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ORIGINAL ARTICLE

Low Rate of Invasive Fungal Infections During Induction and Consolidation Chemotherapy for Adults with De Novo Acute Myeloid Leukemia Without Anti-mold Prophylaxis: Single-Center 2002–2018 Empirical/Pre-emptive Approach

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Abstract Broad-spectrum antifungal prophylaxis is currently considered the standard of care for adults with de novo AML for the prevention of invasive fungal infections (IFIs), especially invasive pulmonary aspergillosis (IPA). Because fluconazole has been used in our center as anti-yeast prophylaxis, we sought to analyze in detail the incidence of IFIs over a 17-year period, as well as their impact on outcome. A standardized protocol of patient management, including serum galactomannan screening and thoracic CT-guided diagnostic-driven antifungal therapy, was used in all patients. A total of 214 consecutive adults with de novo AML who were treated in 3 CETLAM (Grupo Cooperativo para el Estudio y Tratamiento de las Leucemias Agudas y Mielodisplasias) protocols from 2002 to 2018 were included. The 90-day incidence of any IFI (including possible cases) was 11% (95% CI 4–15%), most cases occurred during induction chemotherapy (8%, 95% CI 4–12%), and most cases were probable/proven IPA (8%, 95% CI 3–13%). Developing an IFI during induction and consolidation had no impact on 1-year survival. A case–control study with 23 cases of IPA and 69 controls identified induction/re-induction chemotherapy, chronic pulmonary disease and age > 60 years/poor baseline performance status as potential

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pretreatment risk factors. The current study proves that inpatient induction and consolidation chemotherapy for de novo AML can be given in areas with "a priori" high-burden of airborne molds with fluconazole prophylaxis, while the selective use of anti-mold prophylaxis in patients at very high risk may further reduce the incidence of IFI in this specific clinical scenario.

Keywords Acute myeloid leukemia · Invasive fungal infections · First-line chemotherapy · Antifungal prophylaxis

Introduction

Invasive fungal diseases (IFIs) are life-threatening opportunistic infections, and patients with newly diagnosed acute myeloid leukemia (AML) have traditionally been considered to be the group at highest risk for developing IFI during the prolonged severe pancytopenia that occurs after intensive induction and consolidation chemotherapy [1, 2]. Currently, many centers routinely use posaconazole or voriconazole prophylaxis in all newly diagnosed AML patients with the aim of having an incidence of possible, probable and proven IFI of $< 10\%$ during induction. While such prophylaxis is usually also used after high-dose cytarabine-based consolidation, the risk of IFI appears to be lower than after induction and thus prophylaxis could be omitted [3–6]. However, each center should know its incidence of IFI in these patient groups in order to determine whether broad-spectrum antifungal prophylaxis (AFP) is truly required, rather than using anti-yeast prophylaxis with fluconazole and a diagnostic-driven approach to patients with prolonged fever of unknown origin (PFUO) [7, 8]. Our center is located in Barcelona, an area of Southern Europe with a high level of mold spores in the environment [9], and thus, AML patients here are automatically considered as high risk for invasive mold infections, mostly invasive pulmonary aspergillosis (IPA) [6]. Centers from Barcelona [10] and nearby Valencia [11, 12] have reported a reduction in the rate of IPA from 15 to 25% with fluconazole prophylaxis in high-risk patients, such as de novo AML, to 5–10% after the introduction of posaconazole or voriconazole prophylaxis, as also reported by centers throughout Europe [6].

In contrast to other centers from Barcelona, our adult hemato-oncology unit has traditionally had a low incidence of IPA in the inpatient ward during the early phase after allogeneic [13–15] and autologous stem cell transplantation ($< 3\%$ day $+ 100$ incidence). We have also had a low rate of IFIs during the initial induction and consolidation chemotherapy in adult patients with AML. However, patients have been routinely screened with biweekly serum galactomannan since 2002, and a symptom-driven diagnostic algorithm has been used [7, 16–18], with the rapid and routine use of thoracic computerized tomography (T-CT) in patients with prolonged fever of undetermined origin during neutropenia (PFUO) and CT-guided bronchoalveolar lavage (BAL) sampling when indicated.

In this study, the main objective was to analyze the incidence and potential risk factors for the development of a possible/probable/proven IFI (especially IPA) in our center in patients with de novo AML undergoing frontline intensive induction and consolidation chemotherapy between January 2002 and December 2018. Other objectives included analyzing the impact of developing an IFI on the 1-year overall survival (OS), analyzing the rates and survival impact of developing bacteremia and exploring potential risk factors for developing IPA in our patient cohort.

