, are often combined in patients with haematological malignancies [3]. A poor outcome is often described during the course of IFD [2,4–6]. Primary prophylaxis using fluconazole has been proven to prevent *Candida* infections and to reduce mortality

in patients with haematological cancers [7]. With fluconazole prophylaxis, the incidence of candidaemia has declined, but an increase of bloodstream infections due to non-*albicans Candida* species, including fluconazole-resistant *C. glabrata* and *C. krusei*, has been reported [8]. *Aspergillus* spp. and other moulds, including zygomycetes and *Fusarium* spp., have become more frequent causes of IFD [2,5,9]. New agents were subsequently developed for IFD prophylaxis, such as posaconazole (PSC), which was demonstrated to be superior to fluconazole for IFD prevention in two large controlled trials [10,11] and is currently recommended as primary prophylaxis for patients with acute myeloid leukaemia (AML) and chemotherapy-induced neutropenia and for AHSCT recipients with graft-versus-host disease (GVHD).

Posaconazole is an extended-spectrum triazole, with potent antifungal activity against most *Candida* spp., *Aspergillus* spp., *Fusarium* spp., mucormycetes and endemic fungi [12]. Therapeutic drug monitoring is advocated as inter- and intra-variability in absorption might affect drug efficacy [13]. Breakthrough IFD during the exposure period of PSC prophylaxis was reported in 2% of neutropenic patients [10] and 2.4% of AHSCT recipients [11] in the two pivotal controlled trials. However, other studies show disparate results, with IFD incidence varying from 0 to 17% [14–22].

The principal aim of our study was to investigate in the 'real-life' setting the incidence of breakthrough IFD occurring in patients with haematological cancers receiving PSC primary prophylaxis. Secondary objectives were to identify risk factors for breakthrough IFD, and to describe clinical, microbiological and radiological features of IFD and their outcome.

Material and Methods

We conducted a retrospective study in which we reviewed the medical records of all adult patients consecutively treated by PSC as primary prophylaxis at the Hôpital Saint-Louis from January 2007 to December 2010. Saint-Louis Hospital is a 650-bed tertiary hospital with major clinical activities in haematology. Adult patients with AML, GVHD following AHSCT, idiopathic aplastic anaemia or myelodysplastic syndrome were included if they received antifungal primary prophylaxis with PSC for at least 7 days.

Patients treated with systemic antifungal agents (PSC, liposomal amphotericin-B, caspofungin or voriconazole) at our institution were identified from the computerized database of the central pharmacy, as these drugs are provided using restrictive nominative formularies both for hospitalized patients and outpatients (in France, PSC is only available through hospital pharmacies). Physicians have to provide the reason for PSC prescription on this form (either prophylaxis or curative treatment). Patients treated with PSC and subsequently with another systemic antifungal agent or maintained on PSC as a curative treatment for a suspected IFD were studied to detect breakthrough IFD. Breakthrough IFD was defined as occurrence of a proven or probable IFD according to EORTC/MSG revised definitions [21] while on PSC prophylaxis (PP) or within 15 days after discontinuation of PP. Medical charts were retrieved to assess indication for PSC treatment and to confirm that subsequent systemic antifungal agents were used for treating proven or probable IFD. In order to complete the database, this first list was cross-matched with the electronic medical record-based information that codes medical activity and patients' diagnoses. All patients treated with PSC, and for whom a coding for 'opportunistic mycoses' was notified, were studied. For patients still receiving PSC at the end of 2010, follow-up was continued for up to 4 additional months.

Data recorded were: demographics, underlying haematological disease and phase of the disease, date of PSC prophylaxis initiation and discontinuation, and PSC plasma concentrations (PPCs) on the fifth day of treatment or after. Cumulated duration of PSC prophylaxis was calculated by adding duration of all courses of PSC prophylaxis. In the case of IFD, additional data were studied: clinical presentation, biological data, chest computed tomography scan (CT), direct and indirect mycological diagnosis, treatment of IFD and outcome.

