Posaconazole prophylaxis during front-line chemotherapy of acute myeloid leukemia: a single-center, real-life experience

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ABSTRACT

Background
Posaconazole is effective as primary antifungal prophylaxis of invasive fungal diseases in patients with acute myeloid leukemia.

Design and Methods
The impact of primary antifungal prophylaxis administered during front-line chemotherapy for acute myeloid leukemia was evaluated by comparing 58 patients who received oral amphotericin B (control group) to 99 patients who received oral posaconazole (posaconazole group). The primary endpoint was the incidence of proven/probable invasive fungal diseases. Secondary endpoints included incidence of invasive aspergillosis, survival at 4 and 12 months after the diagnosis of acute myeloid leukemia and costs.

Results
Proven/probable invasive fungal diseases were documented in 51.7% of patients in the control group and in 23.2% in the posaconazole group (P=0.0002). Invasive aspergillosis was documented in 43% of patients in the control group and in 15% in the posaconazole group (P=0.002). No survival difference was observed in patients aged over 60 years. In patients aged 60 years or less, a statistically significant survival advantage was observed at 4 months, but no longer at 12 months, in the posaconazole group (P=0.03). It was calculated that in the posaconazole group there was a mean 50% cost reduction for the antifungal drugs.

Conclusions
Primary antifungal prophylaxis with posaconazole during front-line chemotherapy was effective in preventing invasive fungal diseases in a "real-life" scenario of patients with acute myeloid leukemia, resulted in an early but transitory survival advantage in younger patients and was economically advantageous.

Key words: acute myeloid leukemia, antifungal prophylaxis, posaconazole, invasive fungal diseases, aspergillosis.

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The online version of this article has a Supplementary Appendix.

Introduction

Invasive fungal diseases (IFD), in particular invasive aspergillosis (IA), are a leading cause of morbidity and mortality in patients with acute myeloid leukemia (AML).[1-5] Primary antifungal prophylaxis (PAP) is a commonly used strategy, because the diagnosis of IFD is often difficult to obtain quickly enough to implement an early therapeutic intervention. Based on the results of a randomized controlled trial, posaconazole has been recommended as the drug of choice in AML patients undergoing induction chemotherapy.[6-11] A predefined diagnostic strategy implemented at our Institution in AML patients has allowed us to document a high number of IFD, particularly IA.[12] This epidemiological evidence prompted us to use posaconazole as mold-active PAP in AML patients throughout their whole front-line intensive chemotherapy. We evaluated the effect of this prevention strategy in a "real-life" scenario of AML.

Design and Methods

Patients and prophylactic strategies

Between February 28, 2006 and January 31, 2010, 162 consecutive patients older than 18 years newly diagnosed with non-M3 AML were submitted to remission-induction chemotherapy.[13-16] Five patients already affected by an IFD at the time of AML diagnosis were excluded from the analysis. Front-line treatment included induction chemotherapy, followed by reinduction chemotherapy in patients who did not achieve complete remission after first induction, and consolidation chemotherapy in patients who achieved complete remission after induction or reinduction chemotherapy. Patients were hospitalized in double-bed rooms without high efficiency particulate air (HEPA) filtration and positive pressure. Antibacterial prophylaxis consisted of oral ciprofloxacin (500 mg/bid).

From February 2006 to March 2007 (first period), 47 consecutive patients received oral non-absorbable amphotericin B (oral-AmB) (2,000 mg/day) as PAP. Since April 2007 (second period), the use of systemic PAP with oral posaconazole (200 mg/tid) became a standard practice during front-line chemotherapy. However, posaconazole was replaced by oral amphotericin B in patients receiving midostaurim, a selective inhibitor that targets the fms-like tyrosine-kinase 3 (FLT3) activating mutations frequently found in AML, in view of a possible drug-drug interaction with triazoles.[17] During the second period, 99 patients received oral posaconazole and 11 patients received oral-AmB (due to concomitant midostaurim therapy in 8 cases and to the lack of posaconazole at our pharmacy in 3 cases). Overall, along the 5-year period, 58 patients received oral-AmB PAP (control group) and 99 patients received posaconazole PAP (posaconazole group). In both groups, patients received the same PAP (oral-AmB or posaconazole) starting from induction chemotherapy and during the following front-line chemotherapy cycles, or until the development of an IFD or the use of any antifungal therapy. In patients who developed a breakthrough IFD during PAP, after a response to antifungal treatment was obtained, tailored secondary antifungal prophylaxis (SAP) was instituted during the following chemotherapy cycles.