Patients and Methods

Patient Inclusion

The study includes adult patients from the Hospital de la Santa Creu i Sant Pau, Barcelona, Catalonia (Spain, EU). The inclusion period started when all microbiologic and T-CT procedures were routinely available in all patients on a daily basis (detailed below). Patients included herein were all consecutive patients who were included in three consecutive therapeutic protocols for de novo AML within the CETLAM (Grupo Cooperativo para el Estudio y Tratamiento de las Leucemias Agudas y Mielodisplasias) group from 2002 to 2018, assuring 1-year follow-up in all cases at the time of final analysis (January 2020). The three CETLAM protocols were based on a $3 + 7$ (idarubicin + Ara-C) induction and re-induction (if

necessary) and intermediate or high-dose Ara-C-based consolidation. Details of the chemotherapy used in these protocols are shown in supplemental Table S1 (CETLAM-03 study registered in www.clinicaltrials.gov: NCT01723657; and CETLAM-12: NCT01700413). Patients with secondary or therapy-related AML (n = 84) and patients who received salvage chemotherapy for relapsed AML (n = 105) were excluded, in order to have a more homogeneous population of patients.

All patients signed an informed consent for each of the three CETLAM protocols in which they were included, which complied with national ethical regulations as well as the principles laid in the 1964 Declaration of Helsinki.

Antimicrobial Strategies and Combined Empirical and Diagnostic-Driven AFT

According to our institutional protocols, all patients with de novo AML received anti-yeast prophylaxis with fluconazole during the first 7 days after starting induction or consolidation chemotherapy and until myeloid recovery (granulocyte + monocyte count in peripheral blood $> 0.5 \times 10^9$/L), or until empirical broad-spectrum antifungal therapy (EAFT) for PFUO or targeted antifungal therapy (mostly for IPA) was started. Twice weekly serum galactomannan (sGM) was started at the onset of leucopenia. Patients who developed PFUO during pancytopenia (defined as daily axillary temperature of $>$ 38 °C for 4 or more days) were switched to empirical broad-spectrum EAFT (mostly low-dose lipid formulation of amphotericin B [18, 19] or caspofungin, but for a maximum of 5 days, until results from a diagnostic workup were available. Whenever EAFT was started or if two consecutive sGM were positive ($>$ 0.5) or changes in lung parenchyma were suspected by chest X-ray, T-CT was performed. If T-CT identified bronchopulmonary findings suggestive of infection (whether specific for an IPA or nonspecific findings), a BAL was performed for cytological, bacteriological, viral, parasitic and fungal testing of the affected lung segment(s); GM was also tested in BAL samples (bGM), and a value $>$ 1.0 was considered a positive value. Whenever necessary for sampling bronchial or peribronchial lesions, transbronchial biopsies were done unless contraindicated.

Patients who met the criteria for a probable/proven IPA were treated with intravenous voriconazole, later switched to the oral formulation. If contraindicated, lipid formulations of amphotericin B (AmB) at 3 mg/kg/day [20, 21] or caspofungin [22] were used according to the patients' renal function. If patients met the clinical and radiological criteria for an invasive mold infection but had no evidence of aspergillosis in serum or BAL studies (a situation commonly referred to as a possible IPA [23], while the proper term is a possible invasive mold infection or IMI), treatment with high-dose lipid AmB (5 mg/kg) or posaconazole was used to cover potential molds resistant to voriconazole such as mucormycosis (although none occurred in the current study). Patients with yeast fungemia were treated with caspofungin or lipid formulations of AmB until the species and its antifungal susceptibility were known. The supportive care and clinical/radiological/microbiological follow-up of patients with an IFI followed established guidelines [24].

Patients were hospitalized mostly in rooms with high-efficiency particulate air (HEPA) filtration, and absence of air spores was regularly checked in the room air, the common hall and waiting areas in the inpatient adult onco-hematology unit; water was filtered from taps, showers and other water sources with filters, mostly Kleenpak™ Disposable Tap Filters (PALL Corporation, Port Washington, NY state).

Definitions

AML Risk Categorization and Response to Induction Chemotherapy

Diagnostic AML samples from all patients were prospectively or retrospectively studied with cytogenetic and molecular techniques and classified into favorable, intermediate and adverse risk categories, as shown in detail in Table 1. Standard criteria for cytological complete remission with hematological recovery (CR) or incomplete peripheral blood count recovery (CRi) were used. Patients who achieved a $<$ 50% reduction in bone marrow (BM) blasts and improvement in blood counts were considered as partial remission (PR) after first induction chemotherapy and received re-induction with the same protocol [25]. Refractory patients had persistent BM blasts

Table 1 Patient characteristics with de novo AML treated with induction and consolidation chemotherapy from 2002 to 2018 (unless specified otherwise, values refer to number of patients, and % in parentheses)