Prophylactic PSC was orally administered at the recommended daily dose of 600 mg, ideally with high-fat meals, acid beverage or enteric feedings. Galactomannan blood index was measured systematically once a week in the bone marrow transplantation unit and chest computed tomography (CT) was performed before the transplantation. No routine diagnostic procedure was performed in other units unless an IFD was suspected. IFD onset was defined as the day of occurrence of new clinical or radiology symptoms. Neutropenia was defined as an absolute leukocyte count $<1000/mm^3$ and/or neutrophil count $<500/mm^3$. When possible, bronchoscopy was performed in the case of suspected pulmonary infection. Bronchial aspirate and bronchoalveolar lavage (BAL) were analysed for bacteria, viruses, parasites and fungal pathogens. Galactomannan antigen was detected using a sandwich immunocapture ELISA (Platelia Aspergillus, Bio-Rad, Marne la Coquette, France). If there was suspicion of IFD, it was measured in the serum. Results were expressed as an index of positivity and were considered positive if ≥ 0.5 in the serum, according to the manufacturer's recommendations. The min-

imal inhibitory concentrations (MICs) of azole were determined according to the Antifungal Susceptibility Testing Subcommittee of the European Committee on Antimicrobial Susceptibility Testing (AFST-EUCAST) definitive document [22]. Plasma concentrations of PSC (PPC) were quantified as described previously [23]. The limit of quantification was set at 0.05 mg/L. PPC was expected to be above 0.5 mg/L, according to local recommendations. This threshold and the first IQR of PPC were studied for the risk factor analysis. At the time of the study, there was no formal protocol to monitor PPC in our institution. However, a comment was systematically added by the pharmacist to the results of the PPC, encouraging the prescribers to increase the doses of PSC when the PPC was low, to check adherence and to look for potential absorption issues.

Data were presented as mean (standard deviation), median (range) or count (per cent). The incidence rate of IFD and potential risk factors were analysed using Poisson models accounting for duration of exposure (i.e. cumulated duration of PSC prophylaxis until IFD or end of treatment for each patient). Analyses were carried out using the R statistical software version 2.15.2 (The R Foundation for Statistical Computing, Vienna, Austria).

Results

From January 2007 to December 2010, 354 patients treated for haematological cancer received PSC according to the computerized database of the central pharmacy (Fig. 1). Among these patients, 84 were excluded because they received PSC for <7 days (n = 29), for curative treatment (n = 44), for secondary prophylaxis (n = 2) or while being treated for another underlying disease (n = 9). Finally, 270 patients (122 women) received PSC as primary prophylaxis and were included in this study (Table 1). The mean age was 48.6 years (range, 19–87 years). One hundred and sixty-eight patients (62.2%) were treated with high-dose chemotherapy for acute myeloid leukaemia and 96 (35.6%) were treated for GVHD following an AHSCT. Median PSC treatment duration was 1.4 months (range, 0.2–17.9). One hundred and forty-eight patients (54.8%) had at least one PPC test. Among these patients, the median number of PPCs was two (IQR, one to three). Thirty-one patients (20.9%) had a first PPC under 0.3 mg/L, and 35 (23.6%) had a first PPC between 0.3 and 0.5 mg/L.

While on PSC primary prophylaxis, 40 patients (14.8%) switched to another antifungal therapy (AFT) because an IFD was suspected. No patients were maintained on PSC as a curative treatment for a suspected IFD. Clinical charts of all these patients were reviewed to confirm or exclude proven or probable breakthrough IFD. Among these 40 patients, 31 were excluded from the analysis for the following reasons: PSC stopped for more than 15 days, n = 20; no IFD, n = 6; possible IFD, n = 4; missing data, n = 1 (Fig. 1). Finally, proven (n = 6) or probable (n = 3) breakthrough IFD was diagnosed in nine cases (3.3%). The incidence rate of IFD per 100 person-month during PSC prophylaxis was 1.68 (95% CI, 0.81–3.03). All patients were still on PSC prophylaxis at the onset of IFD. No IFD was diagnosed during the four additional months