Diagnostic strategy and antifungal treatment

Since February 2006, a survey of IFD in AML patients undergoing intensive chemotherapy has been prospectively conducted using a predefined diagnostic and therapeutic strategy regardless of type of antifungal prophylaxis.[12] A microbiology laboratory and a radiology service dedicated to patients with hematologic diseases were available at our center. In the event of febrile neutropenia (temperature >38°C recorded twice or >38.5°C recorded once), a baseline diagnostic work-up based on three blood cultures (Sygnal System, Oxoid, Hants, UK), and other microbiological and radiological examinations, if clinically indicated, was performed. Empirical antibacterial therapy was started and eventually modified according to the microbiological or clinical data. Patients with persisting fever after 4 days of antibacterial therapy or patients with fever relapsing after 48 h of defervescence, as well as patients with other clinical findings possibly related to an IFD, underwent an intensive diagnostic work-up that included three blood cultures, galactomannan (GM) serum detection by Platelia *Aspergillus* assay (Bio-Rad Laboratories, Marnes-La-Couquette, France) over 3 consecutive days, computed tomography of the chest and other examinations as indicated. In the event of a negative intensive diagnostic work-up and persistent fever, the intensive diagnostic work-up was repeated. In patients with radiological evidence suggestive of IFD and negative GM, it was repeated.

The diagnosis of IFD was made in accordance with the revised European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) definitions published in 2008.[18] Considering that the study was started before the revised definitions were published, the classification of the cases was retrospectively redefined. According to the above definitions, the diagnosis of probable pulmonary IA was made from the documentation of one of the following radiological findings - dense, well-circumscribed nodular lesion(s) with or without a halo sign, air-crescent sign, and cavitary lesion - associated with the isolation of the mold from the respiratory tract or a positive GM test from serum or respiratory specimens (bronchoalveolar lavage or sputum). Two consecutive positive serum samples with an index ≥0.5 or a single positive serum sample with an index ≥0.8, or a positive respiratory sample with an index ≥1 were required for a diagnosis of probable IA.

Antifungal therapy was chosen according to the documentation derived from the baseline and intensive diagnostic work-ups. Patients with persisting fever and with clinical and/or microbiological findings suspected to be related to a fungal infection but which together were not sufficient to define a diagnosis of IFD according to the revised EORTC/MSG definitions received preemptive antifungal therapy. Empirical antifungal therapy was reserved to patients with persisting febrile neutropenia, a negative intensive diagnostic work-up and worsening clinical condition. Patients who responded to antifungal therapy underwent further chemotherapy treatments while under tailored SAP.

Death was attributed to the IFD in patients who failed to respond to therapy (i.e., who had stable disease or disease progression) and in patients with a partial response to therapy who died as the result of an acute event involving any of the sites of infection or of an unknown cause.

Ethical statement

This study was approved by the institutional review board and informed consent for the use of clinical data for scientific purposed had been obtained from the patients. This was a non-interventional cohort study and the collection and storage of data were performed by the investigators directly involved in the patients' care using current techniques of ensuring privacy; ethics committee approval was not, therefore, necessary.

Analyses

The aim of our study was to evaluate the efficacy of an overall antifungal prophylaxis strategy (constituted by PAP and eventually

SAP), administered during front-line chemotherapy for AML, by retrospectively comparing two groups of patients who differed for the PAP employed. The primary endpoint was the incidence of proven/probable IFD during front-line chemotherapy. Secondary endpoints included: (i) the overall incidence of proven/probable and possible IFD, the incidence of proven/probable IA and the use of pre-emptive and empiric antifungal therapy during front-line chemotherapy; (ii) overall survival at 4 and 12 months after the diagnosis of AML; (iii) mortality attributable to IFD; (iv) occurrence of severe toxicity or side effects related to PAP; (v) difference in the costs considering duration of time spent in hospital and antifungal drugs administered either for primary or secondary prophylaxis or for therapy during the first 12 months after the diagnosis of AML. The calculation of costs of an inpatient stay for chemotherapy, autologous stem cell transplantation (SCT), and allogeneic SCT was based on data collected for an analysis performed by the Lazio regional section of the Italian Society of Hematology (SIE Lazio) with an Activity Based Costing method. According to these data, it was calculated that in 2009 the mean daily costs of hospitalization during front-line chemotherapy, autologous SCT and allogenec SCT were € 1102, € 1500, and € 1660, respectively (*unpublished data* - provided by Luciana Annino, chief of the SIE Lazio). For the calculation of the costs of intensive care unit stay we used data derived from an analysis performed in Italy in 2000.[19] The mean daily cost of hospitalization in an intensive care unit at that time was € 1895, which corresponds to €2296 in 2009 according to the calculation of inflation in Italy.