	Time period and CETLAM protocol		
	2002–2011[a] (LAM-99 and LAM-03)	2012–2018[a] (LAM-12)	*P*
Total number of patients (n = 214)	129	85	
Age, median years [range]	49 [18–71]	59 [19–72]	< 0.001
Age ≥ 60 years	35 (21)	42 (49)	< 0.001
Age ≥ 50 years	63 (49)	62 (73)	< 0.001
Male gender	59 (54)	43 (51)	Ns
Median leukocytes at diagnosis (x 10^9/L) [range]	14.5 [0.2–410]	9 [0.3–209]	Ns
> 50 × 10^9/L	37 (29)	14 (17)	0.04
Poor performance status (ECOG ≥ 2)	29 (18)	30 (35)	< 0.01
Cytogenetic molecular risk group[b]			< 0.01
Favorable	45 (35)	17 (20)	
Intermediate	32 (25)	38 (45)	
Adverse	52 (40)	30 (35)	
Post-chemotherapy outcomes			
Final response to induction	101 (78)	66 (78)	ns
CR/CRi after induction-1	79 (61)	49 (58)	
Initial PR/CR/CRi after induction-2	22 (17)	17 (20)	
Refractoriness	13 (10)	14 (17)	
Induction (early) death	15 (12)	5 (6)	
Duration of neutropenia during Ind-1/Ind-2, median days [range]	20 [12–62]	21 [13–59]	Ns
Received consolidation	97/101 (96)	57/66 (86)	ns
Duration of neutropenia during consolidation, median days [range]	15 [10–28]	11 [6–21][c]	0.01
Time on the study (from Ind-1 to end consolidation), median days [range]	88 [29–112]	82 [26–108]	ns
Received alloHSCT in first CR as defined by the protocol (mostly intermediate and adverse risk groups)	70 (54)	54 (64)	0.02
Total cases IFI during induction(s)/consolidation	13 (10)	14 (16)	< 0.03
Possible/probable/proven IFIs	0/10/3	2/9/3	
Total cases of IFI during Ind-1/Ind-2	9	11	
Total cases of IFI during consolidation	2	1	
Total cases of IFI in chemorefractory AML (n = 27)	2	2	
Possible/probable/proven IPA (total cases; %)	0/10/1 (11; 9%)	2/9/1 (12; 14%)	< 0.04
Probable/proven IPA	Same as above	10 (12%)	0.1
Other proven IFIs (yeast fungemias)[d]	2 (1.6)	2 (2.4)	ns

CETLAM, Grupo Cooperativo para el Estudio y Tratamiento de las Leucemias Agudas y Mielodisplasias (www.cetlam.com); ECOG PS, Eastern Cooperative Oncology Group performance status; CR, complete remission; CRi, bone marrow CR but with incomplete hematologic recovery, usually either incomplete platelet or neutrophil recovery; Ind-1/Ind-2, first induction chemotherapy/second induction (or re-induction) chemotherapy; IFI, invasive fungal infection; IPA, invasive pulmonary aspergillosis; alloHSCT, allogeneic hematopoietic stem cell transplantation

[a]See supplemental Table 1 for details on the induction and consolidation chemotherapy regimens used in CETLAM protocols LA_99, LAM-03 and LAM-12

[b]In brief, favorable category included the CBF t(8;21)(q22;q22.1) and inv(16)(p13.1q22) or t(16;16)(p13.1;q22; normal karyotype (NK) with mutated NPM1 without FLT3-ITD; and biallelic mutated CEBPA. Intermediate category included NK with wild-type NPM1 and without FLT3-ITD; t(9;11)(p21.3;q23.3); and any cytogenetic abnormalities not classified as favorable or adverse category. Adverse category included t(6;9)(p23;q34.1); t(v;11q23.3) or KMT2A(MLL) rearrangements; t(9;22)(q34.1;q11.2) or BCR-ABL1; inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); −5 or del(5q); −7; −17/abn(17p); complex karyotype or monosomal karyotype; wild-type NPM1 with FLT3-ITD; or mutations in RUNX1, ASXL1 or TP53

[c]Pegylated filgrastim after consolidation was used during this time period only

[d]Fungemia by *Trichosporon beigelii, Candida glabrata (x2) and Candida tropicalis*

during pancytopenia or did not even reach criteria for PR, while early death was defined as death during pancytopenia without evidence of BM blasts during the first 28 days after chemotherapy [25].

Definition of IFI

All data regarding the occurrence of possible, probable or proven IFI have been captured prospectively by the first author (R.M.) on a daily basis since 1991 (ongoing in 2020), and thus, the diagnostic certainty of an IFI was in fact prospectively studied in all patients. Diagnosis and classification of an IFI was performed according to the 2008 European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC/MSG) definitions [23]. Of note, for the patients included herein, the same definitions are applicable using the EORTC/MSG 2019 revised definitions, since all probable cases of IPA had a single sGM $\geq$ 1.0 and/or a single sGM $\geq$ 0.7 plus bGM $\geq$ 0.8, as modified in the mycological evidence of IPA in the recent updated definitions [26].