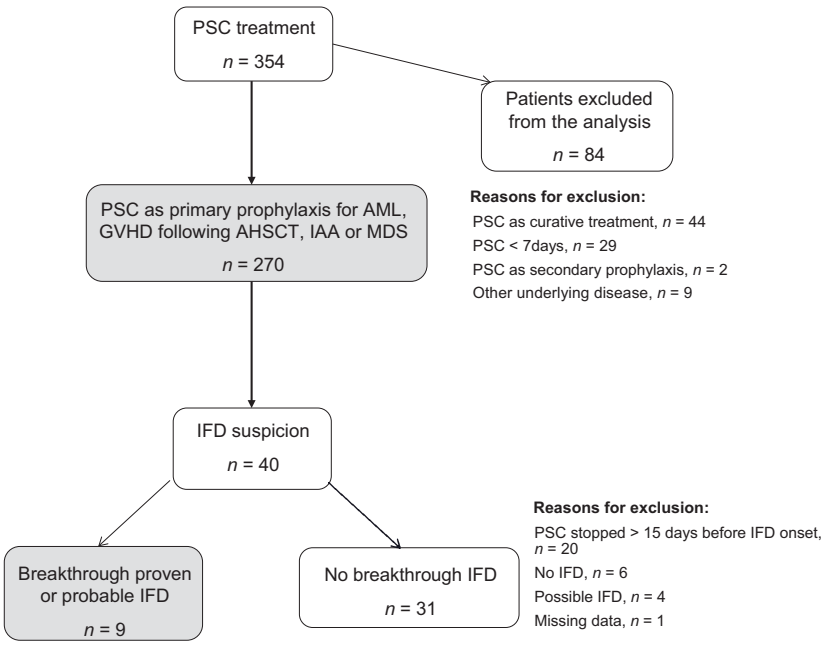

FIG. 1. Study flow-chart of patients included in the study. PSC, posaconazole; AML, acute myeloid leukaemia; GVHD, graft-versus-host disease; AHSCT, allogenic haematopoietic stem cell transplantation; IAA, idiopathic aplastic anaemia; MDS, myelodysplastic syndrome; IFD, invasive fungal infection.

TABLE 1. Characteristics of the patients on PSC primary prophylaxis and of PSC plasma concentration testing (PPC)

Characteristics	All *n* = 270	IFD *n* = 9	No IFD *n* = 261
Age, mean (SD) years	48.6 (14.5)	45.9 (15.3)	48.7 (14.5)
Women, no. (%)	122 (45.2)	4 (44)	118 (45.2)
Disease, no. (%)			
AML	168 (62.2)	8 (89)	160 (61.3)
GVHD following AHSCT	96 (35.6)	1 (11)	95 (36.4)
IAA	4 (1.5)	0	4 (1.5)
MDS	2 (0.7)	0	2 (0.8)
PSC prophylaxis duration, median (range) months	1.4 (0.2–17.9)	0.7 (0.2–7.4)	1.4 (0.2–17.9)
Number of patients with PPC testing, no. (%)	148 (54.8)	7 (78)	141 (54)
Median number of tests (IQR)	2 (1–3)	2 (1–2)	2 (1–3)
First PPC, median (IQR) mg/L	0.57 (0.32–0.86)	0.35 (0.18–0.54)	0.60 (0.34–0.88)
First PPC<0.3 mg/L, no. (%)	31 (20.9)	3 (43)	28 (19.9)
First PPC ≥ 0.3 and <0.5 mg/L, no. (%)	35 (23.6)	2 (29)	33 (23.4)
Median of all PPCs, median (IQR) mg/L	0.60 (0.36–0.86)	0.43 (0.27–0.71)	0.60 (0.36–0.87)
Last PPC before IFD, median (IQR) mg/L		0.43 (0.35–0.57)	

IFD, invasive fungal disease; SD, standard deviation; AML, acute myeloid leukaemia; GVHD, graft-versus-host disease; AHSCT, allogenic haematopoietic stem cell transplantation; IAA, idiopathic aplastic anaemia; MDS, myelodysplastic syndrome; PSC, posaconazole; PPC, PSC plasma concentration.

of follow-up in the patients on PSC prophylaxis at the end of 2010. No additional cases were found with the database that coded patients' diagnoses.

Patients with breakthrough IFD are described in Table 2; 55.6% were male and the median age was 47 years (range, 18–65). Underlying diseases or conditions were AML (n = 7, 7/7 on induction therapy), acquired aplastic anaemia (n = 1) and GVHD following allogenic blood transplantation (n = 1). All patients had a central venous catheter and received broad spectrum antibiotics in the month before the onset of IFD. All except one patient (an AHSCT recipient) were neutropenic at the onset of IFD. Median duration of neutropenia before the onset of IFD was 17 days (range, 10–31). The nadir value of absolute neutrophil count was <100/mm^3 in all patients. Total lymphocyte count at the time of IFD in the AHSCT recipient with GVHD was 300/mm^3.