The cost of the antifungal drugs was calculated analytically considering the exact amount of antifungals administered to each patient during the 12 months from AML diagnosis as reported in the inpatient and outpatient medical records. We used the following prices of the antifungal drugs, as reported by the pharmacy of our hospital for 2009:

- amphotericin B (Fungilin, Bristol-Myers Squibb s.r.l.), oral suspension, 60 mL bottle, 500 mg/5 mL, one bottle sufficient for 3 days of prophylaxis: € 5;
- posaconazole (Noxafil, Schering Plough s.p.a.), oral suspension, 105 mL bottle, 40 mg/mL, one bottle sufficient for 7 days of prophylaxis: € 697
- liposomal amphotericin B (Ambisome, Gilead Sciences s.r.l.), 50 mg vial: € 146.8
- voriconazole (Pfizer Italia s.r.l) intravenous, 200 mg vial: € 123.65; oral, 200 mg tablet: € 41.55
- caspofungin (Cancidas, Merk Sharp & Dohme) 50 mg vial: € 437.9; 70 mg vial: € 561.3.

Survival rates were estimated using the Kaplan-Meier method and compared by the χ^2 and log-rank tests. Descriptive statistics included absolute and relative frequencies for categorical data, and median, mean and range for numerical measurements. The analyses were performed using SPSS software for Windows, version 17.0.

Results

Patients' characteristics and antifungal prophylaxis

The characteristics of the patients in the control group (58 cases) and in the posaconazole group (99 cases) are detailed in Table 1. The two groups had similar demographic, biological and clinical characteristics at diagnosis and during AML treatment. Overall, patients in the control group and in the posaconazole group received 112 and 195 front-line chemotherapy cycles, respectively (mean 1.9 and 2.0 cycles per patient, respectively). Details on PAP and SAP administered during the front-line chemotherapy in the two groups are shown in *Online Supplementary Figures S1A and S1B.*

Primary endpoint

The incidence of proven/probable IFD and the causative pathogens are detailed in Table 2. During the front-line chemotherapy, proven/probable IFD occurred in 30 of 58 patients (51.7%) in the control group and in 23 of 99 patients (23.2%) in the posaconazole group (absolute risk reduction, –28.5%; 95% CI, –12.9 to –42.8; *P*=0.0002). The incidence of IFD in patients who received oral-AmB PAP did not vary significantly along the time of the study: 26 of 48 (54.2%) and 4 of 11 (36.4%) patients were diagnosed with a proven/probable IFD during the first and second period of the study, respectively (*P*=0.33).

Secondary endpoints

Incidence of proven/probable and possible invasive fungal diseases, proven/probable invasive aspergillosis and use of pre-emptive and empiric antifungal therapy

The overall incidence of proven/probable, and possible IFD during front-line chemotherapy was 62.1% among the patients in the control group and 30.3% in the patients in the posaconazole group (absolute risk reduction, –31.7%; 95% CI, –15.7 to –45.8; *P*<0.0001). IA was the most common IFD (40 of 53 IFD, 75.5%) and there were fewer cases of IA in the posaconazole group (15.1% versus 43.1%, absolute risk reduction -27.9%: 95% CI, -13.4 to -42.0, *P*=0.0002). The infection was documented microbiologically thanks to a GM assay in 32 of 40 (80%) cases, 19 of 25 (76%) cases in the control group and 13 of 15 (87%) cases in the posaconazole group. GM from two or more serum samples with an index ≥0.5, from a single serum sample with an index ≥0.8 and from a respiratory specimen with an index ≥1 was detected in 16, two and one cases, respectively, in the control group, and in eight, three and two cases in the posaconazole group, respectively. The median value of the serum GM peak was 1.2 (range, 0.5 - 4.4) in the control group and 1.0 (range, 0.7 - 3) in the posaconazole group (*P*=0.8).

Pre-emptive or empirical antifungal therapy was administered to nine (15.5%) patients in the control group and to two (2.0%) patients in the posaconazole group (absolute risk reduction, –10.5%; 95% CI, –10.1 to –22.2; *P*=0.04) (Table 2).