Definition of Bloodstream Bacterial Infections

Bacteremia was defined by the isolation from blood cultures in a febrile patient of any gram-negative bacilli (GNB) or gram-positive cocci (GCP) species, except for coagulase-negative staphylococci (CNS), which were not included in the present analysis since they very rarely, if ever, lead to death. Multidrug resistant GNB included extended-spectrum beta-lactamase producing (ESBL) enterobacteria, *Klebsiella pneumoniae*-type carbapenemase-producing (KPC) enterobacteria and MDR *Pseudomonas aeuginosa,* which have become a major threat to immunocompromised patients worldwide [27, 28].

Statistical Methods

Duration of each patient in the study encompassed from the start of a first induction and a re-induction (if PR achieved) or a cycle of consolidation chemotherapy until hematological reconstitution, death from any cause during pancytopenia or evidence of chemoresistance and start of salvage chemotherapy or investigational therapy. Post-consolidation IFIs or those occurring during salvage chemotherapy or after transplantation were not studied.

Continuous variables were compared with Student's *t* test (for normally distributed variables) or the Mann–Whitney U test (for non-normally distributed variables). Categorical variables were evaluated with the Chi-square or two-tailed Fisher's exact test. For exploring potential risk factors for developing an IPA, a case–control study was done. For each case of IPA, three controls were used, matched only by the closest time of start of the last chemotherapy given (induction or consolidation). Because of the small number of cases, only univariate Odds Ratios (95% confidence intervals, or CIs) for developing IPA with respect to controls were studied.

The probability of 1-year OS was estimated from the time of start of chemotherapy using Kaplan–Meier curves, while the incidence of developing an IFI was calculated using cumulative incidence estimates, taking into account the competing risks (early death without an IFI or treatment discontinuation due to the patients' or physicians' decision).

Univariate analyses of the association of various clinical risk factors with 1-year OS were calculated with the log-rank test and Kaplan–Meier survival curves. Variables which occurred after treatment was started (achievement of CR/CRi, refractory AML, development of an IFI and development of bacteremia) were analyzed as time-dependent covariates. Multivariate analysis of OS was performed by Cox proportional hazards regression, with inclusion of those variables with a P value less than 0.1 in the prior univariate testing. Tests of significance were two sided, with a significance P level of .05 or less. All statistical analyses were performed using SPSS version 19.0 (SPSS, Chicago, IL), with the exception of the cumulative incidence analyses, which were carried out with NCSS 2004 (Number Cruncher Statistical System, Kaysville, UT).

Results and Discussion

Patient Characteristics and AML Response

Details on patient and AML characteristics are shown in Table 1. The median time on the study was 86 days (range 29–112 days); this time period encompassed a first induction (± re-induction) or a cycle of

consolidation chemotherapy, as previously described. Because the rate of patients who developed an IFI was slightly higher in the 2012–2018 period than in the 2002–2011 period (including 14% possible/probable/proven IPA vs. 9%, respectively), Table 1 shows the patient characteristics in the two time periods. Of note, variables which increase the risk of IPA (such as age > 60 years old and poor performance status) were much higher in the 2012–2018 period.

The proportion of patients who achieved CR/CRi after induction or re-induction, as well as those who received consolidation chemotherapy, was similar in both time periods. The CR/CRi rate was 78% in both time periods.

Occurrence of IFD and Impact on Survival

Overall, 27 cases of possible, probable and proven IFI occurred, mostly IPA (n = 23), as shown in detail in Tables 1 and 2. The 90-day incidence of any IFI was 11% (95% CI 7–15%), with probable/proven IPA occurring in 8% (95% CI 3–13%). The incidence of IFI was higher after induction/re-induction therapy [8% (95% CI 4–12%)] than after consolidation treatment [1% (95% CI 0.1–3%)]. The incidence was higher in the 27 patients who were refractory to induction therapy [14% (95% CI 7–21%)]. Of the 27 patients with an IFI, only 3 (11%) died from this complication, while the other 24 patients had a satisfactory response to AFT. Nineteen of these 24 patients were assigned to receive an allogeneic hematopoietic stem cell transplantation (AlloHSCT) in first CR/CRi. All but 2 cases received the planned alloHSCT, with a delay of > 1 month due to the IFI in 4/17 patients. Because response to induction chemotherapy is well known to be the strongest predictor of OS in patients with AML, while it is also a major protective factor for developing and dying from an IFI [29–32], we analyzed the 1-year OS according to the AML response and having an IFI; the survival depended mostly on the post-induction AML

Table 2 Incidence of invasive fungal infections and their outcome during induction and consolidation chemotherapy