Invasive fungal diseases were candidaemia (*C. glabrata*, n = 2), pulmonary aspergillosis (n = 3), disseminated fusariosis (n = 2) and pulmonary mucormycosis (n = 2). Amongst the six patients with positive mycological culture, five isolates were tested for susceptibility to antifungal agents and four out of five isolates had high MIC to PSC, including both *Candida* isolates. Liposomal amphotericin B was the most common first-line antifungal treatment (eight out of nine patients). Median duration of PSC prophylaxis at IFD onset was 18 days (range, 7–126).

Posaconazole plasma concentration could be retrieved in seven patients. The first PPC sample was retrieved 10 days or more after PSC initiation in four out of seven patients (median, 10 days; range, 6–17). Median first PPC was 0.35 mg/L (IQR, 0.18–0.54). The first PPC was under 0.5 mg/L in five patients (71.4%), among whom three patients (42.9%) had a first PPC under 0.3 mg/L. In two of these last three patients (P4 and P7) the daily dose of PSC had been increased from 600 to 900 mg because the first PPC was under 0.3 mg/L while on a 600 mg daily regimen. The second PPC sample was higher in these two patients (respectively, 0.76 and 0.46 mg/L) but IFD had already occurred by this time. Strikingly, the first PPC was performed late after the initiation of PP in these two patients; respectively, after 14 and 17 days of prophylaxis. Four patients (44.4%) were treated by proton pump inhibitor, which might have an impact on PSC absorption. Two patients (22%) had diarrhoea and one had mucositis (11%) the week before IFD onset.

Median duration of follow-up after IFD was 5 months (range, 1–36). Five patients were cured at the end of the curative antifungal therapy, one had improved but died of refractory AML, and three died because of severe IFD (two with proven or probable aspergillosis and one with disseminated fusariosis). Four additional non-IFD-related deaths were reported. One patient with candidaemia experienced a relapse of candidaemia 2 months after the first episode, while on PSC secondary prophylaxis.

Compared with patients on PSC prophylaxis who did not experience IFD, the single significant risk factor was a first PPC<0.3 mg/L (RR, 7.90; 95% CI, 1.32–47.3; p 0.024; Table 3). The prevalence of breakthrough IFD was higher among leukaemic patients on induction chemotherapy than among AHSCT recipients with GVHD (4.2 vs. 1%; p 0.058), but the difference did not reach statistical significance. No significant difference in the incidence of IFD was found between patients who had PPC testing (n = 148/270; 54.8%) and others (RR, 1.93; 95% CI, 0.40–9.29; p 0.41).

Discussion

Breakthrough proven or probable IFD in 270 patients receiving PSC prophylaxis occurred in 3.3% of cases in this 4-year retrospective study, in 4.2% of neutropenic patients

TABLE 2. Patients with proven and probable IFD on PSC prophylaxis

Patient Gender Age, years	IFD	Underlying disease	Neutropenia <500/mm^3, duration (days) at IFD onset and evolution after IFD	PP duration (days)	PP regimen	First PPC (mg/L) (days after PSC initiation/ days before IFD)	Negative factors of PSC absorption	Clinical and biological signs	Chest CT scan signs	Positive mycological results	Negative mycological results[a]	Antifungal therapy	Outcome
P1 F 18	Proven pulmonary aspergillosis (*Aspergillus terreus*) MIC: AmB >4, VRC 1, PSC 0.125	AML on induction therapy	Yes (10) PNN<500/mm^3 until death	9	300 mg BID	0.17 (6/4)	Diarrhoea	Fever, cough, thoracic pain, dyspnoea	Area of consolidation and bilateral nodules surrounded by ground-glass attenuation	GI-B >0.5 Pulmonary biopsy+ (DM +, culture +, histology +)	TA- BAL-	L-amB then VRC + CAS	IFD-related death
P2 M 48	Proven disseminated fusariosis (*Fusarium solani*) MIC: AmB 8, VRC ≥8, PSC ≥8	AML on induction therapy	Yes (14) PNN>500/mm^3 at D+6	18	300 mg BID	0.35 (10/8)		Fever, skin lesions, myalgia	Bilateral nodules surrounded by ground-glass attenuation	Cutaneous biopsy+ (DM +, culture +, histology +)		VRC	IFD cured, AML relapse, death from AML
P3 F 39	Proven disseminated fusariosis (*Fusarium solani*) MIC: AmB 2, VRC 4, PSC ≥8	AML on induction therapy	Yes (15) PNN<500/mm^3 until death	13	300 mg BID	NA		Fever, skin lesions, myalgia, hepatic cytolysis	NA	BC+ Cutaneous biopsy+ (DM −, culture +, histology +)		L-amB	IFD-related death
P4 M 47	Proven pulmonary mucormycosis	AML on induction therapy	Yes (20) PNN>500/mm^3 at D+6	24	300 mg TID	0.20 (7/10)	Mucositis	Fever, thoracic pain	Area of consolidation and bilateral nodules surrounded by ground-glass attenuation	Pulmonary biopsy+ (DM +, culture −, histology +)	GI-B- TA- BAL-	L-amB and surgery	IFD cured, death from AML
P5 F 30	Candidaemia (*C. glabrata*) MIC: FLC 32, VRC 4, PSC 2, CAS 0.125	GVHD following AHSCT for B-ALL (treated by CS and IST)	No	126	300 mg BID	0.82 (13/113)	PPI	Fever	NA	BC+		L-amB	IFD cured, candidaemia relapse, death from bacterial pneumonia
P6 F 59	Candidaemia (*C. glabrata*) MIC FLC 32, VRC 0.25, PSC 0.25, CAS 0.06	AML on induction therapy	Yes (13) PNN>500/mm^3 at D+8	7	200 mg TID	0.43 (6/1)	PPI	Fever	Proximal pulmonary embolism, excavated nodules and area of consolidation (probable septic pulmonary embolism)	BC+		L-amB + 5FC then CAS	IFD cured
P7 M 62	Probable pulmonary mucormycosis (*Rhizopus sp.*) MIC NA	AML on induction therapy	Yes (32) PNN>500/mm^3 at D+12	32	300 mg TID	0.00 (17/7)	PPI	Fever, cough, haemoptysis, sinusitis	Bilateral nodules surrounded by ground-glass attenuation	TA+ (DM+ culture+)	GI-B- BAL- Sinus biopsy-	L-amB	IFD improvement, death from AML