The flowcharts of chemotherapy cycles, prophylaxis treatments and proven/probable IFD documented in the two periods are shown in *Online Supplementary Figures S1A and S1B*. The rates of proven/probable IFD during induction, reinduction and consolidation chemotherapy and according to antifungal prophylaxis are detailed in Table 3. A lower incidence of proven/probable IFD in the posaconazole group than in the control group was observed over total chemotherapy cycles (11.8% *versus* 26.8%; *P*=0.0015) and over induction chemotherapy cycles (13.1% *versus* 39.6%; *P*=0.0003). Excluding the chemotherapy cycles in which the patients received SAP, a proven/probable IFD was documented in 13.1% and 36.6% of overall cycles while under posaconazole PAP and oral-AmB PAP, respectively (*P*<0.0001). No patients in either group developed a further proven/probable IFD during a successive chemotherapy cycle while under SAP.

Mortality

Twenty-nine of the 58 patients (58%) in the control

group and 52 of the 99 patients (52.5 %) in the posaconazole group died within 12 months after the diagnosis of AML. In the overall population, Kaplan-Meier analysis of the time to death from any cause at 4 and 12 months after AML diagnosis did not show significant survival differences between the two groups (Figure 1A). Likewise, when patients were divided according to age, no survival difference was observed between the control group and the posconazole group in patients aged over 60 years (Figure 1B). However, in patients aged ≤60 years there was a significant survival advantage in the posaconazole group at 4 months after AML diagnosis (88.1% *versus* 71.8%; *P*=0.03), although this difference was no longer evident at 12 months (54.2% *versus* 59.0%; *P*=0.9) (Figure 1C).

Table 1. Characteristics of the patients and the underlying malignancies at baseline and during the treatment phase: comparison of patients who received oral amphotericin B primary antifungal prophylaxis (control group) with patients who received oral posaconazole primary antifungal prophylaxis (posaconazole group).

Characteristic	Control group (58 pts)	Posaconazole group (99 pts)	*P*
Age years			0.7
Mean and SD	55.2±12.0	54.2±13.9	
median (range)	55 (24-77)	58 (20-75)	
N. of pts aged ≤60 years (%)	39 (67.2)	59 (59.6)	
N. of pts aged >60 years (%)	19 (32.8)	40 (40.4)	
Gender, n. of pts (%)			0.9
Male	29 (50)	48 (48.5)	
Female	29 (50)	51(51.5)	
WBC at diagnosis, n. of pts (%)			0.5
≤ 50×10^9/L	45 (77.6)	82 (82.8)	
> 50×10^9/L	13(22.4)	17 (17.2)	
WHO performance status (PS) at diagnosis, n. of pts			0.76
PS 0-1	43 (74.1)	77 (77.8)	
PS 2-4	15 (25.9)	22 (22.2)	
Secondary leukemia ^, n. (%)	13 (22.4)	27 (27.3)	0.6
Cytogenetic risk group, n. of pts (%)#			0.8
Favorable	4 (8.3)	10 (12.5)	
Intermediate	38 (79.2)	61 (76.2)	
Unfavorable	6 (12.5)	9 (11.2)	
Molecular biology, n. of pts (%)§			0.5
FLT3-ITD+	12 (11.1)	12 (16.5)	
NPM+	4 (6.7)	9 (9.9)	
First line chemotherapy protocol, n. of pts (%) *			0.8
Daunorubicin-cytarabine-etoposide (DCE)	29 (50.0)	50 (50.5)	
Mitoxantrone-cytarabine-etoposide (MICE)	18 (31.0)	34 (34.3)	
Daunorubicin-cytarabine (3+7)	9 (15.5)	10 (10.1)	
Fludarabine-cytarabine-G-CSF (FLAG)	2 (3.4)	5 (5.1)	
Front-line chemotherapy cycles			0.9
Total cycles	112	195	
N. of pts who received induction chemotherapy (%)	58 (100)	99 (100)	
N. of pts who received reinduction chemotherapy (%)	7 (12.1)	22 (22.2)	
N. of pts who received consolidation chemotherapy (%) [total cycles]	36 (62.1) [47]	67 (67.7) [74]	
Mean n. of cycles per patient (range)	1.9 (1-4)	2.0 (1-4)	
Duration of neutropenia (PMN <500/mm^3) after front-line chemotherapy cycles			0.9
8-21 days, n. of cycles (%)	71 (63.4)	126 (64.6)	
>21 days, n. of cycles (%)	41 (36.6)	69 (35.4)	
Mean	20±12	21±13	
Achievement of complete remission after induction or reinduction chemotherapy, n. (%)	37 (63.8)	65 (65.7)	0.9
Leukemia relapse within 12 months after diagnosis, n. (%)	13 (35.1)	22 (33.8)	1
Early death with aplastic marrow after induction, n. (%)	6 (10.3)	6 (6.1)	0.4
Stem cell transplant performed within 12 months after diagnosis, n. of pts (%)			
Autologous transplant	9 (15.5)	18 (18.2)	0.8
Allogeneic transplant	7 (12.1)	14 (14.1)	0.8