Day 90 CumInc of any IFI (95% CI)	11% (7–15)
Day 90 probable/proven IFI/IPA (95% CI)	9% (4–14)/8% (3–13)
Day 30 CumInc of any IFI during Ind-1/Ind-2 (95% CI)	8% (4–12)
Day 30 CumInc of any IFI during consolidation (95% CI)	1% (0.1–3)
Day 90 CumInc of IFI in chemorefractory AML (95% CI)	14% (7–21)
Outcome of patients with an IFI (number of patients and % in parentheses).	
Response to AFT and continued to planned HCT with < 1 month delay	13 (48)
Response to AFT and continued to planned HCT with > 1 month delay	4 (15)
Response to AFT but did not proceed to planned HCT	2 (7)
Response to AFT but not a candidate for HCT in first remission	5 (19)
Died from or with the IFI	3 (11)
1-year survival by AML response and development of an IFI	**Probability (95% CI)**
AML in CR/CRi and no IFI (n = 147)	81 (75–87)*
AML in CR/CRi and developed IFI (n = 20)	75 (56–94)*
No response and no IFI (n = 23)	44 (24–64)*
No response and developed IFI (n = 7)	29 (1–62)*

CumInc, Cumulative Incidence; 95% CI 95% confidence interval; IFI, invasive fungal infection; IPA, invasive pulmonary aspergillosis; CR, complete remission; CRi, bone marrow CR but with incomplete hematologic recovery, usually either incomplete platelet or neutrophil recovery; Ind-1/Ind-2, first induction chemotherapy/second induction (or re-induction) chemotherapy; AFT, antifungal therapy

*$P < 0.001$ for the comparison of these two groups

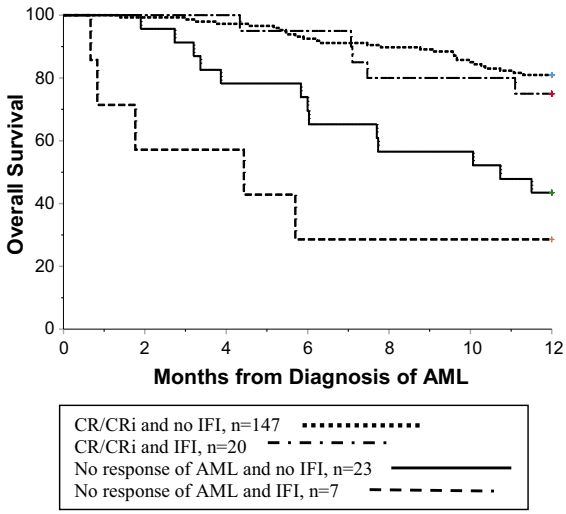

Fig. 1 One-year overall survival according to the development of an invasive fungal infection during induction or consolidation and the achievement of complete remission with (CR) or without (CRi) complete hematologic recovery

response, irrespective of the presence or absence of an IFI (Table 2 and Fig. 1).

Severe Bacterial Bloodstream Infections and Outcomes

The rate of GNB bacteremia was 25%, with a 90-day overall mortality of 24%, while 27% of patients developed GPC bacteremia (excluding CNS), with a 90-day overall mortality of 16%. Details of the types of bacterial infections are shown in Table 3. There were 14 cases of MDR GNB bacteremia, 12 enterobacteria and 2 *P. aeruginosa*.

Variables with an Impact on 1-Year Overall Survival

The OS at 1 year was 70% (95% CI 65–75%) in all 214 patients. Table 4 shows the Cox regression analysis for survival. In multivariate analysis, higher OS was found in patients treated in the later LAM-12 protocol, in those in the favorable/intermediate cytogenetic/molecular risk groups, in patients who achieved CR/CRi to induction/re-induction therapy and in patients who did not develop bacteremia during the study period. (The latter two variables were analyzed

Table 3 Incidence of bloodstream bacterial infections and their outcome during induction and consolidation chemotherapy (% in parentheses)

Type of bacteremia	Number of cases (% of total patients)	90-day mortality (% of cases)
Total patients with at least one bacteremia	96 (45)[a]	19 (20)
Bacteremia by GNB	54 (25)[a]	13 (24)
Enterobacteria bacteremia	39 (18)	9 (23)
Non-MDR enterobacteria	27 (13)	4 (15)
MDR enterobacteria	12 (5)	5 (42)
ESBL enterobacteria	8	3
KPC enterobacteria	4	2
GNFGNB	15 (7)	4 (26)
P. aeruginosa	10 (5)[b]	3
S. maltophila	5 (2)	1
GPC bacteremia (excluding CNS)	57 (27)[a]	9 (16)
E. faecium or E. fecalis	32 (15)	5 (16)
Viridans-group streptococci	17 (8)	1 (5)
S. aureus	8 (3)	3 (38)

GNB, gram-negative bacilli; MDR, multi-drug resistant; ESBL, extended-spectrum beta-lactamase; KPC, *Klebsiella pneumoniae*-type carbapenemase; GNFGNB, glucose non-fermenting GNB; GPC, gram-positive cocci; CNS, coagulase-negative staphylococci