TABLE 2. (*Continued*)

Patient Gender Age, years	IFD	Underlying disease	Neutropenia <500/mm^3, duration (days) at IFD onset and evolution after IFD	PP duration (days)	PP regimen	First PPC (mg/L) (days after PSC initiation/ days before IFD)	Negative factors of PSC absorption	Clinical and biological signs	Chest CT scan signs	Positive mycological results	Negative mycological results[a]	Antifungal therapy	Outcome
P8 M 65	Probable pulmonary aspergillosis	IAA (treated with IST + ALG)	Yes (27) PNN<500/mm^3 until death	24	300 mg BID	0.65 (10/14)		Fever, cough, haemoptysia	Bilateral nodules surrounded by ground-glass attenuation	GI-B >0.5	TA-BAL-	VRC then L-amB then VRC + CAS	IFD-related death
P9 M 41	Probable pulmonary aspergillosis	AML on induction therapy	Yes (20) PNN>500/mm^3 at D+3	16	300 mg BID	NA	PPI and diarrhoea	Fever	Bilateral nodules surrounded by ground-glass attenuation	GI-B >0.5	TA-BAL-	L-amB then VRC	IFD cured

G, gender; IFD, invasive fungal disease; AmB, amphotericin B; FLC, fluconazole; VRC, voriconazole; PSC, posaconazole; CAS, caspofungin; PP, PSC prophylaxis; PPC, PSC plasma concentration; PPI, proton pump inhibitor; CT, computer tomography; AML, acute myeloid leukaemia; GI, galactomannan index (threshold 0.5); GI-B, GI in serum; DM, direct microscopy; TA, tracheal aspiration; L-amB, lipid amphotericin B formulation; NA, not available; BC, blood culture; AHSCT, allogenic haematopoietic stem cell transplantation; ALL, acute lymphoid leukaemia; GVHD, graft-versus-host disease; CS, corticosteroid; IAA, idiopathic aplastic anaemia; IST, immunosuppressive therapy; ALG, anti-lymphocyte globulin.
[a]Mycological negative results: negative direct microscopy and culture.

with AML and 1% of AHSCT recipients. These results are consistent with the two randomized clinical trials that found a 2% incidence rate in neutropenic patients [10] and 2.4% in AHSCT recipients [11], and suggest that breakthrough IFD is also rare in real-life conditions in patients with standard follow-up. A recent study found also a 3.8% incidence of probable and proven IFD in 260 leukaemic patients on PSC prophylaxis [24]. Several observational studies focused on routine use of PSC prophylaxis and found breakthrough IFD rates ranging from 0 to 17% [14–22]. These discrepancies might be explained by the number of patients studied (most of the time <100), a shorter or no minimal duration of PSC prophylaxis (although steady state is usually achieved between 5 and 7 days of treatment) and less stringent IFD diagnosis criteria (possible IFD was not included in our study). They may also reflect the heterogeneity of patients, chemotherapy and transplantation practices, IFD diagnostic modalities and PPC monitoring.