^ *Leukemia preceded by a myelodysplastic phase or another malignancy.* #*Available for 48 and 80 patients in the first and second group, respectively. Patients were stratified according to cytogenetic risk. The favorable-risk karyotype group included patients with t(8;21) and inv(16); the unfavorable-risk group included patients with chromosome 5 and 7 aberrations, inv(3), t(3;3), t(9;22), 11q23 rearrangements and complex karyotypes (> 3 abnormalities); the intermediate risk group included patients with a normal karyotype or cytogenetic lesions not included in the other groups.* §*Available for 45 and 91 patients in the first and second group, respectively.* **see references 13-16.*

Table 2. Invasive fungal diseases in patients with AML during front-line chemotherapy: comparison of patients who received oral amphotericin B primary antifungal prophylaxis (control group) with patients who received oral posaconazole primary antifungal prophylaxis (posaconazole group).

Invasive fungal disease (IFD)	Control group (58 pts)	Posaconazole group (99 pts)	*P* value	Absolute Reduction (95% CI)
	Number of patients (%)			
Patients with proven/probable IFD	30 (51.7)	23 (23.2)	0.0004	-28.5% (-12.9 to -42.8)
Mould				
Invasive aspergillosis	25 (43.1)*	15 (15.1)°	0.0002	-27.9% (-13.4 to -42.0)
Aspergillus flavus	1	1		
A.fumigatus	3	0		
Aspergillus species^	21	14		
Zygomycosis	3 (5.2)*	1 (1.0)		
Rhizopus orhizae	0	1		
Rizomucor pusillis	1	0		
Mucor species	1	0		
Cunninghamella species	1	0		
Yeast				
Invasive candidiasis	3 (5.2)*	5 (5.0)°	1	+0.12% (-9.5 to 7.0)
Candida albicans	2	3		
C. tropicalis	1	0		
C. guilliermondii	0	2		
Geotrichum capitatum	1 (1.7)	2 (2.0)		
Pneumocystis jirovecii	1*	1		
Patients with possible IFD	6 (10.3)	7 (7.1)	0.55	+1.9% (+9.3 to -8.6)
Total patients with IFD	36 (62.1)	30 (30.3)	<.0001	-31.7% (-15.7 to -45.8)
Pre-emptive or empiric antifungal therapy	9 (15.5)	5 (2.0)	0.04	-10.5% (-0.1 to -22.2)

**Three patients with a diagnosis of invasive aspergillosis were diagnosed with* P.jiroveci *infection (1 case), zygomycosis, (1 case) and candidemia (1 case) during the same chemotherapy-induced neutropenia period. °One patient with a diagnosis of invasive aspergillosis was diagnosed with a concomitant invasive candidiasis. ^ In these cases a diagnosis of invasive aspergillosis was obtained by a positive test for aspergillus galactomannan antigen (see text for details) or by compatible histopathological findings.*

The mortality rate at 100 days after the diagnosis of proven/probable IFD was 36.7% (11 out of 30 patients) in the control group and 43.5% (10 out of 23 patients) in the posaconazole group (*P*=0.08). Of 81 deaths that occurred within 12 months of the diagnosis of AML, 10 (12.3%) were considered to be related to a proven/probable IFD: 17.2% (5 of 29) in the control group and 9.6% (5 of 52) in the posaconazole group (*P*=0.48). In the remaining 71 patients, death was related to leukemia progression (63 cases; 77.8%), bacterial infections (4 cases) and other causes (4 cases). Mortality was attributable to IFD in five of 58 (8.6%) patients in the control group and in five of 99 (5.0%) patients in the posaconazole group (*P*=0.17).

At 12 months after the diagnosis of AML, death had occurred in 32 of 53 patients (60.4%) who developed a proven/probable IFD during front-line chemotherapy and in 49 of 104 patients (47.1%) who did not (*P*=0.13).

Toxicity and side effects

There was no severe toxicity related to any PAP. Posaconazole was discontinued within 7 days in three of 99 patients (3.0%) due to poor oral compliance related to mucositis and vomiting after chemotherapy.

Costs of hospitalization and antifungal drugs

The mean hospitalization time during the first 12 months after AML diagnosis was 94 days (range, 19-182) in the control group and 89 days (range, 16-196) in the posaconazole group (*P*=0.6). The presumptive costs of hospital stay and the exact costs of antifungal drugs are detailed in Table 4. A mean cost reduction per patient of € 5,399 for the hospital stay and of € 10,763 for the antifungal therapy was calculated in favor of patients in the posaconazole group.