[a]Fifteen patients developed separate bacteremias by both a GNB and a GPC during induction and consolidation chemotherapy, and thus, the number of bacteremias (n = 111) is higher than the number of patients (n = 96)

[b]Includes 2 cases of MDR *P. aeruginosa*

Table 4 Multivariate analysis of the 1-year survival

	Overall analysis[b]		
	1-year survival (95% CI)	HR (95% CI)	Mutivariate *P*
Time period and CETLAM protocol			
LAM-99 and LAM-03 (2002–2011)	62 (55–70)	1	0.02
LAM-12 (2012–2018)	73 (64–84)	0.7 (0.2–0.9)	
Cytogenetic/molecular risk group			
Favorable	81 (71–91)	1	0.03
Intermediate	72 (62–79)	0.9 (0.8–1.2)	
Adverse[b]	48 (40–56)	0.4 (0.2–0.7)	
Patient age			
Below 60 years	73 (60–81)	NA	0.3
$\geq$ 60 years	60 (50–70)		
Leukocytes at diagnosis			
Below 50×10^9/L	72 (66–78)	NA	0.1
$\geq 50 \times 10^9$/L	57 (44–70)		
Achieved CR/CRi[a]	[c]	0.25 (0.01–0.5)	< 0.01
IFI during induction/consolidation[a]	[d]	1.1 (0.6–1.5)	0.8
GNB or GPC bacteremia during	[e]		0.02
Induction/consolidation[a]		2.4 (1.3–3.8)	

HR, hazard Ratio; 95% CI 95% confidence interval; CR, complete remission; CRi, bone marrow CR but with incomplete hematologic recovery, usually either incomplete platelet or neutrophil recovery; CETLAM, Grupo Cooperativo para el Estudio y Tratamiento de las Leucemias Agudas y Mielodisplasias (www.cetlam.com); IFI, invasive fungal infection; GNB, gram-negative bacilli; GPC, gram-positive cocci

[a]Analyzed as a time-dependent covariate

[b]Multivariate analysis was done in the whole patient population, without censoring patients with an adverse prognosis at the time of allogeneic transplantation

[c]With time-dependent covariates survival from day 1 of treatment to day 365 is not adequately shown with a Kaplan–Meier survival estimate. However, for descriptive purposes only, the 1-year survival in patients with and without CR was 81% versus 44%

[d]With time-dependent covariates, survival from day 1 of treatment to day 365 is not adequately shown with a Kaplan–Meier survival estimate. However, for descriptive purposes only, the 1-year survival in patients with and without an IFI was 64% versus 71%

[e]With time-dependent covariates, survival from day 1 of treatment to day 365 is not adequately shown with a Kaplan–Meier survival estimate. However, for descriptive purposes only, the 1-year survival in patients with and without bacteremia was 62% versus 74%

as time-dependent covariates.) However, developing an IFI during induction/consolidation did not have an impact of 1-year survival (with IFI 64% (95% CI 49–75%) vs. 71% (64% CI 49–78%) without IFI, $P = 0.8$).

Case–Control Study and Potential Risk Factors for IPA

To explore potential risk factors associated with the development of proven/probable IA during/after consolidation therapy, we compared the clinical characteristics of the 23 patients diagnosed with IPA with those of the 69 contemporary control patients. As shown in Table 5, in univariate analysis, cases had a higher OR of being post-induction/re-induction chemotherapy (vs. consolidation), suffered more from a chronic bronchopulmonary disease or had a heavy smoking history than controls, had a poor baseline performance status, developed more prolonged and profound neutropenia and monocytopenia after the last chemotherapy received than controls (as defined in Table 5) and showed a trend toward having an age $\geq$ 60 years. A trend toward a higher OR of being diagnosed a lower respiratory tract infection (LRTI) by a conventional respiratory virus was also found,

Table 5 Univariate analysis of case–control study of potential risk factors for developing invasive pulmonary aspergillosis during induction and consolidation chemotherapy