Four out of the nine breakthrough IFD cases (45%) in our study were mucormycosis or fusariosis, whereas these diseases only accounted for <20% of IFD in patients with haematological disease and AHSCT recipients according to observational studies performed before PSC was widely used as primary prophylaxis [1,2,5], and in the two pivotal randomized trials of PSC prophylaxis [10,11]. Similarly, a recent Austrian retrospective study reported six cases of mucormycosis among 11 proven IFDs occurring in 95 patients on PSC prophylaxis [16]. Limited efficacy of PSC for *Rhizopus* spp., *Rhizomucor* spp. and some species of *Fusarium* [12] might explain these findings. Concerns have been raised about an eventual shift of the IFD spectrum towards uncommon moulds since the use of PSC prophylaxis, but data are still limited and, as IFDs are rare events even in haematological settings, it is difficult to have solid evidence of a significant increase in the incidence of mucormycosis and fusariosis. In our study, a high proportion of isolates had high MIC to PSC, which suggests a selection of resistant fungi by PSC prophylaxis. As in the literature, IFD-related deaths were common in our study and occurred in patients with aspergillosis and fusariosis.

We found that a PPC <0.3 mg/L was associated with a significant increased risk of IFD (RR, 7.90; 95% CI, 1.32–47.3; p 0.024). In some patients, the first PPC testing was performed late, 10 days or more after PSC initiation, and we could postulate that some breakthrough IFD might have been prevented by monitoring PPC earlier and targeting a higher level of PPC. Several studies suggest also that low PSC plasma concentrations are associated with an increased risk of IFD [14,15]. However, a French study did not detect any breakthrough IFD among 36 patients on PSC prophylaxis, despite a low PPC (<0.5 mL) in 44% of cases [25].

TABLE 3. IFD incidence and risk factor analysis

Variable	Estimate	p-value
Suspicion of IFD, no. (%)	40 (14.8)	
Breakthrough IFD, no. (%)	9 (3.3)	
Total no. of person-month	535.2	
IFD incidence rate (95% CI), per 100 person-month	1.68 (0.81–3.03)	
Test for constant rate		0.68
Analysis of risk factors, RR (95% CI)		
Age, years	0.99 (0.95–1.04)	0.76
Male gender	0.72 (0.19–2.68)	0.62
GVHD vs. AML	0.13 (0.017–1.07)	0.058
First PPC categories		
≥0.5 mg/L	1	(Reference)
≥0.3 and <0.5 mg/L	3.80 (0.54–27.0)	0.18
<0.3 mg/L	7.90 (1.32–47.3)	0.024

IFD, invasive fungal disease; GVHD, graft-versus-host disease; AML, acute myeloid leukaemia; PPC, PSC plasma concentration.

Anyway, accurate target concentrations for PSC prophylaxis remain controversial. PSC bioavailability is highly variable and depends on several factors: concomitant high-fat meals and acid beverages enhance PSC plasma concentrations, whereas diarrhoea, mucitis and several medications such as anti-acid proton pump inhibitors might reduce PSC absorption [15,25–27]. For all these reasons, therapeutic drug monitoring is advocated by most authors [15,25,28], even if the plasma concentration imperfectly reflects what happens in infected tissues [29]. Other risk factors for IFD in patients on PSC prophylaxis might influence the effectiveness of PSC prophylaxis: increased age and lack of response to induction chemotherapy, as reported by a monocentric prospective study [30], or genetic variants, as suggested by several studies (review in Ref. [31]).

Our study has some limitations. The retrospective and single-centre design of this study could have underestimated the incidence of IFD among patients on PSC prophylaxis. Indeed, some patients could have been diagnosed and treated for a breakthrough IFD in another hospital, or could have died due to another reason while having an undiagnosed breakthrough IFD. Moreover, our study had limited power to identify IFD risk factors, because of the small number of patients with breakthrough IFD. In addition, the phase of the disease at the time of PSC prophylaxis was not described in patients without IFD, whereas we acknowledge that it would have been relevant information. The occurrence of IFD before initiation of PSC prophylaxis in patients treated for AML is very unlikely as PSC is usually initiated within days after the occurrence of neutropenia, but we can not absolutely rule out a baseline IFD in these patients. Last, not all patients had PPC testing, but no significant difference in the incidence of IFD was found between patients who had PPC testing and others.