Discussion

The incidence of IFD in AML patients undergoing intensive chemotherapy at our center appears to be high compared to that reported for some recent multicenter studies.[2-4,7] Several factors need to be considered. First, this was a "real-life" experience which included very high-risk patients who are usually excluded from clinical trials: all consecutive AML patients were included, 38% of patients were elderly (>60 years), 25% of patients had secondary leukemia, several patients had an unfavorable performance status at AML diagnosis. Second, lack of HEPA filtration and positive pressure rooms and frequent hospital construction work may have contributed to the epidemiological impact of infections by airborne filamentous fungi, even though environmental problems do not justify the number of documented infections by endogenous yeasts. Third, the clinically-driven diagnostic approach with dedicated microbiological and radiological services implemented at our center was sensitive and prompt. This diagnostic approach reduced the underdiagnosis of IFD and the use of empirical antifungal therapy which are associated with less intensive strategies.[12] In contrast to multicenter studies, in single center experiences in which a prede-

Table 3. Proven/probable IFD according to phase of the front-line chemotherapy and antifungal prophylaxis.

	Induction chemotherapy	Reinduction or consolidation chemotherapy	Total cycles
	N. of IFD/n. of cycles (%)		
Control group			
Total cycles	23/58 (39.6)a	7/54 (13.0)c	30/112 (26.8)e
Primary antifungal prophylaxis	23/58 (39.6)	7/24 (29.2)	30/82 (36.6)g
Secondary antifungal prophylaxis	/	0/30 (0)	0/30 (0)
Posaconazole group			
Total cycles	13/99 (13.1)b	10/96 (10.4)[d]	23/195 (11.8)f
Primary antifungal prophylaxis	13/99 (13.1)	10/76 (13.1)	23/175 (13.1)m
Secondary antifungal prophylaxis	/	0/20 (0)	0/20 (0)

a versus *b: absolute reduction -26.5%; 95% CI -12.4 to -40.4;* P=*0.0003; c* versus *d: absolute reduction -2.5%; 95% CI -14.9 to +7.6;* P=*0.8; e* versus *f: absolute reduction -15.0%; 95% CI :-5.9 to -24.6;* P =*0.0015; g* versus *m: absolute reduction -23.4%; 95% CI :-12.2 to -35.0;* P<*0.0001.*

Table 4. Cost in euros of hospital stay and of antifungal treatments used in the two groups in the 12 months after the diagnosis of AML.

	Control group (58 patients)	Posaconazole group (99 patients)	Difference in costs per patient
Hospital stay			
Induction or consolidation chemotherapy	5,341,380	8,532,790	
Autologous SCT	400,500	756,000	
Allogeneic SCT	547,800	916,320	
Intensive care unit	18,370	27,550	
Total cost	6,308,050	10.232.660	
Mean cost per patient	108,759	103,360	-5399
Antifungal treatments			
Primary prophylaxis			
Total cost	3,000[a]	272,000[b]	
Mean cost per patient	52	2,747	+2,695
IFD treatment and secondary prophylaxis			
Total cost	1,220,000[c]	750,000[d]	
Mean cost per patient	21,034	7,576	-13,458
All antifungal treatments			
Total cost	1,223,000	1,022,000	
Mean cost per patient	21,086	10,323	-10,763

[a]oral amphotericin B: total 1800 days of prophylaxis; [b]oral posaconazole: total 2657 days of prophylaxis; [c]intravenous voriconazole: 610 days of treatments; oral voriconazole: 3805 days of treatment; liposomal amphotericin B: 336 days of treatment; caspofungin: 207 days of treatment; posaconazole: 50 days of treatment; [d]intravenous voriconazole: 468 days of treatment; oral voriconazole: 2405 days of treatment; liposomal amphotericin B: 465 days of treatment; caspofungin: 218 days of treatment.

fined diagnostic strategy in a real-life scenario was applied, the documentation of IFD was high.[20-22] In a study conducted in the Netherlands, 30% of 269 consecutive AML patients were diagnosed with IA.[20] A prospective cohort trial in a single center in Cologne, which confirmed the posaconazole prophylaxis efficacy in a standard clinical setting of AML patients,[21] showed a high incidence of IFD during induction chemotherapy in patients not receiving prophylaxis (19.5% of proven/probable IFD and 22% of pulmonary infiltrates indicative of IA). In a further study, 62% of patients under fluconazole PAP and 38% of those under posaconazole PAP developed a proven/probable or possible IFD during first induction chemotherapy for AML.[22] In all these experiences, the authors commented that they did not expect such a high incidence of IFD revealed by the implementation of a rigorous diagnostic standard and prospective documentation.