Baseline variables and Post-chemotherapy time-dependent covariates	Cases with IPA	Controls	Univariate Odds Ratio (95% CI)	*P*
Baseline variables				
Total Number of patients	23	69		
Post-induction chemotherapy status	16 (69)	29 (42)	–	
Post-consolidation chemotherapy status	2 (9)	38 (55)	2.3 (1.4–4.1)	0.01
Post-re-induction chemotherapy status	5 (22)	2 (3)	–	
Patient age $\geq$ 60 years	15 (65)	27 (39)	1.8 (0.9–3.6)	0.07
Achieved CR/CRi	18 (78)	57 (83)	NA	ns
Suffered from chronic bronchopulmonary disease or smoking > 10 years/> 10 cigarettes per day	9 (39)	10 (14)	3.6 (1.2–11.6)	0.02
Baseline poor performance status (ECOG $\geq$ 2)	11 (48)	13 (19)	3.1 (1.3–6.7)	0.01
Post-treatment (time-dependent) variables				
Neutropenia $< 0.2 \times 10^9$/L for > 14 days during current induction or consolidation	16 (70)	21 (30)	2.8 (1.01–10.1)	0.05
Monocytopenia $< 0.2 \times 10^9$/L for > 10 days during current induction or consolidation	20 (87)	17 (25)	4 (1.8–9.5)	0.01
Had a LRTI by a respiratory virus diagnosed during induction or consolidation (before IPA in "Cases")	5 (22)	2 (3)	3.4 (0.91–9.8)	0.07
Developed bacteremia during current induction or consolidation	6 (26)	14 (21)	NA	ns
Remained for > 4 days outside the hematology ward (outside protected air environment) during post-treatment pancytopenia	5 (22)	26 (38)	NA	ns

ECOG, Eastern Cooperative Oncology Group performance status; CR, complete remission; CRi, bone marrow CR but with incomplete hematological recovery, usually either incomplete platelet or neutrophil recovery; IPA, invasive pulmonary aspergillosis; LRTI, lower respiratory tract infection; ns, not statistically significant; NA, not applicable due to the lack of even a statistical trend

although this comparison is biased by the fact that only 12/69 (17%) controls underwent a BAL as opposed to 18/23 (78%) patients with an IPA. No differences were apparent in the proportion of patients with AML in CR during treatment, development of bacteremia or being admitted/housed during this course of pancytopenia outside the HEPA-filtered inpatient ward for > 4 days.

Discussion

During induction and consolidation chemotherapy, widespread use of fluconazole has reduced the overall incidence of *Candida* infections markedly with IMI, mostly aspergillosis, now being the major IFI in patients with AML. Primary antifungal prophylaxis with posaconazole, voriconazole or an echinocandin has reduced the incidence of IPA to < 5–10% in centers with previously high rates of this infection [6], including centers from our geographical area [10–12]. Using broad-spectrum prophylaxis in all patients, however, has many potential caveats, and individual centers may choose not to use it, provided they have a low incidence of probable/proven IPA (specifically, < 10% [5, 7] and can assure early diagnosis of this infection and a low mortality with a strictly implemented diagnostic-driven therapeutic approach. Our strategy has relied on allowing a few days of EAFT in neutropenic AML patients with PFUO or suspicion of a possible IFI [18], while a diagnostic-driven strategy is done in parallel [7, 16, 33]. We consider that withholding EAFT in a very high risk symptomatic patient would be justified only if there were single rapidly available serologic, antigenic or molecular tests which could exclude the presence of an IFI with a certainty (or negative predictive value) of > 95% in symptomatic patients at high risk of developing an IFI. This justifies the real-world use of EAFT for a few days until all the available tests have

been run and the presence of an IFI can then be excluded, including high-resolution thoracic radiology [1, 6, 7, 18]. During (or even before) this period of EAFT, sGM screening has a high positive predictive value and a high negative predictive value in these high-risk patients provided the test is done at least two days each week and patients do not receive prophylaxis with a mold-active antifungal (i.e., posaconazole) [34]. In addition, T-CT has a very high negative predictive value, and a normal result excludes a pulmonary IMI. On the other hand, early T-CT shows specific findings for an IPA in most cases [16], and sGM and/or a positive bGM will lead to the early diagnosis and treatment of an IPA [7, 31].

Our results show that without posaconazole-based prophylaxis, and in a real-life clinical setting, the incidence of probable/proven IFI considering both induction and consolidation chemotherapy for de novo AML is 9% (8% of IPA), with a very low IFI-related mortality (3/214 patients) and, more importantly, no evidence that developing an IFI had an independent impact on 1-year OS. This latter finding is probably due to the small patient numbers, and we would like to emphasize that most studies do indeed find that developing an IFI, especially an IPA, has a negative impact on mid and long-term survival in patients with AML [6, 31, 32, 35, 36]. We did, in fact, find that the 1-year survival was better in more recently treated patients, who had a higher rate of possible/probable/proven IPA (14% vs. 9%; see Table 1). On the other hand, variables with a strong impact on survival were having adverse cytogenetic/molecular abnormalities and being refractory to induction/re-induction chemotherapy. We did not censor patients at the time of alloHSCT, since this would have falsely biased the survival of patients with the highest risk of dying from their AML; during the entire study period, patients who were considered to be at high risk of AML progression and death were assigned to receive an alloHSCT in first CR/CRi (detailed in Supplemental Table 1S). If such patients were censored at the time of alloHSCT, being by definition still alive, then their 1-year survival would be estimated to be much higher than their actual survival. To analyze the impact of receiving an alloHSCT on survival, we introduced this variable as a time-dependent covariate in the final multivariate analysis shown in Table 4. The overall result did not change, and receiving an alloHSCT had no impact on the 1-year OS (data not shown). We did, however, explore the impact that developing an IFI had on receiving a planned alloHSCT; of the 19 patients who responded to antifungal therapy and were to receive an alloHSCT, only 2 (11%) did not receive this planned therapy solely due to the development of an IFI (details in Table 2).