In conclusion, these data demonstrate that IFD is rare in patients on PSC prophylaxis in real-life conditions, but is associated with a poor outcome. A high frequency of PSC-resistant fungi amongst breakthrough IFDs is suggested by our results. In our study, a PPC below 0.3 mg/L was associated with a significant increased risk of IFD, supporting the usefulness of therapeutic drug monitoring. We suggest that, in the case of a low PPC, prompt actions in order to increase PPC should be initiated: optimization of adherence to treatment, avoidance of drug interactions, administration with 'fatty meals', and/or increasing PSC dosage, in addition to closer PPC monitoring. New PSC galenics (i.e. intravenous formulations and oral tablets) may help to improve adherence to PSC treatment and PSC absorption, and thus to optimize the effectiveness of antifungal prophylaxis in high-risk haematological patients. Further larger studies are needed to identify specific risk factors for IFD in patients on PSC prophylaxis, and to define the optimal PSC plasma concentration.

Acknowledgements

The authors wish to thank the nurses, microbiologists, radiologists and clinicians who actively participated in the patients' care.

Author's Contribution

NL and ML contributed to the study design, case analysis, patients' management and manuscript preparation. NL contributed to the data collection. ER, GS, AB and EA contributed to the study design and patients' management. ST and HS contributed to pharmacy database management and the PPC assessment. RP contributed to the statistical analysis. SB contributed to the mycological analysis. JMM contributed to the study design, patients' management and manuscript preparation. All authors read and approved the final manuscript.

Transparency Declaration

All authors declared that there are no conflicting interests. No grants were received by the authors for this study.

References

1. Pagano L, Caira M, Rossi G *et al.* A prospective survey of febrile events in hematological malignancies. *Ann Hematol* 2012; 91: 767–774.