The high incidence of IFD prompted us to employ posaconazole PAP not only during induction but also during reinduction and consolidation treatment considering the significant number of IFD documented during these phases (*Online Supplementary Figure S1A*). In fact, we did not compare two PAP regimens in AML patients during induction chemotherapy as other authors did;[10,21,22] rather, the aim of our study was to compare two prevention strategies with PAP and eventually SAP during the whole front-line chemotherapy and to evaluate their effect on survival in the long term, over a 1-year period.

Our study confirmed that posaconazole PAP effectively reduced IFD, including IA, in a real-life scenario of consecutive AML patients undergoing front-line chemotherapy. Compared to the rates in the control group, in patients in the posaconazole group there was an absolute reduction in proven/probable IFD of 26.5% (from 39.6% to 13.1%) during induction chemotherapy and of 28.5% (from 51.7% to 23.2%) during the whole front-line AML chemotherapy. The historical comparison of two populations of patients represents a limitation of our analysis considering that factors other than PAP, such as variability in the epidemiology of environmental molds, may have contributed to the reduction of IFD. On the other hand, the high rate of IFD documented in patients in the second period who did not receive posaconazole PAP (mainly because of the contraindication of being under midostaurim therapy) seems to confirm the primary role of the pharmacological intervention. Despite the significant reduction of IFD, the rate of breakthrough infections while under posaconazole PAP continued to be relevant (13.1% of the cycles). Considering that almost all fungal pathogens isolated during posaconazole PAP were susceptible *in vitro* to the triazole (*unreported data*), the possibility of reduced absorption, with sub-therapeutic serum concentrations of posaconazole, must be considered.[23-25] Many factors, such as the development of mucositis, impaired dietary intake and use of proton pump inhibitors may cause interindividual pharmacokinetic variability in AML patients, and therapeutic drug monitoring may be required.[23-28]

Unlike previous studies showing impaired sensitivity of the GM assay in patients on antifungal therapy,[29] in our experience GM retained a major role in the diagnosis of IA also in patients receiving posaconazole. Assuming that reduced absorption of posaconazole, leading to subtherapeutic serum concentrations, could explain the occurrence of breakthrough IA, normal production and spread of GM by the fungal pathogen in these cases seems to be likely.

In contrast to the results of a multicenter study[7] but in accordance with the results of another study carried out by the Cologne group,[21] in our experience the use of posaconazole PAP had no impact on the survival of the overall population. However, in our series a significant survival advantage at 4 months after the diagnosis of AML was observed in younger patients who received posaconazole PAP, even though this advantage was lost at a later