Although the study was primarily focused on IFIs, we also analyzed the rates of bacteremias during the pancytopenic phase after induction and consolidation chemotherapy, since MDR bacteria, especially GNB, are currently one of the largest threats worldwide in the treatment of patients with hematological malignancies [27, 28]. Although we did not focus on MDR GNB, due to their small numbers in the current study, most patients (45%) developed bacteremia by a GNB (25%) and/or a non-CNS GPC (27%) during induction or consolidation. Most importantly, developing bacteremia was found to have an impact on the 1-year OS, while it had no apparent impact on the development of an IFI.

Due to the retrospective nature of the study, we did not have detailed patient characteristics at the onset of induction chemotherapy in addition to those included in the CETLAM database, especially for patients who did not develop an IFI. Thus, we could not do a risk factor analysis for the development of IPA (the most common IFI) in all 214 patients. A case–control study was thus done by the first author (R.M.), matching three controls per each case of IPA according to the time chemotherapy was started (induction, re-induction or consolidation). In univariate analysis, receiving induction/re-induction chemotherapy increased the OR of developing IPA, with respect to consolidation chemotherapy. This has been described in several studies [4, 6, 37], and although it would suggest that posaconazole prophylaxis can be omitted from the consolidation cycles, one should consider whether patients will be treated as outpatients during consolidation or as inpatients during the whole phase of pancytopenia, as in most of our patients. In a fully outpatient high-dose Ara-C-based consolidation, contrarily to the results reported herein, we consider that posaconazole prophylaxis should be given since patients will be exposed to high concentrations of airborne fungal spores, which is the case in our geographic area [9]. Other variables found to increase the OR of IPA include having a chronic respiratory disease and/or history of heavy smoking, having a poor performance status, elderly age, longer duration

of neutropenia and monocytopenia, and having a LRTI by a respiratory virus during the post-chemotherapy pancytopenia. All of these factors have been described as risk factors for IPA [1, 9, 13].

We are considering introducing in our center prophylaxis with a mold-active antifungal (posaconazole, voriconazole, isavuconazole, lipid formulations of AmB or an echinocandin) on a case-by-case basis; thus, inpatients with AML who are receiving induction or re-induction chemotherapy and have an age of > 60 years or have a poor performance status, suffer from any chronic bronchopulmonary condition or have a smoking history or any other known risk factor for heavy pulmonary colonization by fungal spores, such as farming, construction work, gardening or composting [1, 38, 39]. Such a differentiated approach would help target patients most likely to benefit from antifungal treatment while limiting unnecessary antifungal use that might only serve to add antifungal-related toxicities or life-threatening drug interactions. In this respect, isavuconazole is generating great interest among clinicians; its good oral bioavailability, its broad-spectrum of activity (similar to posaconazole) and, especially, its better safety profile (weaker drug interactions mediated by the P450 cytochrome, lower hepatotoxicity and shortening rather prolongation of the QTc interval) are surely appealing. Recent studies in AML induction [43] and after allogeneic transplantation [44] have proven the good tolerability and high feasibility as primary AFP, although the rate of possible/probable/proven IFI was 18% after AML induction and day + 100 post-allogeneic transplantation, respectively [43, 44]. In addition, high rates of breakthrough IFIs have been found in another recent study [45]. It is thus obvious that universal prophylaxis with isavuconazole will not be a solution to primary AFP.

Targeted prophylaxis is in line with current recommendations [5, 7], and time will tell whether the incidence of IPA (and other IFIs) in patients with AML during induction and consolidation can be reduced even further in our center.

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Authors' Contributions RM was involved in conception and execution of the research reported in the paper, the integrity and analysis of the data, inpatient care and writing the various versions of the manuscript. In addition, he also had the task of data management and statistical analyses. AG and MS collaborated in patient care, performed most data management, contributed to the conception and execution of the research reported in the paper, and participated in writing or interpreting relevant parts of the manuscript. All other co-authors contributed to the conception and execution of the research reported in the paper, collaborated in patient care and participated in writing or interpreting relevant parts of the manuscript.

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Compliance with Ethical Standards

Conflict of interest None of the authors reports any conflict of interest for this manuscript.

Ethics Approval Due to the retrospective nature of the study, ethical approval was waived by our center's Ethical Committee. However, all patients signed an informed consent for each of the 3 CETLAM protocols in which they were included, which complied with national ethical regulations as well as the principles laid in the 1964 Declaration of Helsinki.

Consent for Publication All authors agree to the submission of the manuscript in its actual format.

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