2. Kontoyiannis DP, Marr KA, Park BJ *et al.* Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clin Infect Dis* 2010; 50: 1091–1100.
3. Worth LJ, Slavin MA. Bloodstream infections in haematology: risks and new challenges for prevention. *Blood Rev* 2009; 23: 113–122.
4. Lortholary O, Gangneux J-P, Sitbon K *et al.* Epidemiological trends in invasive aspergillosis in France: the SAIF network (2005–2007). *Clin Microbiol Infect* 2011; 17: 1882–1889.
5. Neofytos D, Horn D, Anaissie E *et al.* Epidemiology and outcome of invasive fungal infection in adult hematopoietic stem cell transplant recipients: analysis of Multicenter Prospective Antifungal Therapy (PATH) Alliance registry. *Clin Infect Dis* 2009; 48: 265–273.
6. Hahn-Ast C, Glasmacher A, Mückter S *et al.* Overall survival and fungal infection-related mortality in patients with invasive fungal infection and neutropenia after myelosuppressive chemotherapy in a tertiary care centre from 1995 to 2006. *J Antimicrob Chemother* 2010; 65: 761–768.
7. Marr KA, Seidel K, Slavin MA *et al.* Prolonged fluconazole prophylaxis is associated with persistent protection against candidiasis-related death in allogeneic marrow transplant recipients: long-term follow-up of a randomized, placebo-controlled trial. *Blood* 2000; 96: 2055–2061.
8. Sipsas NV, Lewis RE, Tarrand J *et al.* Candidemia in patients with hematologic malignancies in the era of new antifungal agents (2001–2007): stable incidence but changing epidemiology of a still frequently lethal infection. *Cancer* 2009; 115: 4745–4752.
9. Lanternier F, Dannaoui E, Morizot G *et al.* A global analysis of mucormycosis in France: the RetroZygo Study (2005–2007). *Clin Infect Dis* 2012; 54: S35–S43.
10. Cornely OA, Maertens J, Winston DJ *et al.* Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *N Engl J Med* 2007; 356: 348–359.
11. Ullmann AJ, Lipton JH, Vesole DH *et al.* Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. *N Engl J Med* 2007; 356: 335–347.
12. Torres HA, Hachem RY, Chemaly RF, Kontoyiannis DP, Raad II. Posaconazole: a broad-spectrum triazole antifungal. *Lancet Infect Dis* 2005; 5: 775–785.
13. Lipp H-P. Posaconazole: clinical pharmacokinetics and drug interactions. *Mycoses* 2011; 54 (suppl 1): 32–38.
14. Hoenigl M, Raggam RB, Salzer HJF *et al.* Posaconazole plasma concentrations and invasive mould infections in patients with haematological malignancies. *Int J Antimicrob Agents* 2012; 39: 510–513.
15. Dolton MJ, Ray JE, Marriott D, McLachlan AJ. Posaconazole exposure-response relationship: evaluating the utility of therapeutic drug monitoring. *Antimicrob Agents Chemother* 2012; 56: 2806–2813.
16. Auberger J, Lass-Flörl C, Aigner M *et al.* Invasive fungal breakthrough infections, fungal colonization and emergence of resistant strains in high-risk patients receiving antifungal prophylaxis with posaconazole: real-life data from a single-centre institutional retrospective observational study. *J Antimicrob Chemother* 2012; 67: 2268–2273.
17. Low CY, Ong WC, Wong E *et al.* Real-world experience with posaconazole prophylaxis in high-risk hematological patients in Singapore: a prospective audit. *J Infect* 2013; 66: 291–294.
18. Vehreschild JJ, Rüping MJGT, Wisplinghoff H *et al.* Clinical effectiveness of posaconazole prophylaxis in patients with acute myelogenous leukaemia (AML): a 6 year experience of the Cologne AML cohort. *J Antimicrob Chemother* 2010; 65: 1466–1471.
19. Hahn J, Stifel F, Reichle A, Holler E, Andreesen R. Clinical experience with posaconazole prophylaxis–a retrospective analysis in a haematological unit. *Mycoses* 2011; 54 (suppl 1): 12–16.
20. Winston DJ, Bartoni K, Territo MC, Schiller GJ. Efficacy, safety, and breakthrough infections associated with standard long-term posaconazole antifungal prophylaxis in allogeneic stem cell transplantation recipients. *Biol Blood Marrow Transplant* 2011; 17: 507–515.
21. De Pauw B, Walsh TJ, Donnelly JP *et al.* Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 2008; 46: 1813–1821.
22. Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST). EUCAST definitive document EDef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts. *Clin Microbiol Infect* 2008; 14: 398–405.
23. Pennick GJ, Clark M, Sutton DA, Rinaldi MG. Development and validation of a high-performance liquid chromatography assay for voriconazole. *Antimicrob Agents Chemother* 2003; 47: 2348–2350.
24. Pagano L, Caira M, Candoni A *et al.* Evaluation of the practice of antifungal prophylaxis use in patients with newly diagnosed acute myeloid leukemia: results from the SEIFEM 2010-B registry. *Clin Infect Dis* 2012; 55: 1515–1521.
25. Lebeaux D, Lanternier F, Elie C *et al.* Therapeutic drug monitoring of posaconazole: a monocentric study with 54 adults. *Antimicrob Agents Chemother* 2009; 53: 5224–5229.
26. Tonini J, Thiébaut A, Jourdil JF *et al.* Therapeutic drug monitoring of posaconazole in allogeneic hematopoietic stem cell transplantation patients who develop gastrointestinal graft-versus-host disease. *Antimicrob Agents Chemother* 2012; 56: 5247–5252.
27. Vaes M, Hites M, Cotton F *et al.* Therapeutic drug monitoring of posaconazole in patients with acute myeloid leukemia or myelodysplastic syndrome. *Antimicrob Agents Chemother* 2012; 56: 6298–6303.
28. Ananda-Rajah MR, Grigg A, Slavin MA. Making sense of posaconazole therapeutic drug monitoring: a practical approach. *Curr Opin Infect Dis* 2012; 25: 605–611.
29. Conte JE Jr, Golden JA, Krishna G *et al.* Intrapulmonary pharmacokinetics and pharmacodynamics of posaconazole at steady state in healthy subjects. *Antimicrob Agents Chemother* 2009; 53: 703–707.
30. Michallet M, Sobh M, Morisset S *et al.* Risk factors for invasive aspergillosis in acute myeloid leukemia patients prophylactically treated with posaconazole. *Med Mycol* 2011; 49: 681–687.
31. Van der Velden WJFM, Blijlevens NMA, Donnelly JP. Genetic variants and the risk for invasive mould disease in immunocompromised hematology patients. *Curr Opin Infect Dis* 2011; 24: 554–563.