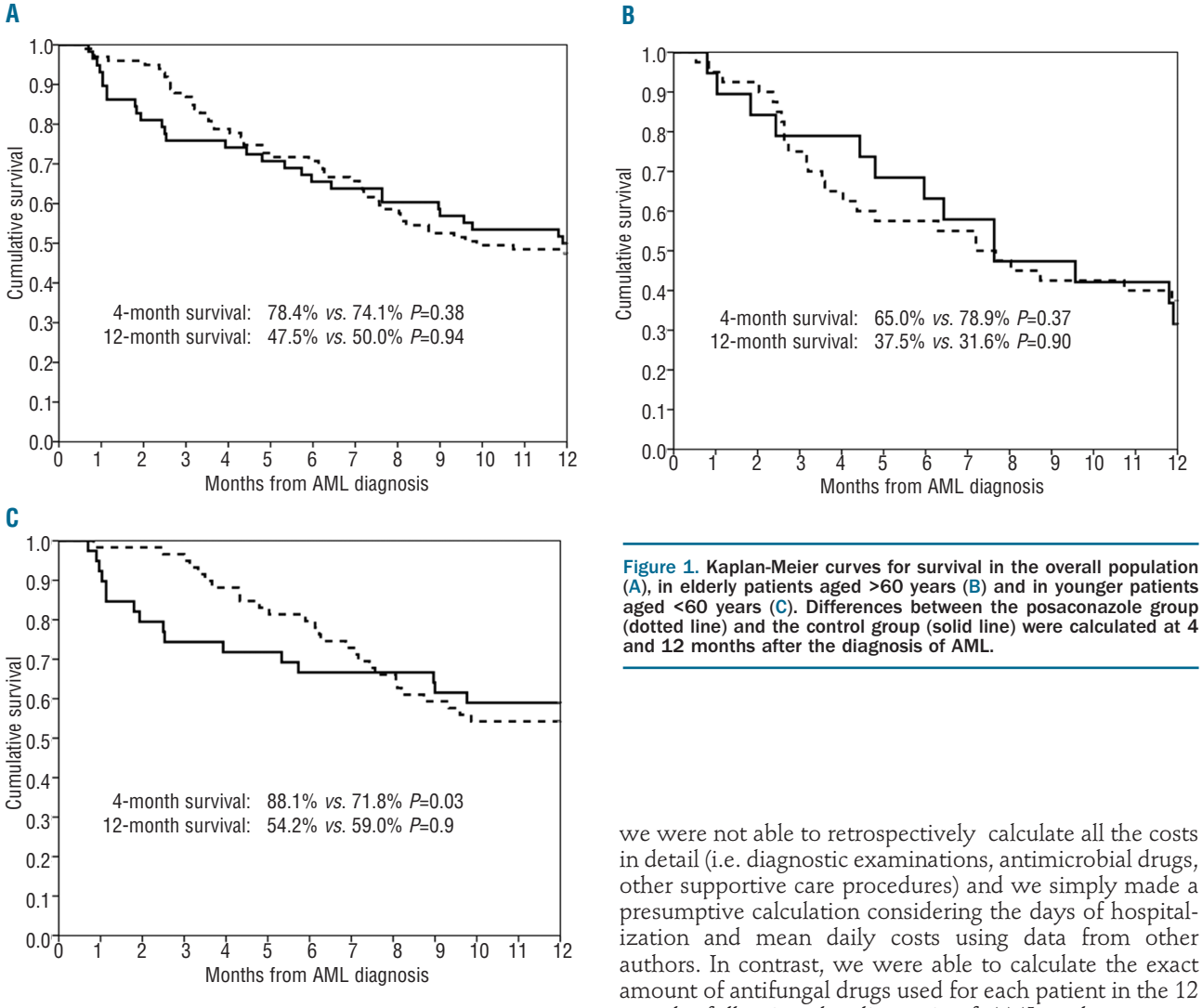

Figure 1. Kaplan-Meier curves for survival in the overall population (A), in elderly patients aged >60 years (B) and in younger patients aged <60 years (C). Differences between the posaconazole group (dotted line) and the control group (solid line) were calculated at 4 and 12 months after the diagnosis of AML.

follow-up. The 12-month mortality rate of patients who developed a proven/probable IFD did not differ from that of patients who did not; in fact, in most patients who died, the primary cause of death was leukemia progression, and not the IFD. Likewise, the time spent in hospital in the first 12 months after the diagnosis of AML was not different between the two groups. All these findings seem to show that in a high-risk, real-life setting the biological and clinical characteristics of the underlying malignancy and of the antileukemic treatments are dominant factors able to overcome the outcome advantages of any strategy to prevent infectious. In contrast, considering subpopulations of patients at standard risk, such as younger patients, the effect of proper IFD prevention on survival may become apparent.

A major problem of the use of the new antifungal agents in prophylaxis is their cost. On the other hand, the costs of managing an IFD may be even more substantial. Recent reports suggest a good cost-benefit ratio of prophylaxis with posaconazole compared to other triazoles among neutropenic and transplanted patients.[30-35] In our study, we did not observe that PAP had a significant impact on duration or costs of hospitalization. However, this analysis suffered from important limitations in that we were not able to retrospectively calculate all the costs in detail (i.e. diagnostic examinations, antimicrobial drugs, other supportive care procedures) and we simply made a presumptive calculation considering the days of hospitalization and mean daily costs using data from other authors. In contrast, we were able to calculate the exact amount of antifungal drugs used for each patient in the 12 months following the diagnosis of AML and posaconazole PAP was associated with a 50% mean reduction of the costs of antifungal drugs. Importantly, it should be considered that the advantage we observed in our experience may be less evident in settings in which the epidemiological impact of IFD is lower.

In conclusion, our experience highlights the importance of the knowledge of local epidemiology in order to define a good antifungal strategy, confirms the efficacy of posaconazole in the prevention of IFD, with a transitory survival advantage in younger patients, and suggests potential economic advantages of correct antifungal prophylaxis in a high-risk, real-life population outside of controlled trials. Breakthrough infections despite prophylaxis exposed the possible problem of posaconazole gastrointestinal absorption and led us to design a project aimed at the therapeutic drug monitoring of posaconazole.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

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